As the COVID19 pandemic ignited in the United States the Norris Laboratory quickly responded by expanding essential BSL3 capacity at the University of Florida, enabling antiviral and pathophysiological studies of the SARS-CoV-2 virus at the Emerging Pathogens Institute. In addition to SARS-CoV-2 research, the cellular and molecular pathogenesis of Bacillus anthracis in natural hosts, from amoeba to animals, and its evolution are being investigated. The lab operates within the Spatial Epidemiology and Ecology Research Lab headed by Dr. Jason Blackburn to assist with genomics of the anthrax causing species B. anthracis and Bacillus cereus biovar anthracis (Bcbva). The montage on the cover illustrates how the COVID19 pandemic SARS-CoV-2 virus has not only dominated our lives but has become an important research subject at the Emerging Pathogens Institute. Plaque formation by the SARS-CoV-2 virus is shown in the red circular images. In the gray image, spores of B. anthracis can be seen inside Acanthamoeba castellanii shortly after phagocytosis. This image is juxtaposed with circular whole genome SNP trees of insect and animal isolates of the emergent anthrax pathogen Bcbva. The chemical structure shown is a sugar containing the monosaccharide residue anthrose at its tip. Most Bacillus anthracis coat the outside of their spore with inumerable copies of this antigenic sugar. Several B. anthracis strains from Africa and now other places in the world possess mutations rendering the spore surface free of anthrose, potentially increasing lethality of this dangerous bacterium.

UF | Emerging Pathogens Institute
UNIVERSITY of FLORIDA

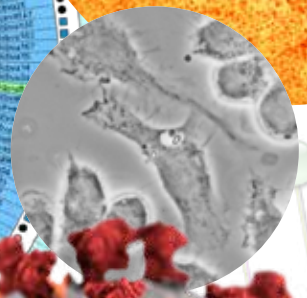
EPI RESEARCH DAY

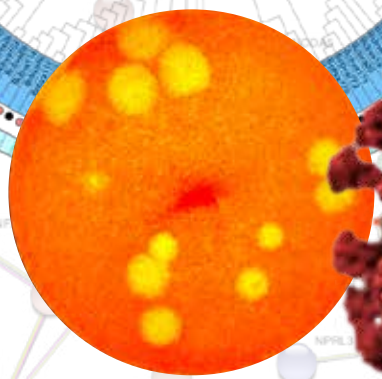
BOOK OF ABSTRACTS

2021

EMERGING PATHOGENS INSTITUTE

RESEARCH DAY





BOOK OF ABSTRACTS

FEBRUARY 2021

Table of Contents

Letter from the Director 2

Schedule of Events 3

Keynote Speakers 4

Enteric and Foodborne Pathogens 5-22
Abstracts 1-11

Influenza and Respiratory Viruses 23-39
Abstracts 12-22

Parasitic and Fungal Diseases 40-45
Abstracts 23-26

Tuberculosis and Mycobacterial Diseases 46-48
Abstracts 27-28

Vector-Borne Diseases 49-79
Abstracts 29-52

Other Bacterial Pathogens 80-94
Abstracts 53-63

Other Viral Pathogens 95-113
Abstracts 64-75

Other Topic Areas 114-136
Abstracts 76-91

Index 137-145

Letter from the Director

Welcome to the fourteenth annual EPI Research Day! It is also the first virtual Research Day, reflecting ongoing concerns about SARS-CoV-2. While we will all miss the close contact of previous years, we are hopeful you will like the virtual format, and take full advantage of the range of interactions possible at a virtual level. As you look though the abstracts and view the associated posters, you should get a feel for the wide range of emerging pathogens-related research conducted by EPI members and collaborators at the University of Florida. In keeping with the interdisciplinary nature of EPI, authors come from eight different UF Colleges. We are also pleased to welcome investigators from outside of UF, including collaborators from other Universities and state and federal agencies.

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

Dr. Andrew P. Dobson is a Professor in the Department of Ecology and Evolutionary Biology at Princeton. His research focuses on the population and community ecology of infectious diseases in a variety of endangered and fragile ecosystems: the Serengeti in East Africa, the coastal salt marshes and grasslands of California; the forest fragments of Malaysia and Bangladesh, and the eyes of the finches in the back yards of New England. He also works on the interaction between climate variability and the transmission of malaria and cholera in India and Bangladesh.

Dr. Philippe Sansonetti is currently an emeritus professor at Institut Pasteur Paris, professor at the College de France, and head of The Center for Microbes, Development & Health at Institut Pasteur, Shanghai. He was a pioneer in the field of cellular microbiology, with landmark work in deciphering the molecular and cellular mechanisms of Shigella pathogenesis; he has applied similar approaches to decipher the symbiotic mechanisms established between a host and its gut microbiota.

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

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

Schedule of Events

9:00 AM – 10:30 AM	Poster Session 1 (odd poster numbers) *Presenters: Start a meeting in your designated Poster Room*
10:30 AM – 12:00 PM	Poster Session 2 (even poster numbers) *Presenters: Start a meeting in your designated Poster Room*
12:00 PM – 1:00 PM	Lunch Break
1:00 PM – 1:10 PM	Introductions *Dr. J. Glenn Morris* *Director, EPI*
1:10 PM – 3:15 PM	Keynote Speeches *Dr. Andrew P. Dobson* *Dr. Philippe Sansonetti*

1:10 – 2:10pm

Andy Dobson, D.Phil.

Professor
Princeton University

"*Ecology, Economics and evolution of Emerging Pathogens*"

2:10 – 3:10pm

Philippe J. Sansonetti, M.D.

Professor, Collège de France
Emeritus Professor, Institut Pasteur Paris
Head of The Center for Microbes, Development and Health, Institut Pasteur Shanghai

"*Microbes without borders: Tensions on the 20th century paradigm of public health*"

01. ANALYSIS OF INNATE IMMUNE RESPONSES TO EXOSOMES FROM SALMONELLA-INFECTED MACROPHAGES

Lisa E. Emerson - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Jatin Sharma** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Austin Sheppe** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Carol Hoffman** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Gustavo Maegawa** - Department of Pediatrics, College of Medicine, University of Florida; **Joseph Larkin III** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **David W. Pascual** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Mariola J. Edelmann** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Introduction: Non-typhoidal Salmonella (NTS) is an emerging public health concern causing over 95-million infections globally each year. In other words, one in every ten people can expect to be infected with NTS each year and incur symptoms including diarrhea, nausea, vomiting, gastrointestinal cramping, and fever. Infection is typically self-limiting but can become a severe, invasive infection called invasive NTS (iNTS), which has a case-fatality rate of 20%. Despite a high disease burden and the number of deadly infections, an NTS prevention strategy has not been developed. Salmonella is spread by ingesting contaminated food or water, and once ingested, Salmonella can infect a variety of host cells, including macrophages. Once Salmonella inside the macrophage, the macrophage begins to secrete exosomes that contain Salmonella

antigens. Exosomes are extracellular vesicles, which are 30-150 nm in diameter, and are used by the host for intercellular communication. While exosomes formed during infection have been shown to contain Salmonella antigens, the immunological role of exosomes has not been characterized.

Methods: To study this, we performed two experiments. We collected exosomes 24- and 48- hours after Salmonella infection of RAW264.7 macrophages. Next, we treated mice with 40 µg of exosomes intranasally or PBS for control animals. After 24 hours, we sacrificed and dissected the mice, made a single-cell suspension of lung tissue, and analyzed the lung cell subsets using flow cytometry. In the second experiment, we used the harvested exosomes to treat Bone Marrow-Derived Macrophages (BMDMs) and analyzed polarization markers iNOS, TNFα, IL-10, and Arg-1 using RT-qPCR.

Results: In the first experiment, we found that populations of interstitial macrophages, CD11b+ dendritic cells, and CD103+ dendritic cells were activated. Alveolar macrophages did not show an increase between control and exosome-treated mice. The second experiment displayed polarization towards an M1 response with markers iNOS and TNFα significantly increased between control and exosome treated groups.

Conclusions: Overall, our study suggests that exosomes alone are capable of eliciting an innate immune response. BMDMs were polarized towards an M1 response after exosome treatment, and subsets of innate immune cells were activated in the lungs 24 hours after exosome treatment. This is a novel finding, and the immunological role of exosomes from infected cells warrants further exploration.

02. CAMPYLOBACTER COLONIZATION, ENVIRONMENTAL ENTERIC DYSFUNCTION, STUNTING, AND ASSOCIATED RISK FACTORS AMONG YOUNG CHILDREN IN RURAL ETHIOPIA: A CROSS-SECTIONAL STUDY FROM THE CAMPYLOBACTER GENOMICS AND ENVIRONMENTAL ENTERIC DYSFUNCTION (CAGED) PROJECT

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

Livestock farming provides a possible mechanism by which smallholder farmers can meet their household need for animal source foods (ASF), which may reduce the risk of stunting. However, direct/indirect contacts with domestic animals may increase colonization by Campylobacter spp., which has been associated with Environmental Enteric Dysfunction (EED) and stunting. A cross-sectional study involving 102 randomly selected children between 12 and 16 months of age was conducted in rural eastern Ethiopia to establish prevalence rates of Campylobacter colonization, EED, and stunting, and evaluate potential risk factors. Data were collected between September and December 2018. The prevalence of EED and stunting was 50% (95% CI: 40% - 60%) and 41% (95% CI: 32% - 51%), respectively. Among enrolled children, 56% had consumed some ASF in the previous 24 hours; 47% had diarrhea and 50% had fever in the past 15 days. 54%, 63%, 71% or 43% of households owned at least one chicken, cow/bull, goat, or sheep; 54 (53%) households kept chickens indoors overnight and only half of these confined the animals. Sanitation was poor, with high levels of unimproved latrines and open defecation. Most households had access to an improved source of drinking water. The prevalence of Campylobacter colonization was 50% (95% CI: 41% - 60%) by PCR. In addition to the thermotolerant species C. jejuni, C. coli and C. upsaliensis, non-thermotolerant species related to C.

hyointestinalis and C. fetus were frequently detected by Meta-total RNA sequencing (MeTRS). Current breastfeeding and ASF consumption increased the odds of Campylobacter detection by PCR, while improved drinking water supply decreased the odds of EED. No risk factors were significantly associated with stunting. Further studies are necessary to better understand reservoirs and transmission pathways of Campylobacter spp. and their potential impact on child health.

03. COMPARATIVE ASSESSMENT OF BIOFILM FORMATION AMONG VARIOUS SEROTYPES OF VIBRIO CHOLERAE

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; **Mohammad Shahnoor Hossain** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Vanessa Rouzier** - Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Mohammed Rashid** - Emerging Pathogens Institute, 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; **Jean William Pape** - Emerging Pathogens Institute, University of Florida; **J. Glenn Morris, Jr.** - 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: Toxigenic Vibrio cholerae, a water-borne pathogen can cause a profuse diarrheal disease Cholera and remains a major public health concern globally. Survival and persistence of V. cholerae in aquatic reservoirs largely depend on its ability to form biofilm in association with biotic and/or abiotic surfaces in those reservoirs. Recently, we have isolated diverse serotypes of V. cholerae O1 strains from aquatic

reservoirs in Haiti. In the present study, we aim to evaluate the comparative biofilm forming ability between clinical and diverse environmental serotypes of V. cholerae isolated in Haiti.

Method: Biofilm assay was performed following the standard biofilm measurement method using 24-well cell culture plate. Data were plotted and statistical analyses were performed in GraphPad prism 8.

Results: Regardless of origin, our results indicate that Ogawa and poly positive serotypes of V. cholerae strains have comparatively higher biofilm forming capacity (~6 and ~8 fold higher, respectively) than Inaba serotype. We also found that non-toxigenic strains are better biofilm producers (~2 fold higher biofilm formation) than the toxigenic strains, and the environmental strains have comparatively higher biofilm forming ability (~3 and ~5 fold higher for environmental Ogawa and poly positive respectively) compared to the clinical isolates. Our preliminary findings suggest that V. cholerae strains show wide variation in biofilm forming ability based on their serotypes and origin indicating that different serotypes might have responded to distinct (vps-dependent and vps-independent) biofilm forming mechanism. Our results also indicate that, the gene expressions for several important biofilm related genes are high among the environmental non-toxigenic ogawa and environmental non-toxigenic Inaba strains isolated from Haiti.

Conclusions: We suggest that higher biofilm former strains/serotypes would better adapt to environmental persistence promoting the transmission of cholera in Haiti and potentially globally. Further studies are required to unravel the genetic basis of serotype dependent variation of biofilm formation among the isolates. Periodic emergence and reemergence of cholera pose a major public health concern all over the world. Understanding the genetic basis of serotype dependent variation of biofilm in V. cholerae will help us to design better intervention strategies to reduce cholera cases and associated public health burden.

04. DEVELOPMENT OF A CHOLERA RAPID DIAGNOSTIC TEST THAT TARGETS BOTH VIBRIO CHOLERAE AND VIBRIOPHAGE

Md. Abu Sayeed - Department of Environmental and Global Health, University of Florida; **Ashraful Islam Khan** - Infectious Disease Division, International Centre for Diarrhoeal Disease Research, Bangladesh; **Yasmin Ara Begum** - Infectious Disease Division, International Centre for Diarrhoeal Disease Research, Bangladesh; **Firdausi Qadri** - Infectious Disease Division, International Centre for Diarrhoeal Disease Research, Bangladesh; **Eric J. Nelson** - Department of Environmental and Global Health, University of Florida

Introduction: Cholera is an acute watery diarrheal disease that can cause severe dehydration and death. It is an important public health problem in Asia, Africa and Latin America with 3-5 million cases globally and over 10,000 deaths annually. Rapid and accurate diagnosis of cholera is critical to effectively mitigate cholera outbreaks. Early detection of the etiologic agent Vibrio cholerae is difficult because outbreaks often occur in settings without laboratories. Rapid diagnostic tests (RDTs) offer attractive solutions yet have limitations. Several immunochromatography or lateral flow immunoassay-based RDTs targeting V. cholerae O1 specific antigens exist. However, the odds of an RDT testing positive are reduced by nearly 90% when lytic vibriophage (ICP1) are present in the stool sample. We hypothesize that adding a monoclonal antibody for ICP1 as a proxy for V. cholerae will increase diagnostic reliability when vibriophage are present.

Methodology and Results: Reverse vaccinology was used to identify immunogenic vibriophage proteins. A total of 48 conserved ICP1 open reading frames (ORFs) were screened using VaxiJen and IEDB to identify probable immunogenic proteins and epitopes, respectively. Out of the 48 candidates, we found 16 conserved ORFs with high predictive scores. To assure geographic generalizability, a subset of the ORFs (n=6) were detected in ICP1 positive stools from both Bangladesh and South Sudan; non-cholera controls stools were negative for these ORFs. Two final candidates were chosen for expression and monoclonal production: ORF75 (baseplate protein) and head protein (ORF122). After expression in and purification from BL21 cells, mAbs are now being raised in a

commercial murine model to these proteins. After further selection, one mAb will be used to develop a new RDT that will be tested in a diagnostic clinical study (n=100 cholera patients; 50 with ICP1 and 50 without ICP1) to test for significant differences in sensitivity and specificity between RDTs with and without the mAb for ICP1.

Conclusion: If successful, a more reliable RDT may significantly advance cholera elimination efforts and clinical decision-support. Recognizing that RDTs may fail because of lytic bacteriophage and pivoting to using mAbs for phage as a proxy for the primary pathogen target will likely impact diagnostic development for other bacterial diseases with similar challenges.

05. DISSEMINATION MECHANISMS OF NEW DELHI METALLO-B-LACTAMASE GENES IN HOSPITALIZED PATIENTS

Yuting Zhai - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Shinyoung Lee** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Lin Teng** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Zhengxin Ma** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Nicole B. Hilliard** - Infection Control, University of Florida Health/Shands Hospital; **Kartikeya Cherabuddi -** College of Medicine, University of Florida; **Kenneth H. Rand** - College of Medicine, University of Florida; **J. Glenn Morris, Jr.** - Emerging Pathogens Institute, College of Medicine, University of Florida; **Nicole M. Iovine** - College of Medicine, University of Florida; **Kwang Cheol Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Background: New Delhi metallo-β-lactamase (NDM) producing Enterobacteriaceae is a major clinical concern worldwide. We characterized NDM-positive pathogens isolated from patients and

assessed the dissemination patterns of the blaNDM genes in a hospital setting.

Methods: Eleven NDM positive Enterobacteriaceae (three Enterobacter hormaechei, six Klebsiella pneumoniae and two Escherichia coli) were isolated from nine patients over a one-year period. A combination of short- and long-read whole genome sequencing was used for genome analysis. Clinical treatment history of patients was linked with genetic features of individual isolates to investigate the dissemination patterns of the blaNDM genes and NDM-positive strains.

Results: blaNDM in clonal K. pneumoniae were transmitted between two patients. In other instances, an identical IncC plasmid encoding NDM-1 was transmitted between E. coli and K. pneumoniae isolated from the same patient, and the same IncX3 plasmid, carrying blaNDM-1 or blaNDM-5, was harbored in E. hormaechei. Varying patterns of insertion sequence (IS) elements were identified as a critical transmission mechanism in association with blaNDM genes.

Conclusions: Multiple transmission patterns were identified in hospitalized patients, including dissemination of clonal bacterial strains carrying resistance genes, and horizontal transfer of resistance genes among divergent bacterial strains. Controlling spread of NDM is complex: while attention to standard infection control practices is critically important, this needs to be matched by aggressive efforts to limit unnecessary antimicrobial use, to minimize the selection for and risk of transfer of “high mobility” resistance genes among Enterobacteriaceae.

06. FLORIDA CLINICAL CASES OF SALMONELLA ENTERITIDIS ARE GENETICALLY LINKED WITH CHICKEN ISOLATES ACROSS USA: RETROSPECTIVE ANALYSIS, 2017-2018

Nitya Singh - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Xiaolong Li** - Department of Environmental and Global Health, Emerging Pathogens Institute, University of Florida; **Elizabeth M Beshears** - Bureau of Public Health Laboratories, Florida Department of Health; **Jason L. Blanton** - Bureau of Public Health Laboratories, Florida Department of Health; **Jamie DeMent** - Bureau of Public Health Laboratories, Florida Department of Health; **Arie Havelaar** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

The state of Florida reports a high burden of non-typhoidal Salmonella enterica with an incidence that is approximately two times higher than the national incidence. Serotype Enteritidis has been reported as the most prevalent in Florida and across the USA. We used a combination approach comprising core-genome MLST (Multilocus sequence typing) profiling/ hierarchical-clustering (HC) and SNP-based phylogeny to retrospectively explore the genetic relatedness of S. Enteritidis isolates from clinical (176 isolates) and poultry (24 isolates) sources in Florida, collected during 2017-2018. Because a substantial proportion of poultry meat consumed in Florida is produced in other states, we also included 1317 poultry isolates from other states, collected during the same period. Within Florida, we detected four common HC5 clusters (<= 5 allelic difference) with >2 clinical isolates, comprising 55 clinical and 21 poultry isolates. We found 492 poultry isolates collected across the USA in 8 HC5 clusters with Florida clinical isolates. Four clusters included clinical, and poultry isolates from Florida as well as poultry isolates from other states. The other four clusters included clinical isolates from Florida and only poultry isolates from other states. As S. Enteritidis is a highly clonal organism, to further identify closely related clusters, we used a small range of 0-4 SNP distance as cutoff for close genetic relatedness and observed between two and six such clusters within six HC5 clusters.

Geospatial and time-series analysis of these SNP clusters, stratified by state and collection source demonstrated that, while temporally linked to human isolates, the poultry isolates were distant in time and across locations throughout the US. This suggests the vertical transmission of Salmonella clones from higher levels in the breeding pyramid to production flocks that caused widespread and sporadic occurrence of genetically similar isolates from poultry sources in different states and at different time points. Our results suggest that hierarchical clustering is an effective method to identify case clusters that are more distant in time and place than traditional outbreaks, but may have been infected from a common source. The potential of this new approach to source identification would be strengthened by more systematic surveillance of Salmonella in non-human reservoirs. Current data for Florida is very limited. Isolates in NCBI were mainly deposited by FSIS, thereby biasing the available data to animal source foods. Isolates from other foods and non-clinical sources from Florida are rare in the public domain and would benefit from more investigation.

07. GEOGRAPHICAL DISTRIBUTION AND SPACE-TIME CLUSTERING OF HUMAN INFECTIONS WITH MAJOR SALMONELLA SEROTYPES IN FLORIDA, 2017-2018

Xiaolong Li - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Nitya Singh** - Department of Animal Sciences, Emerging Pathogens Institute, University of Florida; **Jason Blackburn** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Elizabeth Beshearse** - Food and Waterborne Disease Program, Florida Department of Health; **Jason L. Blanton** - Bureau of Public Health Laboratories, Florida Department of Health; **Jamie DeMent -** Food and Waterborne Disease Program, Florida Department of Health; **Arie Havelaar** - Department of Animal Sciences, Emerging Pathogens Institute, University of Florida

A diversity of Salmonella enterica serotypes contribute to cases of nontyphoidal salmonellosis which is the leading reported foodborne illness in Florida. The spectrum of Salmonella serotypes circulating in Florida has been identified; however, the geographical characteristics and spatial patterns of the major serotypes are poorly understood. Here, we examined the geographical distribution of 803 whole-genome sequenced Salmonella isolates within seven major serotypes (Enteritidis, Newport, Javiana, Sandiego, Braenderup, Typhimurium, and i 4,[5],12:i:-) collected during 2017-2018. Metadata for these isolates were obtained from Florida Department of Health, and the EnteroBase platform was used for serotype prediction. The crude incidence rate of isolates for each serotype was smoothed with the Spatial Empirical Bayes method at the county level using GeoDa before disease mapping. Subsequently, we evaluated space-time clustering of isolates at the zip code level by employing retrospective discrete Poisson and multinomial scan statistic models in SaTScan software. Infections with serotypes Enteritidis, Newport and Braenderup were distributed across Florida, while areas with high incidence rate were concentrated in the central and south for Enteritidis, in the northwest and northeast for Newport, and in the southwest for Braenderup. Infections with Sandiego were distributed

across Florida, except the Panhandle area. In contrast, high incidence areas for Typhimurium were concentrated in the Panhandle. Serotype i 4,[5],12:i: was limited to the south of Florida. The incidence rate for Javiana was relatively higher in the north compared to the south, with cases clustered in the northeast. Space-time clusters were detected for each major serotype during 2017-2018. Multinomial scan statistic found that infection with Javiana posed a higher risk in the north and southwest in the fall of 2017, compared to other major serotypes. This serotype-specific clustering analysis will assist in determining distinct reservoirs and environmental determinants for the major serotypes in Florida.

08. HIGH-THROUGHPUT LOW-COST NL-QPCR FOR ENTEROPATHOGENS: A PROOF-OF-CONCEPT AMONG HOSPITALIZED PATIENTS IN BANGLADESH

Katelyn Flaherty - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Jessica A. Grembi** - Department of Medicine, College of Medicine, Stanford University; **Vasavi V. Ramachandran** - Department of Pediatrics, College of Medicine, Stanford University; **Farhana Haque** - University College London; **Selina Khatun** - Ministry of Health and Family Welfare, Government of Bangladesh; **Stacey Maple -** Stanford University; **Torben Becker** - Department of Emergency Medicine, College of Medicine, University of Florida; **Gary K. Schoolnik** - Department of Medicine, College of Medicine, Stanford University; **Andrew J. Hryckowian** - Department of Medicine, College of Medicine, University of Wisconsin-Madison; **Eric J. Nelson** - Department of Pediatrics, College of Medicine, University of Florida

Background: Diarrheal disease is a leading cause of morbidity and mortality globally, especially in low- and middle-income countries. High-throughput and low-cost approaches to identify etiologic agents are needed to guide public health mitigation. Nanoliter-qPCR (nl-qPCR) is an alternative to more expensive methods yet is nascent in development and without a proof-of-concept among hospitalized patients.

Methods: A census-based study was conducted among hospitalized patients admitted at two government hospitals in rural Bangladesh during a diarrheal outbreak period. DNA was extracted from stool samples and assayed by nl-qPCR for common bacterial, protozoan, and helminth enteropathogens as the primary outcome. Microbial data were analyzed for clinical and geospatial correlates of pathogen detection as secondary outcomes.

Results: A total of 961 patients were enrolled; 827 patients were included in the analysis. Enteropathogens were detected in 69% of patient samples; More than one enteropathogen was detected in 32%. Enteropathogens commonly detected by median cycle threshold (CT) were EAEC (26.0%), STEC (18.3%), ETEC (15.5% ST, 2.2% LT), Shigella spp. (14.8%), and Vibrio cholerae (tcpA 10.2%).

Conclusions: This study demonstrates a proof-of-concept for nl-qPCR as a high-throughput low-cost method for enteropathogen detection among hospitalized patients. The rates of detection were consistent with similar studies that use more expensive approaches.

09. POTENTIAL ANTIMICROBIAL RESISTANCE MITIGATION IN LIVESTOCK INDUSTRY THROUGH PRODUCTION SYSTEM MANAGEMENT AND ANIMAL BREEDING

Peixin Fan - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Lin Teng** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Zhengxin Ma** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Shinyoung Lee** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Corwin Nelson** - Department of Animal Sciences, College of Agricultural and Life Sciences, University of Florida; **Joseph Driver** - Department of Animal Sciences, College of Agricultural and Life Sciences, University of Florida; **Mauricio Elzo** - Department of Animal Sciences, College of Agricultural and Life Sciences, University of Florida; **Christina Boucher** - Department of Computer and Information Science and Engineering, University of Florida; **Kwang Cheol Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Introduction: The rapid spread of antimicrobial resistance (AMR) is an urgent global threat in public health. The gastrointestinal tract microbiota of farm animals is a reservoir of AMR, which can transfer to humans through the food chain. However, an effective AMR mitigation strategy is currently lacking at the pre-harvest level.

Purpose: The purpose of this study is to seek factors for AMR load in food-producing animals throughout the production lifecycle for developing potential AMR mitigation strategies.

Methods: We collected fecal samples from a beef cattle cohort (n = 278) raised without antibiotic exposure at different growth stages from birth, preweaning and postweaning stages on pasture, to the end of fattening stage in feedlot. The gut resistome was investigated using both culture-dependent and -independent (metagenomic sequencing) approaches.

The gut microbiota composition was detected using the 16S rRNA gene amplicon sequencing.

Results: We found that AMR in the gut of newborns was highly concentrated, but it was reduced in mature cattle grazing on pasture. However, AMR increased at the fattening stage in the feedlot. The change in the gut resistome was associated with the development of gut microbiota that was affected by mobile genetic elements, which greatly contributes to the AMR transmission. We identified critical bacteria for AMR increase in feedlot operation, which harbored specific transposons carrying antimicrobial resistance genes. Notably, the prevalence of AMR bacteria were strongly influenced by host genetics and inversely interacted with bacteria that were enriched in cattle grazing on pasture.

Significance: Our results highlight the advantage of cattle grazing system on reducing the AMR in beef cattle, and shed light on AMR mitigation in feedlot operation by modification of microbiome through animal breeding and management.

10. PROTECTIVE IMMUNE RESPONSES GENERATED BY EXOSOMES FROM SALMONELLA INFECTED MACROPHAGES

Hailey Barker - Department of Molecular Genetics and Microbiology, Undergraduate Research, College of Agricultural and Life Sciences, University of Florida; **Lisa E. Emerson** - Department of Molecular Genetics and Microbiology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Mark Ou** - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida; **Carol Hoffman** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **David W. Pascual** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Mariola J. Edelmann** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Introduction: Salmonella enterica serovar Typhimurium is a Gram-negative intracellular bacterium that is a causative agent of foodborne

infections. Salmonella can infect macrophages and survives within a Salmonella-containing vacuole. Our laboratory found that Salmonella-infected macrophages release extracellular nano-vesicles called exosomes that have immunomodulatory functions. Exosomes generated by infected macrophages package proteins, nucleic acids, metabolites, and lipids, including Salmonella antigens. We hypothesized that exosomes packaging pathogen-derived antigens are sufficient on their own to stimulate an immune response. While our previous work has characterized the antigen contents from infected macrophages, the extent to which the exosomes generate a protective immune response remains unexplored. IgA and IgG are immunoglobulins produced by the immune system as a response to an antigen, and immunoglobulin responses to specific antigens may indicate immune protection against the organism. The objectives of this project are to determine if exosomes produced by macrophages during Salmonella infection can generate pathogen-specific IgA and IgG responses and if the immune response is sufficient to protect against salmonellosis.

Methods: To study this, we divided 24 mice into three groups: exosome (EXO), positive control (ΔAroA), and negative control (PBS). Mice were treated intranasally with 40 µg of exosomes from Salmonella-infected cells (previously frozen), a protective dose of the vaccine strain of Salmonella (ΔaroA), or intranasally with 30 µl of PBS. Mouse blood and stool were collected every two weeks and analyzed for IgG and IgA responses. After four treatments, mice were given a challenge dose of UK-1 Salmonella and monitored for 14 days.

Results: Fecal IgA and serum IgG titers showed similar responses between the EXO and ΔaroA treatment groups and no measurable titers among PBS mice. Following the challenge, the PBS group mice had a mean survival of 5 days, the EXO group had a mean survival of 6 days, and ΔaroA had a mean survival of 11 days.

Conclusions: In short, the immune protection conferred by exosomes from Salmonella-infected cells has not yet been defined. This study aims to investigate conferred immune protection by monitoring fecal IgA and serum IgG titers and finally challenging mice with pathogenic Salmonella.

Results are suggestive of some conferred immune protection from exosomes derived from Salmonella-infected cells. Future work will be done with fresh exosome preparations instead of frozen samples, which are expected to have improved efficacy.

11. TRANSMISSION OF ANTIMICROBIAL RESISTANT GENES AT THE WILDLIFE-LIVESTOCK INTERFACE

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

Introduction: The spread of antimicrobial resistance (AMR) is a major concern for animal and human health and leads to economic costs. It is increasingly postulated that wildlife could play an important role in the emergence and transmission of antimicrobial resistant microorganisms. However, the occurrence and transmission of AMR at the wildlife-livestock interface remain elusive.

Purpose: The purpose of this study was investigating the transmission of antimicrobial resistant genes (ARGs) at the wildlife-livestock interface to help gain perception of developing migration strategies of AMR.

Methods: We collected 364 fecal samples from cattle, feral swine and environments. Cefotaxime resistant bacteria (CRB) were isolated by plating on MacConkey agar containing cefotaxime (4 μg/mL). CRB carrying either CTX-M or CMY-2 gene were selected for whole genome sequencing to characterize extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase-producing bacteria. Furthermore, microbiota transmission between cattle and feral swine was investigated using the 16S rRNA gene

amplicon sequencing. The gut resistome and ARG transmission was detected using shotgun metagenomics sequencing.

Results: A high prevalence (33.5%) of ESBL- and AmpC β-lactamase-producing Escherichia coli was detected in feral swine. All isolates were multi-drug resistance, harboring various ARGs and robust virulence factors. Besides, similar microbiota structure was observed between cattle and feral swine reflected by bacterial phylum composition and co-occurring OTUs. Importantly, the proportion of ARGs that conferred tetracycline resistance was positively associated with the relative abundance of bacteria belonging to Bacteroidetes and Synergistetes in both cattle and feral swine. Moreover, clonal spread of AmpC-producing bacteria was observed between feral swine and cattle.

Significance: Our results provide critical knowledge to better understand the ARB and ARGs transmission at the wildlife-livestock interface which should be controlled to mitigate the potential spread of AMR.

**12. CRISPR-MEDIATED IDENTIFICATION OF SARS-COV-2 HOST-CELL DEPENDENCIES
ENABLES REPURPOSING DRUGS FOR ANTI-VIRAL DISCOVERY**

Michael Norris - Department of Geography, Emerging Pathogens Institute, University of Florida; **Andrew Bluhm** - Department of Geography, Emerging Pathogens Institute, University of Florida

Currently the world is still in the throes of a pandemic caused by the SARS-CoV-2 virus. Utilizing the new and validated technology of genome wide CRISPR screens, we aim to expand the understanding of virulence mechanisms while providing targets for therapeutic development to combat this new disease, COVID-19. To that end, we leveraged the power of CRISPR technology to create whole genome knock out (Brunello) or activation libraries (Calabrese) for use in CRISPRko and CRISPRa infection screens to identify host factors that are required for SARS-CoV-2 infection or restrict SARS-CoV-2 infection, respectively. Whole genome CRISPRko libraries were created in HEK293T-hACE2 and Vero E6 cells, while activation libraries were created only in HEK293T-hACE2 cells. These massive libraries were then infected with SARS-CoV-2 and the surviving cells sequenced to identify survivors. In this work, we focused on the knockout survivors of the infection. Once sequenced, we identified genes and pathways required for SARS-CoV-2 viral replication. From there we were able to test drugs known to interfere with those gene functions and/or essential pathways in in vitro assays looking at cytotoxicity as a proxy for cell survival and viral replication. We found drug candidates that were highly effective at reducing SARS-CoV-2-induced cytotoxicity and inhibiting viral replication in cells. Strong single drug candidates were then tested in various combinations to determine a synergistic or additive affect.

13. DEPLOYMENT OF NOVEL SARS-COV-2 TESTING IN FIRST RESPONDERS, FRONT LINE PROVIDERS, AND ESSENTIAL PERSONNEL DURING THE COVID-19 PANDEMIC

Cindy Montero - Department of Emergency Medicine, College of Medicine, University of Florida; **Meenakshi Balakrishnan** - Department of Emergency Medicine, College of Medicine, University of Florida; **Julia Loeb** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Robert Cueto** - Department of Emergency Medicine, College of Medicine, University of Florida; **Maureen Long** - Department of Veterinary Medical Sciences, 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; **Derek Cummings** - Department of Biology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **J. Glenn Morris, Jr.** - Emerging Pathogens Institute, University of Florida; **Lisa Merck** - Department of Emergency Medicine, College of Medicine, University of Florida; **Adrian Tyndall** - Department of Emergency Medicine, College of Medicine, University of Florida

Background: First responders and essential personnel are at high-risk of contracting COVID-19 through occupational exposure. Despite this risk, providers found significant barriers to SARS-CoV-2 PCR testing throughout 2020. The University of Florida (UF) designed novel testing algorithms to measure SARS-CoV-2 virus and antibodies. Tests were deployed to front-line providers and essential personnel. Data on risk factors and disease epidemiology are presented.

Methods: Novel SARS-CoV-2 PCR and antibody testing was made accessible to a cohort of first responders and essential workers across North Florida beginning in April, 2020. Subjects were recruited via advertisement and social media. An electronic interface governed by computer engineering provided remote access to informed consent, surveys, appointment scheduling, and testing results. Through this mechanism, asymptomatic first responders (ex: emergency medicine,

police, fire, EMS, and hospital workers); as well as, essential city personnel (ex: teachers, utility, and transit workers) registered as subjects. Self-report data were collected via survey including: demographics (age/race/sex/employer/occupation/hours per week); medical/travel/exposure history; and perception of access to PPE. All data were compiled using REDCap© 2021. Testing was completed at one of three Emergency Departments via nasal swab/saliva/serum samples collected by trained research personnel. PCR results were reported within 48 hours. Follow-up survey data are collected at scheduled intervals (1, 6, and 12 months). The protocol was approved by UF IRB, Environmental Safety, and Information Security.

Results: Between 4/2020-2/2021, a total of 4,401 tests were collected within the cohort of n=1684 subjects (n=3,055 PCR; 1,347 IgG antibody). Subject demographics were self-reported as: 47.7% female (n=803); age range = 18-76 (mean=39); 7% African American (n=117); 11.1% Hispanic/Latino (n=187); 80.3% Caucasian (n=1353); 8.1% Asian (n=137); 2.4% Multiracial (n=41); 2.1% Other (n=36). Occupations included employees of: medical facilities 65.3% (n=1,099); police/fire/EMS 29.5% (n=497); and city employees/teachers/other 5.2% (n=88). Test results demonstrated a 1.72% prevalence of COVID +PCR and an overall prevalence of COVID +IgG 4.0 % (1.4% unknown positives, 1.3% known positives, 1.4% vaccinated). This rate was less than that reported for Alachua County (5.3% +PCR). In total, there were 33 counties represented across N Florida.

Conclusions: A novel test strategy was deployed to provide rapid access to SARS-CoV-2 monitoring for high-risk, asymptomatic essential workers across North Florida. Access to testing demonstrated good penetrance within the community. Overall, this cohort of first responders / front-line providers demonstrated lower incidence of positive PCR than that quantified in the surrounding community. Low incidence may be related to frequency of serial testing/surveillance within this asymptomatic, high-risk cohort. Analyses of disease expression and the development of immunity are ongoing.

14. DETECTION OF SARS-COV-2 SPECIFIC IGA IN THE BREASTMILK OF COVID-19 VACCINATED, LACTATING HEALTH CARE WORKERS

Vivian Valcarce Luaces - Department of Pediatrics, College of Medicine, University of Florida; **Lauren Stewart Stafford** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Josef Neu** - Department of Pediatrics, College of Medicine, University of Florida; **Nicole Cacho** - Department of Pediatrics, College of Medicine, University of Florida; **Leslie Parker** - Department of Pediatrics, College of Nursing, University of Florida; **David J Burchfield** - Department of Pediatrics, College of Medicine, University of Florida; **Nan Li** - University of Florida; **Joseph Larkin III** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

In 2019, a deadly virus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible for COVID-19, emerged. In December 2020, two mRNA-based COVID-19 vaccines were approved for use in the US and have begun providing immunity to those receiving the vaccination. Certain vaccines given to pregnant and lactating mothers provide immunity to infants through transmission across the placenta and umbilical cord (IgG) and breast milk (IgA). Breastmilk produced by mothers with a history of COVID-19 infection has been found to be a source of anti-SARS-CoV-2 IgA and IgG. This study aims to determine the presence of specific SARS-CoV-2 IgA in the breastmilk of lactating women after the COVID-19 vaccine administration. As such, lactating healthcare workers who received the SARS-CoV-2 mRNA vaccine (Pfizer/BioNtech or Moderna) made up a sample group. Plasma and breast milk were collected at three-time points (pre-vaccination, 14-28 days post the first dose of vaccine, and 7-10 days post-second dose of vaccine). SARS-CoV-2 specific IgA (breastmilk) and IgG (plasma) concentration were then measured by ELISA. We found consistent secretion of SARS-CoV-2 specific IgA in breast milk after COVID-19 vaccination. The second dose of the SARS-CoV-2 vaccine was necessary to elicit a detectable IgA response in breastmilk by ELISA. There is a correlation of the level of SARS-CoV-2

specific IgG in blood with SARS-CoV-2 specific IgA in breastmilk. Our results show that the mRNA-based COVID-19 vaccines from Pfizer/BioNTech and Moderna induce SARS-CoV-2 specific IgA secretion in breastmilk. Further studies are needed to determine the duration of this immune response, its capability to neutralize the COVID-19 virus, transfer passive immunity to breastfeeding infants, and the potential therapeutic use of milk IgA to combat the COVID-19.

15. EMERGING SARS-COV-2 MUTATIONS CIRCULATING IN THE UNITED STATES

Lauren Brinkac - Noblis; **Sheila Diepold** - Tetracore; **Shane Mitchell** - Noblis; **Lee F. Kolakowski** - Tetracore; **William M. Nelson** - Tetracore; **Katharine Jennings -** Noblis

Coronavirus disease 2019 (COVID-19), the infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a global pandemic of unprecedented scale. Despite significant increases in COVID-19 positive cases in the United States (US), the extent of genomic diversity of SARS-CoV-2 in circulation has yet to be fully elucidated. Utilizing Oxford Nanopore Technologies (ONT) complete whole genome sequences (WGS) of SARS-CoV-2 clinical isolates obtained from confirmed COVID-19 cases throughout the US, were generated and analyzed. Twenty-one isolates belong to the potential emerging 20G/8083A clade and co-harbor distinct mutations such as an additional spike protein mutation, Q913H, located within the helical fusion core of the heptad repeat 1 (HR1) region, underscoring the critical need for enhanced genomic surveillance to effectively monitor SARS-CoV-2 evolution.

16. IMPACTS OF CARBON NANOTUBES ON LIPID CONTENT AND LUNG CELL SUSCEPTIBILITY FOLLOWING EXPOSURE TO INFLUENZA A VIRUS

A. O'Connor - Department of Environmental and Global Health, Center for Environmental and Human Toxicology, College of Public Health and Health Professions, University of Florida; **S.T. Humes** - Department of Environmental and Global Health, Center for Environmental and Human Toxicology, College of Public Health and Health Professions, University of Florida; **S. Bisesi** - Department of Environmental and Global Health, Center for Environmental and Human Toxicology, College of Public Health and Health Professions, University of Florida; **L. Ingram** - Department of Pharmaceutical and Biomedical Sciences, University of Georgia; **M. Finnerty** - Department of Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA; **John Lednicky** - Department of Environmental and Global Health, Center for Environmental and Human Toxicology, College of Public Health and Health Professions, University of Florida; **B.S. Cummings** - Department of Pharmaceutical and Biomedical Sciences, University of Georgia; **Tara Sabo-Attwood** - Department of Environmental and Global Health, Center for Environmental and Human Toxicology, College of Public Health and Health Professions, University of Florida

Background: Nanoparticles (NP) are very small particles used in industrial and consumer products but we have limited understanding of how inhalation exposure to NP impacts human health. Research has focused on pulmonary endpoints such as fibrosis and asthma, while the area of viral susceptibility remains less well explored. We have shown that exposure of small airway epithelial cells (SAEC) to one type of NP, single-walled carbon nanotubes (SWNTs), increases host susceptibility to Influenza A virus (IAV) infection, as evidenced by increased viral titers. To elucidate the mechanisms responsible for this result we hypothesized that SWNTs alter lipids that are involved in repressing host antiviral defenses.

Methods: We exposed SAECs to 20 µg/mL SWNTs for 24 h and collected the cells and media for lipidomics analyses (n=6). Lipids were extracted per the Bligh-Dyer method, followed by mass spectrometry (LC-ESI-

MS/MS). To determine the role of ceramides specifically, a ceramide inducer (sphingomyelinase (SMase)) or inhibitor (Imipramine) was added to SAECs prior to infection with IAV (strain A/Mexico/4108/2009 (H1N1). Viral titers were performed using the TCID50 method. Data were evaluated by ANOVA with multiple pairwise comparisons.

Results: We observed enrichment of several classes and species in both the cellular and secreted lipid landscapes. In the cellular lipidome, SWNT exposure resulted in enrichment of phosphatidylcholines (PC), phosphatidyethanolamines (PE), plasmenyl species, sphingomyelins (SM), and oxidized triglycerides (OxTG). In the secreted lipidome, SWNT exposure resulted in increases in PE, oxidized lysophosphatidylethanolamines (OxLPE), plasmenyl species, SM, ceramides (Cer), and OxTG. We also discovered that viral titers were increased and decreased with the addition of SMase and imipramine, respectively.

Conclusions: This work shows that SWNT can alter the lipidome of SAEC and that ceramides seem to play a role in modulating viral titers. Future studies to define the mechanistic pathways involved in SWNT-induced influenza viral infections are underway.

17. LONGITUDINAL MONITORING FOR FEVER: AN INEXPENSIVE COVID-19 SCREENING METHOD

Justin Kim - Department of Medicine, College of Medicine, University of Florida; **Marcus Threadcraft** - Department of Medicine, College of Medicine, University of Florida; **Richard Wenzel** - Department of Medicine, College of Medicine, Virginia Commonwealth University; **Frederick Southwick** - Department of Medicine, College of Medicine, University of Florida

Introduction: Throughout the U.S. the COVID-19 pandemic is surging, and the rise in cases has exceeded the testing capacity in the U.S. As a consequence, residents wait in long lines to be tested, and results for reverse transcriptase polymerase reaction (RTPCR) tests are often delayed for 7- to 10-days. These conditions delay rapid isolation of those who are infected as well as contact tracing. Is there a better way to quickly identify the onset of disease and encourage earlier isolation of potentially SARS-CoV-2 infected individuals? We recommend that longitudinal home monitoring for fever be encouraged as a preliminary screening tool.

Methods: Kinsa Inc. (the maker of the Kinsa Smart Phone Oral and Ear thermometers) provided data which represented a national convenience sample of deidentified raw data including temperatures, location of the measurements using GPS coordinates, age, gender, dates and times of measurements, measurement site (oral, axillary, ear, or rectal), and a symptom checklist. There were 1251 individuals who reported testing positive for SARS-CoV-2 and 1249 control individuals matched for location, gender, and age who had not reported a positive test. 38,901 temperature measurements for the COVID-19 group and 37,420 measurements for the control group were collected over a 10-month period from 2/21/2020 to 12/20/2020. Fever defined as: a rise of 1°C of 1.8 °F in core temperature and/or a fixed oral temperature of ≥ 38 °C or 100.4 °F. Statistical analyses were performed using a two-sided Fisher's exact test.

Results: Fever was present in 62.7% of SARS-CoV-2 test positive individuals. A single temperature check was less sensitive, detecting fever in only 12.8% of cases. When monitored for 72 hours or longer, 74.1% had fever. Symptoms were absent in 19.8% and among asymptomatic cases fever was present in 50.4%; however, if temperature was monitored for > 72 hours fever was detected in 71.4% of COVID+ individuals without symptoms. Comparison to the control population revealed that the specificity of fever for COVID-19 was 0.481 for all those monitored for fever ($p< 0.0001$) and 0.397 in those monitored for > 72 hours ($p< 0.0001$). Eight symptoms demonstrated specificities of greater than 0.800: loss of smell and taste (0.953), trouble breathing (0.978), fatigue (0.867), stuffy nose (0.864), headache (0.802), earache (0.976), chills (0.848), and diarrhea (0.957).

Conclusions: Fever is a common sign of COVID-19 that we recommend be used as a preliminary screen before more specific RTPCR and antigen testing. This approach is: Inexpensive and Convenient, Allows continuous monitoring for the onset of disease, Encourages timely isolation If widely implemented, this approach has the potential to markedly reduce the spread of infection, particularly in households and other closed environments.

18. MULTIPLE RECOMBINATION EVENTS AND STRONG PURIFYING SELECTION AT THE ORIGIN OF SARS COV 2 SPIKE GLYCOPROTEIN INCREASED CORRELATED DYNAMIC MOVEMENTS

Massimiliano Tagliamonte - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Nabil Abid** - Laboratory of Transmissible Diseases and Biological Active Substances, University of Monastir; **Stefano Borocci** - Department for Innovation in Biological, Agro-food and Forest Systems, University of Tuscia; **Elisa Sangiovanni** - Department for Innovation in Biological, Agro-food and Forest Systems, University of Tuscia; **David A. Ostrov** - Department of Pathology, Immunology, and Laboratory Medicine, College of Medicine, University of Florida; **Sergei L. Kosakovsky Pond -** Department of Biology, Temple University; **Marco Salemi** - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Giovanni Chillemi** - Department for Innovation in Biological, Agrofood and Forest Systems (DIBAF), University of Tuscia; **Carla Mavian** - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida

Our evolutionary and structural analyses revealed that the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike gene is a complex mosaic resulting from several recombination events. Additionally, the fixation of variants has mainly been driven by purifying selection, suggesting the presence of conserved structural features. Our dynamic simulations identified two main long-range covariant dynamic movements of the novel glycoprotein, and showed that, as a result of the evolutionary duality, they are preserved. The first movement involves the receptor binding domain with the N-terminal domain and the C-terminal domain 2 and is maintained across human, bat and pangolin coronaviruses. The second is a complex network of long-range dynamics specific to SARS-CoV-2 involving the novel PRRA and the conserved KR*SF cleavage sites, as well as conserved segments in C-terminal domain 3. These movements, essential for host cell binding, are maintained by hinges conserved across human, bat, and pangolin coronaviruses

glycoproteins. The hinges, located around Threonine 333 and Proline 527 within the N-terminal domain and C-terminal domain 2, represent candidate targets for the future development of novel pan-coronavirus inhibitors. In summary, we show that while recombination created a new configuration that increased the covariant dynamic movements of the SARS-CoV-2 glycoprotein, negative selection preserved its inter-domain structure throughout evolution in different hosts and inter-species transmissions.

19. RECONSTRUCT COVID19 EPIDEMIC CURVES IN HUBEI PROVINCE CITIES BY ADJUSTING MOBILE POPULATION BETWEEN DIFFIRENT CITIES

Neda Jalali - Department of Biostatistics, College of Public Health and Health Professions, University of Florida; **Yang Yang** - Department of Biostatistics, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

Background: In the ongoing COVID-19 pandemic, the effect of moving population between different locations has not been fully investigated on the person-to-person transmission rate. In this study, we used an SEIR model to estimate the transmission rates in different cities of Hubei province by adjusting the role of the moving population.

Methods: We used daily migration indexes between different cities of Hubei province collected from mobility data to reconstruct the COVID-19 epidemic curve in each city of Hubei through a Susceptible, Asymptomatic and Infectious, Exposed and Infectious (Pre-symptomatic), Infectious (Symptomatic), Recovered (SAEIR) stochastic model. To optimize the model, we categorized different cities together which had similar epidemic curves based on the total number of cases during the epidemic period and the highest number of cases at the peak of the epidemic. We use a multivariate hypergeometric distribution to sample the number of migrated cases from each compartment. Next, using a multinomial distribution, we sample the number of cases that traveled to other cities corresponding to their disease status. Due to different observed variables in the model, we implemented a PFMCMC algorithm to infer the data.

Findings: Our finding shows that excluding Wuhan, the migrated population is the main source of epidemics in the Hubei province cities. In our study, the total number of estimated asymptomatic cases is lower than other studies, which shows either the large probability of being symptomatic or asymptomatic transmission rate.

Conclusion: The restriction decision made by the China government truly controlled the disease. If this restriction was applied earlier, it could control the emergence of COVID-19 in other cities of Hubei province. Asymptotic cases have a significant role in disease transmission dynamics and need more investigation.

20. SIMULTANEOUS DETECTION OF SARS-COV-2 AND INFLUENZA VIRUSES AT THE POINT-OF-CARE

Carlos Manzanas - Department of Mechanical and Aerospace Engineering, College of Engineering, University of Florida; **Md Mahbubul Alam** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Julia Loeb** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **John Lednicky** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Chang-Yu Wu** - Department of Environmental Engineering Sciences, College of Engineering, University of Florida; **Z. Hugh Fan** - Department of Mechanical and Aerospace Engineering, College of Engineering, University of Florida

Early and accurate detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and other respiratory viruses at the point-of-care (POC) is crucial for reducing the transmission of coronavirus disease 2019 (COVID-19). Adequate access and availability of POC devices for virus detection will significantly reduce the number of people who get infected by these viruses and are desperately needed. To address this need, we have been developing a valve-enabled lysis, paper-based RNA enrichment, and RNA amplification device (VLEAD) for simultaneous

detection of influenza A H1N1 pdm2009 virus and SARS-CoV-2. It is very important to detect these two viruses simultaneously since these viruses can cause contagious respiratory illnesses with similar symptoms in some people. When these two viruses are circulating in the same season, is important to tell them apart and also, some people may harbor dual infections. This 2-plex VLEAD consists of two sets of components fabricated in one platform. Each set includes 4 buffer wells, 1 mixing well, and 1 detection unit. The sample preparation process starts by addition of a sample directly to the mixing well, wherein lysis buffer is immediately discharged. The binding buffer is then released to bind RNA (including the virus RNA) onto the chromatography paper in the detection unit. This is followed by dispensing of two wash buffers to purify the attached RNA. All of these sequential steps are controlled by manipulation of valves, and the reagents can be pre-packaged in the device. As a result, the operation can be carried out at POC. We chose molecular detection by reverse transcription loop-mediated isothermal amplification (RT-LAMP) for its simple thermal management and great sensitivity and specificity. The RT-LAMP assay we use has a limit of detection of 10 genome equivalents for SARS-CoV-2 and 6 for Influenza A H1N1pdm2009 viruses. Detection of amplicons is achieved by recording the color change in the presence of SYBR Green by either naked eye viewing of color development or its detection by use of a smartphone camera. We have demonstrated the functions of the device by detecting heat-inactivated SARS-CoV-2 and UV-inactivated influenza A H1N1pdm2009 viruses. Our efforts will lead to a rapid and highly sensitive POC platform for simultaneous detection of both types of viruses.

21. THE GUT MICROBIOME OF COVID-19 RECOVERED PATIENTS RETURNS TO UNINFECTED STATUS IN A MINORITY-DOMINATED UNITED STATES COHORT

Rachel Newsome - Department of Medicine, University of Florida Shands Cancer Center, Clinical and Translational Science Institute, College of Medicine, University of Florida; **Josee Gauthier** - Department of Medicine, University of Florida Shands Cancer Center, College of Medicine, University of Florida; **Maria C. Hernandez** - Department of Medicine, University of Florida Shands Cancer Center, College of Medicine, University of Florida; **George E. Abraham** - Department of Medicine, College of Medicine, University of Mississippi Medical Center; **Tanya O. Robinson** - Department of Medicine, College of Medicine, University of Mississippi Medical Center; **Haley B. Williams** - Department of Medicine, College of Medicine, University of Mississippi Medical Center; **Meredith Sloan** - Department of Medicine, College of Medicine, University of Mississippi Medical Center; **Sarah C. Glover** - Department of Medicine, College of Medicine, University of Mississippi Medical Center; **Raad Z. Gharaibeh** - Department of Medicine, University of Florida Shands Cancer Center, College of Medicine, University of Florida; **Christian Jobin** - Department of Medicine, University of Florida Shands Cancer Center, College of Medicine, University of Florida

Introduction: Intestinal microbiota influences both susceptibility and severity of bacterial and viral-induced pathogenicity, including respiratory diseases. In this study, we investigated the relationship between intestinal microbiota and SARS-CoV-2-mediated pathogenicity in the United States, majority African American cohort.

Hypothesis: Intestinal microbiota is modulated by SARS-CoV-2 infection and is related to symptom severity and recovery from the disease.

Methods: We conducted a single-institution study, prospectively collecting fecal samples from 50 SARS-CoV-2 infected patients within 3 days of ICU admission and 9 SARS-CoV-2 recovered patients upon testing negative for the virus. Feces of 34 uninfected subjects at the hospital with unrelated respiratory medical conditions were used as controls.

Total fecal RNA/DNA was extracted and microbiota composition was determined using 16S rRNA gene sequencing of the V1-V3 region. The 16S rDNA sequencing reads were processed using DADA2 to generate amplicon sequence variants (ASV). RT-PCR on fecal RNA using two sets of validated primer/probes was performed to establish the presence or absence of SARS-CoV-2 viral RNA.

Results: The fecal microbial composition was found to be significantly different between SARS-CoV-2 patients and controls (PERMANOVA FDR-P=0.004), independent of treatments such as antibiotic exposure. Peptoniphilus, Corynebacterium and Campylobacter were identified as the three most significantly enriched genera in COVID patients compared to controls. Actively infected patients were also found to have a different gut microbiota than recovered patients (PERMANOVA FDR-P=0.003), and the most enriched genera in the COVID-19 patients was Campylobacter, with Agathobacter being enriched in the recovered patients. No difference in microbial community structure between recovered patients and uninfected controls was observed (PERMANOVA FDR-P=0.93), with Phocea being the top genus associated with patients who recovered from COVID-19. Furthermore, no difference in alpha diversity between the three groups was noticed. More importantly, 24 of the 50 COVID-19 patients (48%) tested positive via RT-qPCR for fecal SARS-CoV-2 RNA. A significant difference in gut microbial composition between SARS-CoV-2 positive and negative samples was observed, with Klebsiella and Agathobacter being enriched in the positive cohort and Phocea in the negative cohort. No significant associations between microbiome composition and disease severity or proton pump inhibitor treatment were found.

Conclusion: The intestinal microbiota is sensitive to the presence of SARS-CoV-2, with increased relative abundance of genera (Campylobacter, Klebsiella) associated with GI disease. Further studies are needed to investigate the functional impact of deleterious bacterial genera in SARS-CoV-2 on GI health.

22. WASTEWATER-BASED EPIDEMIOLOGY OF SARS-COV-2 IN GAINESVILLE, FL

Andrew Rainey - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Joseph H. Bisesi Jr.** - Department of Environmental and Global Health, Center for Environmental and Human Toxicology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Tara Sabo-Attwood** - Department of Environmental and Global Health, Center for Environmental and Human Toxicology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Anthony Maurelli** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

Introduction: Wastewater-Based Epidemiology (WBE) is a public health surveillance tool used to conduct community sampling to identify incidence, monitor trends, and determine the prevalence of diseases. WBE has been deployed across the globe during the SARS-CoV-2 pandemic to monitor COVID-19 infections to identify specific locations of infected individuals, and to monitor the trends of COVID-19 infections over time. We have been conducting weekly wastewater surveillance of SARS-CoV-2 in Gainesville, FL to determine how well WBE of SARS-CoV-2 can monitor trends of COVID-19 of a population.

Methods: One liter, 24-hr composite influent wastewater samples were collected from Gainesville, FL water reclamation facilities once a week starting on 5/31/2020. Aliquots (50mL) are adjusted to 25mM $MgCl_2$ and filtered using a 0.45µm electronegative membrane filter disc. Each filter was then added to a 5mL tube containing garnet beads and DNA/RNA shield for viral inactivation. The filter was subjected to bead beating and the solution containing SARS-CoV-2 viral particles was processed for RNA extraction with the Zymo Quick-DNA/RNA Viral MagBead kit, and nucleic acid extracts are purified using the Zymo OneStep PCR Inhibitor Removal Kit. RNA was tested using qPCR for the SARS-CoV-2 CDC N2 target. Total viral genomic copies were calculated using a standard curve consisting of

a serial dilution of a gBlock containing the CDC N2 target sequence. qPCR values were back calculated to total genomic copies/L of wastewater. COVID-19 clinical case data was taken from the Florida Department of Health daily COVID-19 reports using the city level data. Weekly COVID-19 incidence is the total new cases reported for Gainesville at the end of each Sunday. Linear regression was used to determine the relationship between SARS-CoV-2 wastewater levels with clinically confirmed COVID-19 cases.

Results: Weekly COVID-19 cases from May 31, 2020-January 31, 2021 ranged from 9-898 with a mean of 443. Weekly SARS-CoV-2 wastewater data across the same dates ranged from 0-12.73 Log10 genomic copies/L with a mean of 9 Log10 genomic copies/L. There was a significant positive relationship between SARS-CoV-2 wastewater levels and clinically confirmed COVID-19 cases.

Conclusions: WBE of SARS-CoV-2 is a useful tool in monitoring COVID-19 cases on the population level. In the absence of widespread testing, data generated from SARS-CoV-2 wastewater surveillance can effectively track the trends of COVID-19 cases in a mid-sized city (Gainesville, FL) and thus, WBE can be s useful tool for policy makers when determining city-wide COVID-19 control measures.

23. CRYPTOCOCCUS NEOFORMANS PHOSPHOLIPASE IS IMPORTANT FOR SURVIVAL IN THE CENTRAL NERVOUS SYSTEM OF C57BL/6 MICE

Mohamed F. Hamed - Department of Oral Biology, College of Dentistry, University of Florida

Background: The encapsulated fungus Cryptococcus neoformans (Cn) is the most common cause of fungal meningitis, with the highest rate of cryptococcosis found in AIDS patients. Despite antifungal treatment, cryptococcal meningitis is known for its high morbidity and mortality rates due to its difficult eradication from the brain. Cn's capsule contributes directly to its pathogenesis; specifically, its main component, glucuronoxylomannan, has been associated with invasion of the brain. Studies in mouse models of cryptococcal meningoencephalitis have indicated that the portals of Cn invasion into the brain are the cerebral capillaries, which have been confirmed by pathological studies in human brain tissues. However, there are important knowledge gaps regarding the fungal factor(s), regulatory mechanisms, and interactions of Cn with cells of the central nervous system (CNS) supporting this invasive process. Secreted phospholipase B (PLB) activity promotes the survival and replication of Cn inside macrophages and during infection. Although Cn plb mutant is not essential to establish neurological disease in rodents, its interactions with glial cells (microglia and astrocytes) have not been yet described. Hence, we hypothesized that Cn plb mutant strain invades the brain but does not alter the morphology of glial cells in brain tissue.

Methods and Results: Using a murine model of systemic Cn infection and histopathological techniques, we confirmed that Cn plb invades the CNS to a limited extend and causes significantly smaller tissue lesions or cryptococcomas than the wild-type and complemented strains. Additionally, Cn plb strain does not change glial cells morphology.

Conclusion: Cn phospholipase contributes to fungal survival in brain tissue. Identification of the mechanisms by which Cn phospholipase interferes with glial cell functions will provide novel insights into the neurotropism of this deadly infection and may offer new therapeutic opportunities and preventive measures for combating cerebral cryptococcosis, a disease that kills ~200,000 AIDS patients annually around the world.

24. EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS OF LEISHMANIASIS PRESENTING TO AN ACADEMIC MEDICAL CENTER IN FLORIDA – 2016 TO 2020

Coulter N. Small - College of Medicine, University of Florida; **Norman Beatty** - Department of Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida

Introduction: Leishmaniasis is a disease caused by the protozoan parasite of the genus Leishmania. The infection is vector-borne and transmitted after the bite of a "sandfly" within the subfamily Phlebotominae. Leishmaniasis can take on three distinct clinical syndromes, which include Cutaneous Leishmaniasis (CL), Mucosal Leishmaniasis (ML), and Visceral Leishmaniasis (VL). The purpose of this project was to retrospectively analyze the cases of Leishmaniasis at a single academic medical center here in Florida to assess epidemiological and clinical characteristics of this vector-borne disease.

Method: We retrospectively analyzed patients diagnosed with any form of Leishmaniasis who sought care within the University of Florida Health Network located in Gainesville, Florida, between 05/1/2016 to 05/5/2020. Exclusion criteria were patients ≤18 years old or those not diagnosed with Leishmaniasis via molecular detection. Demographic, social, and travel histories were extracted as well as clinical characteristics and treatments were assessed.

Results: Eight individuals were diagnosed with Leishmaniasis; seven (87.5%) having CL, and one (12.5%) with both CL and ML. All patients presented with a non-healing skin lesion; five had a single lesion on an extremity, two had multiple lesions on various extremities and trunk, and

one had extremity lesion with accompanying ulcer in the nares. Seven (87.5%) reported known or suspected exposure to the vector during travel. Six (75%) contracted New World CL (N=5, Leishmania viannia brasiliensis; N=1, L. mexicana and L. panamensis) and one developed New World CL and ML (N=6, Leishmania spp.) while traveling or living in endemic regions of Latin America (Bolivia, Brazil, Mexico, Panama, Peru, Ecuador). One patient contracted Old World CL (L. major) while living in Iraq. Three (37.5%) patients were graduate students, two (25%) patients were university professors, one (12.5%) patient was a military pilot, and two patients lived in an endemic region with frequent travel to United States (U.S). Five individuals (62.5%) were conducting fieldwork in rural ecological settings. Four (50%) patients received care outside of the U.S. prior to presentation at our institution. Five (62.5%) patients saw a clinical cure for their Leishmaniasis during this time period. Three (37.5%) of patients were lost to follow-up.

Conclusions: In Florida we are likely to see New World CL and ML among those who have exposure to endemic regions within Latin America. Occupational risks include conducting fieldwork for research or those who travel to these regions. Those traveling to endemic regions should receive education on preventative measures to avoid sandfly exposure.

25. SURVEY OF GASTROINTESTINAL PARASITES AND PARASITE CONTROL PRACTICES OF FARMED WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) IN FLORIDA, USA

Sydney L. Cottingham - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Heather D. S. Walden** - Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida; **Ramiro Isaza** - Department of Comparative, Diagnostic, and Population Medicine, 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

Information regarding the epidemiology of gastrointestinal (GI) parasites in farmed white-tailed deer (WTD) is scarce, and parasite control strategies and sources of information used by Florida deer farmers have not been described. The objectives of this study were to i) estimate and compare the prevalence and fecal egg counts (FEC) of gastrointestinal parasites in farmed and free-ranging WTD and ii) describe current parasite control practices on Florida deer farms and identify sources of management information referenced by farmed deer producers in the state. Fresh fecal specimens (n=133) were collected from live, captive WTD and hunter-harvested, free-ranging WTD on 8 deer farms and 6 Wildlife Management Areas (WMA) across Florida. Fecal sedimentation and centrifugal flotation methods were used to identify helminth eggs and protozoan oocysts. Eggs per gram of feces (EPG) were quantified using a modified McMasters technique. Eggs and oocysts of 8 GI parasite species were detected in fecal specimens from farmed and free-ranging WTD, and Trichostrongyle-type eggs were the most commonly detected eggs in both herd types. GI parasite prevalence was lower in farmed WTD than free-ranging WTD, and EPG counts in both herd types were low at the time of survey. Farmed deer producers were surveyed to assess their farm management practices, perceptions about GI parasites, and sources of management information. The most common anthelmintic drugs

reported were ivermectin (53%) and fenbendazole (26%), and frequency of administration varied from less than once per year, to over 7 times per year. Eighty four percent of producers agreed or strongly agreed that GI parasites limit farmed deer production in Florida, but 97% indicated that they believed anthelmintic effectively controlled GI parasites. Most producers indicated having little or no knowledge of parasite epidemiology and consulted their peers more often than veterinarians for parasite control recommendations. This work represents the first description of GI parasite prevalence, control strategies, and sources of management information on WTD farms in Florida. These data will be useful to guide future research and extension education initiatives and to develop management guidelines specific to the deer farming industry in Florida.

26. THE POTENTIAL DISTRIBUTION OF PYTHIUM INSIDIOSUM IN THE CHINCOTEAGUE NATIONAL WILDLIFE REFUGE, VIRGINIA

Manuel Jara - Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University; **Kevin Holcomb** - U.S. Fish & Wildlife Service, Chincoteague National Wildlife Refuge; **Xuechun Wang** - Department of Plant Pathology, College of Agricultural and Life Sciences, University of Florida; **Erica M. Goss** - Department of Plant Pathology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Gustavo Machado** - Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University

Pythium insidiosum is a widespread oomycete pathogen that causes pythiosis in mammals. Recent increase in cases reported in North America indicates a need to better understand the distribution and persistence of the pathogen in the environment. The Chincoteague National Wildlife Refuge, located on Assateague Island, Virginia, hosts two grazing areas for horses, also known as the Chincoteague Ponies. In the past 3 years, 12 horses have succumbed to infection by P. insidiosum. We reconstructed the distribution of P. insidiosum in the Refuge based on 136 environmental water samples collected between June and September of 2019. Using an ecological niche model framework, we estimated and

mapped suitable areas for P. insidiosum throughout the Refuge. We found P. insidiosum throughout much of the study area, both within and outside the grazing areas. Our results showed significant monthly variation in the predicted suitability, where the most influential environmental predictors were land-surface water and temperature. June, July, and August were the months with the highest predicted suitability for P. insidiosum across the Refuge, while December through March were less favorable months. These results could be used to make management decisions and support monitoring horses for lesions during high risk months.

27. LONGITUDINAL PROFILING OF THE GUT MICROBIOME DURING AND AFTER RIFAMYCIN-BASED THERAPY FOR LATENT TUBERCULOSIS INFECTION (LTBI).

Marie Nancy Seraphin - Department of Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Julia Bellot** - Department of Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Maria Ukhanova** - Emerging Pathogens Institute, University of Florida; **Massimiliano Tagliamonte** - Emerging Pathogens Institute, University of Florida; **Michael Lauzardo** - Department of Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Charles Peloquin** - Department of Pharmacotherapy and Translational Research, Emerging Pathogens Institute, College of Pharmacy, University of Florida; **Volker Mai** - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

Tuberculosis (TB) and the human microbiome are both of critical importance to human health. Nevertheless, there is currently very little research overlap between the two. TB control efforts rely on prolonged therapy with a combination of antibiotics, including the broad-spectrum first-line anti-TB agent rifamycin. Antibiotics may have a long-term impact on the gut microbiota. However, data for the anti-TB agents are limited. We profiled the gut microbiome of six LTBI cases and six community controls using amplicon sequencing. The LTBI cases received 3 – 4 months of a rifamycin-based regimen for latent infection. Both groups were sampled at baseline, monthly during the LTBI case therapy, and two-month post-therapy. Firmicutes, Actinobacteria, Bacteroidetes, and Proteobacteria were the most abundant phyla in both groups. At baseline, cases and controls differed on the relative abundance of Tenericutes and Proteobacteria. Diversity did not seem to vary significantly between groups at baseline or across sampling intervals; however, rifamycin exposure significantly impacted community richness.

While we could identify an increase in microbial community dissimilarity due to rifamycin exposure, the shift did not significantly differ from what would be expected from random sampling variation.

28. THE LATENT TUBERCULOSIS CARE CASCADE IN FLORIDA: COMPARING THE EXPERIENCES OF A LARGE AND SMALL URBAN COUNTIES.

Patricia Wright - Department of Medicine, Emerging Pathogens Institute, University of Florida; **Darlin Ramirez** - Department of Medicine, Emerging Pathogens Institute, University of Florida; **Stephanie Reynolds-Bigby** - Department of Medicine, Emerging Pathogens Institute, University of Florida; **Marie Nancy Seraphin** - Department of Medicine, Emerging Pathogens Institute, University of Florida; **Michael Lauzardo** - Department of Medicine, Emerging Pathogens Institute, University of Florida

Introduction: Screening for latent tuberculosis infection (LTBI) prevents progression to infectious tuberculosis (TB) disease and is a key strategy to eliminating TB in the United States. In Florida, LTBI diagnosis and treatment falls mainly on county health departments. Although both classified as Urban, Alachua, and Miami-Dade counties are very different in population numbers and characteristics, which allows for the opportunity to explore differences in the LTBI care cascade across the two communities.

Methods: LTBI screening data were collected at TB clinics in health departments in Alachua and Miami-Dade counties to construct a six-month cohort of clients accessing LTBI services. Using the cascade of care framework, we used descriptive statistics to summarize the cohort's movement through the LTBI screening and treatment steps and chi-square statistics to compare rates between the two Counties.

Results: 2,993 clients of the health department met inclusion criteria for our cohort, 711 in Alachua and 2,282 in Miami-Dade. These clients accessed LTBI screening at a health department TB clinic for the first time between January – June 2016. We followed them through the LTBI care cascade until discharge or lost to follow. The cohort is mostly female (51.88%) and of non-US birth origin (62.91%). More than 79 countries are

represented in the dataset, while we lacked country of birth for 19.0%. Of the total cohort, 69.2% were referred to health department TB clinics from community providers with a positive tuberculin skin test or blood test results for follow-up. LTBI prevalence was 81.7% and was significantly higher in Miami-Dade than in Alachua (3.5% versus 59.2%, $\chi 2 = 560.9$, $p<0.001$). Of the 872 clients classified as LTBI, 744 (85.3%) were offered treatment, and 418 (56.2%) accepted to initiate therapy. LTBI treatment completion rate was 49.0%. While both counties had a similar treatment completion rate (55% Alachua, 49% Miami-Dade), only a fraction of clients in both counties completed LTBI therapy among those eligible for treatment (27.6% total: 33.3% Alachua: 27.6% Miami-Dade).

Conclusion: In Florida, the LTBI care cascade differs substantially between Alachua and Miami-Dade Counties, which differences in population demographics may explain. An evaluation of strategies to retain clients in care during the testing and treatment initiation stages of the care cascade may impact LTBI screening program success. Targeting screening efforts towards clients at high risk for LTBI and active TB may also improve retention in care.

29. A DIAGNOSTIC METHOD FOR SPECIFIC DETECTION OF ALPHAVIRAL EXPOSURE

Aya Tal-mason - Department of Medicine, University of Florida; **Ruiyu Pu** - College of Veterinary Medicine, University of Florida; **Amy Vittor** - Department of Medicine, University of Florida

Introduction: In Darién, Panama, arboviral diseases are on the rise and thus a target for epidemiological investigation. Alphaviruses such as Venezuelan equine encephalitis virus (VEEV) and Madariaga virus (MADV, previously known as South American equine encephalitis virus) are of particular public health interest. VEEV has been endemic as a human pathogen in the country since 1960, while is MADV actively emerging in the region of Darién. One method of alphaviral disease surveillance is through assessing population exposure to these viruses.

Methods: A current method of detecting alphaviral exposure is through Enzyme-Linked Immunosorbent Assay (ELISA) that utilizes whole virus as the antigen. This method is apt at determining general alphaviral exposure but is lacking when there is a need to distinguish between viral species. Plaque reduction neutralization tests (PRNT) are the gold standard for species-specific diagnosis, but require high levels of technical expertise, biosafety, and time. We set out to develop a species-specific IgG diagnostic method to detect alphaviral exposure. We utilized 65 samples from a Panamanian cohort, comparing VEEV and MADV results for whole virus ELISAs as well as PRNTs. For the antigen, we utilized VEEV IAB and MADV recombinant envelope protein (Mapp Biopharmaceuticals, Inc.).

Results and Discussion: Results were plotted and a diagnostic cutoff was determined for each test, where both sensitivity and specificity were maximized. MADV assay sensitivity increased from 77.8% to 88.9%, while specificity increased from 74.5% to 91.5%. For the VEEV assay, results

demosnstrate a decrease in sensitivity from 100% to 95.7% and specificity from 95.1% to 92.9% These results imply superiority of the modified recombinant protein ELISA for MADV exposure detection, while the whole virus ELISA remains superior for detection of VEEV exposure. Moreover, in the modified ELISA, an overall decrease in species cross-reactivity was observed. These findings pave the way for a new, species-specific standard for detecting alphaviral exposure.

30. ACTION OF THE MOSQUITOCIDAL PLANT ALKALOID LIRIODENINE ON THE GABAA RECEPTOR

Q. Cocquerel - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **F. Demares** - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Werner Geldenhuys** - West Virginia University; **A.-M. Le Ray** - Université d'Angers, France; **D. Breard** - Université d'Angers, France; **P Richomme -** Université d'Angers, France; **C. Legros** - CNRS UMR6015 – INSERM U1083, Angers, France; **Jeffrey Bloomquist** - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

The plant alkaloid liriodenine has been shown to be biologically active with multiple effects on mammals, fungi and bacteria, but has never been evaluated for insecticidal potency. In this study, liriodenine was applied topically to adult female Anopheles gambiae, and found to be mildly toxic. Its lethality was synergized with both dimethyl sulfoxide (DMSO) and the mono-oxygenase inhibitor, piperonyl butoxide (PBO). In recordings of the ventral nerve cord of larval Drosophila melanogaster (Dm), liriodenine was neuroexcitatory and reversed the inhibitory effect of 3 mM GABA at effective concentrations of 20-30 µM. GABAA receptors in Periplaneta americana acutely isolated neurons isolated from the American cockroach were studied under patch clamp and inhibitory effects with an IC50 value of about 1 µM were observed. In contrast, as expected bicuculline did not reverse the effects of GABA on either the DmCNS or cockroach neurons. In silico molecular models highlighted novel docking poses for liriodenine and bicuculline on the GABA receptor.

This study is the first assessing of the toxicology of liriodenine on insects, and suggests the GABA as a target, with liriodenine acting as an active analog of bicuculline in insects.

31. AN AGENT BASED MODEL OF AFRICAN SWINE FEVER INFECTION AMONG WILD SUIDS AND ORNITHODOROUS MOUBATA TICKS

Emily Gordon - Department of Geography, College of Liberal Arts and Sciences, University of Florida; **Gabriela Hamerlinck** - Department of Geography, College of Liberal Arts and Sciences, University of Florida

African Swine Fever (ASF) is a vectored viral suid disease that poses a significant threat to domestic swine production. It is endemic to Africa, but in recent decades has escaped to Europe and Asia. Empirical evidence of disease dynamics within the Ornithodorous moubata vector and wild suid hosts is limited and current control methods may be ineffective at preventing disease establishment. We present an Agent Based Model (ABM) used to explore underlying vector-host population dynamics before expanding the model to baseline disease dynamics within the system. Using NetLogo as our ABM platform, our SEIR ASF model displays realistic movement of vectors and hosts within a representative environment. Though empirical evidence is sparse for the system as a whole, our model is parameterized using biologically accurate data wherever possible. We have simulated basic disease dynamics emerging from agent interactions. Tick vectors, representing O. moubata, are initialized inside of warthog burrows that randomly populate the environment, and do not move past the confines of the defined burrow. Adult hosts, representing warthogs (Phacochoerus aethiopicus), are also initialized in burrows, but are free to roam the environment. Juvenile hosts (hoglets) remain in close proximity to burrows. Ticks may bite and consequently infect hosts, and viremic hosts may infect a vector that feeds on them. Preliminary simulations indicate that our model approximates observed sylvatic ASF dynamics, and will proceed with a focus on extracting data regarding agent interactions and infection prevalence across time. Our model is the first ABM to describe ASF disease dynamics and could be used to evaluate potential control strategies against ASF. Future iterations will focus on parameterization

and verification of the model to most accurately capture observed patterns of organism behavior.

32. ANTIGENIC SIGNALS IN DENV PROTEINS BEYOND THE SURFACE: WHERE ARE THEY?

Angkana T. Huang - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Bernardo Garcia-Carreras** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Derek Cummings** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Introduction: Antigenic proximity determines the relevance of accumulated immunity in the population against past circulating strains to emerging strains, and thus, is important in understanding transmission dynamics over long time frames. Changes in the surface proteins can affect the binding of antibodies and the ability to neutralize viruses making them plausible targets for study. In contrast, little is explored on whether non-surface proteins also contribute to, are correlated with, or are independent of antigenic properties. Leveraging a dataset of 347 dengue virus (DENV) strains circulating in Bangkok, Thailand between 1994 to 2014, with both whole genome data and antigenic characterization (measured as log2 plague neutralization (PRNT) titers against twenty global reference DENV antisera) available, we assessed this unknown relationship.

Method: Pairwise antigenic distances were calculated for all virus pairs from a 3-dimensional antigenic map constructed using the measured titers. Effect sizes of amino acid substitutions in all sixteen protein products of DENV were estimated using models published in the literature. Correlated substitutions were grouped as clusters to avoid collinearity. Predictions of pairwise antigenic distances were made through summing these estimates. Using 100-fold Monte Carlo cross-validation (10% as test), prediction performances were summarized as the median root mean square error (RMSE) and 95% interquartile range (IQR) of the test sets.

Results: As expected, low RMSE was observed when modelled with substitutions in the envelope protein, E. Random subsets of sites on the polyprotein with the same length achieved an equivalent RMSE suggesting propagation of signals between E and other proteins. To identify proteins with additional antigenic contributions, their RMSE were compared against random sites from the polyprotein and E subsetted to their respective sizes revealing nonstructural protein 2A (NS2A) as the potential candidate. To pin down those contributive sites, substitution effect sizes were estimated for 300 random subsets of 60 sites from NS2A concatenated with E. 63/219 sites on the NS2A were estimated to have nonzero effect for >99% of the time that they were included. These sites were dispersed throughout the length of NS2A.

Discussion: 85% of NS2A mutagenesis mutants were reported to be lethal or defective. Yet, its residue diversity is highest among the DENV proteins. Whether the identified antigenic contributions revealed in this study could explain the excessive maintenance of diversity through balancing selection awaits to be studied.

33. DETECTION OF A TICK-BORNE EXOTIC RICKETTSIA IN AN INVASIVE REPTILE TICK

Carrie De Jesus - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Madison Harman** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Christina M. Romagosa** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida

Invasive species can transport their associated parasites and pathogens from their native to non-native range. In Florida, numerous invasive reptile and amphibian species have established populations and previous studies found that these species can transport their ticks. Amblyomma rotundatum is an invasive tick from South America that has become established in Florida. This hard-bodied tick was introduced into Florida with the cane toad (Rhinella marina). Amblyomma rotundatum is a specialist feeder on amphibians and reptiles and can utilize numerous hosts in the state. Amblyomma rotundatum has previously shown to vector pathogens that can impact the health of reptiles and amphibians. Whether the pathogens A. rotundatum can vector were transported during the invasion process is unknown. We conducted a preliminary study to examine A. rotundatum from cane toads in Florida to determine if exotic pathogens were transported to Florida. We examined 99 cane toads from a population in Homestead, Florida from June to October 2019. We examined each individual toad for the presence of ticks and removed the ticks with forceps if present. For DNA extractions of the ticks, we grouped ticks together by host. DNA was extracted from individual adult and nymphal ticks, and pools of larvae (5). We conducted PCR's to determine the presence of Rickettsia, Anaplasma, Ehrlichia and Borrelia bacteria. Positive PCR results were Sanger sequenced to identify the pathogen species. Out of the toads examined 18/99 (18.2%) were infested with ticks. From the toads infested we collected a total 88 ticks and on average toads were infested with 4.89 ticks. PCR results were

negative for Anaplasma, Ehrlichia and Borrelia. Out of the ticks pooled by host, 11/18 tested positive for R. bellii. Rickettsia bellii is a common Rickettsial species found in numerous tick species in the Americas. Our study found that the R. bellii strain from our specimens matched closest to Central and South American samples in Genbank. Our study indicates that invasive ticks can bring their pathogens with them during an invasion and become established. Understanding the potential pathogens invasive ticks carry can provide valuable insight on the risk of establishment of tick-borne pathogens to native and invasive wildlife. In future studies we plan to screen ticks for additional parasites such as hemogregarines and examine other cane toad populations in Florida.

34. DIVERSITY AND DISTRIBUTION OF TICK-BORNE RICKETTSIA IN ESWATINI

Kimberly Ledger - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida

Worldwide, including in Africa, rickettsioses are recognized as emerging or re-emerging infections. The aim of this study was to screen a diversity of questing tick species for the presence of rickettsial bacteria in Eswatini and explore patterns in the distributions of ticks and rickettsial species. Questing ticks were collected at 26 savanna sites across conservation area, protected ranches, government ranches, and communal rangelands and tested for rickettsial DNA using real-time PCR and direct sequencing of multiple loci. We detected rickettsial DNA in three out of seven tick species (Amblyomma hebraeum, Haemaphysalis elliptica, and Rhipicephalus simus). Notably, we found an average prevalence of 55% for Rickettsia africae in A. hebreaum, 4.2% for Rickettsia conorii in H. elliptica, and 4.4% for Rickettsia massiliae in R. simus. Amblyomma hebreaum were collected at 19 sites, of which we detected R. africae in 13, including sites in all land-use categories. Haemaphysalis elliptica were collected at 18 sites, of which we detected R. conorii in one communal rangeland and one government ranch. Rhipicephalus simus were collected at 18 sites, of which we detected R. massiliae at six sites within

a single conservation area and in one government ranch. These results suggest the widespread presence of A. hebreaum ticks capable of spreading R. africae in Eswatini savannas while the presence of H. elliptica with R. conorii and R. simus with R. massiliae are concentrated to communal areas and conservation areas, respectively. These trends could be a result of stronger reservoir host or environmental constraints on the distribution of R. conorii and R. massiliae as compared to R. africae.

35. ECOLOGICAL COMPARISONS OF ECTOPARASITE COMMUNITIES OF SYLVATIC AND URBAN OPOSSUMS AND RACCOONS IN NORTH-CENTRAL FLORIDA

Sarah Mays Maestas - Department of Entomology and Nematology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Lindsay Campbell** - Department of Entomology and Nematology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Phillip Kaufman** - Department of Entomology, Texas A&M University, College Station, TX

Wildlife are ectoparasite hosts in vector-borne pathogen transmission cycles, and urban adaptation of wildlife may cause changes in vector-borne pathogen ecology and transmission patterns. To compare ectoparasite community compositions in sylvatic and urban environments, raccoons and opossums were live-trapped at a rural research station and the city of Gainesville, FL during 8 trapping sessions across four seasons. Partial redundancy analyses were used to test the hypotheses that host species, sylvatic vs. urban habitat, and collection season explained a significant proportion of the variance in ectoparasite communities. Although the mean number of ectoparasites per animal was similar between the two habitats, the mean number of ticks collected was greater in the sylvatic habitat, while the mean number of fleas collected was greater in the urban habitat. Partial redundancy analysis indicated that there was a significant habitat by season interaction, and

significant effect of host species on ectoparasite community composition ($P < 0.001$). Greater seasonal variability in ectoparasite community composition occurred in sylvatic habitat than in urban habitat, which may have implications for pathogen transmission and maintenance in these environments.

36. ESTER MOIETY STRUCTURAL MODIFICATION OF REPELLENT AND INSECTICIDAL PYRETHROIDS

Gary M. Richoux - Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida; **Liu Yang** - Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida; **Kenneth Linthicum** - USDA, ARS, CMAVE; **Edmund Norris** - USDA, ARS, CMAVE; **Jeffrey Bloomquist** - Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida

One of the most effective ways of reducing the propagation of mosquito vectored diseases is through the prevention of biting and subsequent transmission of pathogens. A highly utilized means of accomplishing this is through the development and deployment of spatially-acting mosquito repellents and insecticides. Significant market and field success has been realized through the implementation of pyrethroid-class compounds such as transfluthrin, metofluthrin, and resmethrin. Unfortunately, heavy use of these repellents and insecticides has led to the emergence of field-dominant strains of pyrethroid-resistant mosquitoes. This resistance necessitates further research into the development of new repellents and insecticides in the pursuit of resistance breaking activities. Because the efficacy of pyrethroid actives is practically unparalleled in modern-day mosquito control, we endeavored to explore the modification of existing pyrethroids to identify structural motifs which might not be affected by the active site mutations eliciting pyrethroid-resistance. Since it's understood that most synthetic pyrethroids contain activity-specific and -dependent chiral centers, we chose instead to focus our efforts on exploring the alkoxy moiety of the ester obtained from the esterification of trans-permethrinic and trans-chrysanthemic acid. To this end, we synthesized and screened 26 compounds for spatially-acting repellency and insecticidal activity against the pyrethroid-susceptible, Orlando (OR),

and pyrethroid-resistant, Puerto Rico (PR), strains of Aedes aegypti mosquito. Screening was completed utilizing the high-throughput benchtop glass tube assay developed within our laboratories. If sufficient repellency or toxicity was observed at our screening concentration of 100 µg/cm2, EC50 or LC50 values were obtained. So far, we have screened a mixture of branched, unbranched, aliphatic, halogenated, cyclic, non-cyclic, and heteroatom-containing esters. Early trends indicate that n-propyl, n-butyl, n-pentyl, cyclobutyl- and cyclopental-substituents exhibit promising repellent and insecticidal activity with minimal resistance. While this activity is not near as potent as observed with transfluthrin, further derivatization of these functional groups offer a promising route to future synthesis. Additionally, a handful of derivatives, such as GMR082 and GMR146 exhibit excellent activity. While we have not yet completed screening of these compounds against the PR mosquito strain, this information will be obtained in the near future

37. EVIDENCES OF SUSCEPTIBILITY AND PERMISSIBILITY OF OVINE TROPHOBLASTS TO ZIKA VIRUS

Ming Lo - Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida; **Valeria Carvalho** - Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida; **Maureen Long** - Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida

Introduction: Infection of Zika virus (ZIKV) during pregnancy is associated with microcephaly. Previous study showed that experimental infection of Zika virus in mid-gestation pregnant ewe resulted in significant smaller head sized in infected fetuses, indicating ovine serves as a good model species for in utero transmission of ZIKV. At least one infected ewe had culturable ZIKV from the placenta, suggesting that ZIKV can replicate in ovine placenta cells. In this study, we tested the hypothesis that ZIKV can infect and replicate in previously established immortalized ovine trophoblasts cell lines (AH-1).

Method: AH-1 cells were seeded in 48-well plate at 7 x 10^4 cells per well and incubated for 24 hours, reaching approximately 80% confluency before ZIKV infection. The cells were washed twice with PBS then infected with 100 µl of Zika virus (Puerto Rico) per well at designated concentrations from 10^6, 10^5, 10^4, 10^3, 10^2 to 1 TCID50. The cells were incubated with the ZIKV inoculum for 1 hour at 37 °C, 5% CO2. After 1-hour incubation, the inoculum was removed and replaced by 0.5 ml of complete maintaining medium. The infected cells were then incubated at 37°C, 5% CO2 and harvested at 24, 48 and 72 hours post infection. Total RNA of infected cells at designated time points were extracted by Trizol. Real-time, one step reverse transcription was applied to quantify the ZIKV polyprotein RNA in the ZIKV infected AH-1 cells.

Results: At ZIKV challenge dose of 10^4 TCID50, the production of ZIKV polyprotein RNA was exponentially increased in the AH-1 cells with Ct values (standard deviation) ranging from 28.2 (0.127), 25.0 (0.062) to 22.2 (0.077) at 24, 48 and 72 hours post infection, respectively. A similar growth pattern was observed at a challenge dose of 10^5 TCID50. The Ct value (standard deviation) went down from 21.8 (0.024), 18.6 (0.091) to 17.4(0.078) at 24, 48 and 72 hours, respectively. The Ct value of all the time points at ZIKV input dose below 10^2 TCID50 were not detectable.

Conclusions: Our results support that AH-1 cell was susceptible and permissive to ZIKV infection. ZIKV Infection at 10^4 or 10^5 TCID50 in AH-1 cells for more than 48 hours yields high ZIKV titers. The results confirmed that the AH-1 cell have all the cell machinery required for ZIKV replication and can be used as an in vitro model to study the effects of hormones on ZIKV replication in the trophoblasts.

38. EXPLORATION OF COVARIATE-CONSTRAINED RANDOMIZATION FOR CLUSTER RANDOMIZED TRIALS IN WHICH MANY CLUSTERS ARE AVAILABLE

Amy M. Crisp - Department of Biostatistics, College of Public Health and Health Professions, University of Florida; **Natalie Dean** - Department of Biostatistics, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

Introduction: A current cluster randomized trial aims to investigate the efficacy of Targeted Indoor Residual Spraying (TIRS) in the prevention of symptomatic disease from Aedes-borne viruses such as dengue, chikungunya, and Zika in children in Merida, Mexico. This study utilizes covariate-constrained randomization in order to achieve balance across two treatment arms of four specified census-level covariates and geographic sector. Covariate-constrained randomization is a process by which the final trial randomization pattern is chosen from a large set of randomization schemes that are all balanced across the treatment arms for chosen covariates. As some selected clusters may be subsequently found unsuitable for the trial, we desire a strategy to substitute new clusters while maintaining covariate balance.

Methods: An algorithm was developed for producing covariate-constrained randomizations for the TIRS trial for which the number of balanced rerandomizations and the average minimum pairwise distance between clusters were maximized. The latter is to achieve greater geographic separation between clusters to minimize contamination. Substitutions were made as needed. Validity of the design—as indicated by the lack of pairs of clusters that always or never appear in the same treatment arm—was confirmed at each stage. Using data on the census tract-level clusters considered for the TIRS trial, randomization simulations were explored with varying numbers of clusters available and clusters selected.

Results: The algorithm we developed successfully produced many randomization schemes that allowed for many rerandomizations, all of which balanced specified covariates while maintaining geographic spread

for 50 clusters selected out of 133 available. These designs are more robust to cluster substitutions. Simulations show that there are limitations to validity as the numbers of clusters available and selected are reduced.

Conclusions: We extend existing methods for covariate-constrained randomization to consider the setting where clusters are selected from a larger set of eligible units, and substitution may be necessary. We also explicitly capture geographic spread between clusters in the procedure to reduce shared boundaries where contamination may occur. These methods have the potential to improve the robustness of cluster randomized trials, while considering feasibility.

39. HOST-PATHOGEN INTERACTIONS IN ZIKV INFECTION OF THE AEDES AEGYPTI MIDGUT

Jasmine B. Ayers - Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Rhoel Dinglasan** - Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida

Zika virus (ZIKV) is an arthropod-borne virus (arbovirus) of the genus Flavivirus that causes acute febrile illness in humans. While acute infection with ZIKV is typically asymptomatic or mild, the virus has been found to cause neurological birth defects such as microcephaly in 5-10% of infants born to mothers with confirmed infection during pregnancy. ZIKV is primarily transmitted between humans by Aedes aegypti. The initial site of viral infection in the mosquito is the midgut epithelium, and the midgut is the first physical defense barrier against virus establishment. The proliferation of arboviruses in the midgut following consumption of an infected blood meal is a prerequisite for subsequent systemic infection of the vector and eventual transmission of the virus when the mosquito takes another blood meal. Interestingly, not all Ae. aegypti can be efficiently infected with and/or transmit flaviviruses, which is sometimes due to a failure to establish viral infection in the midgut. Our recently published work demonstrated that apoptosis of clusters of midgut epithelial cells induced within 2 hours of a ZIKV-infected blood meal suppresses viral replication in the midgut. The aim of our current

research is to elucidate the interactions between virus and mosquito host, which facilitate antiviral immunity in the midgut at understudied early infection timepoints. We utilized infectious clone-derived fluorescent reporter virus, immunofluorescence assay for ZIKV nonstructural protein 1 and midgut cell type markers, and structured illumination pseudo-confocal microscopy to visualize ZIKV midgut infection and determine the virus' cell tropisms within the midgut. To determine the mechanism by which clustered apoptosis is triggered in the midgut, we used rt-qPCR to detect upregulation of candidate immune cytokine genes. Competent mosquito vectors are required to sustain a human outbreak, so defining what makes mosquitoes able to resist infection could facilitate vector-borne disease control efforts, such as generating genetically engineered mosquitoes which are resistant to arbovirus infection.

40. INNOVATIVE AEDES AEGYPTI CONTROL IN ST. AUGUSTINE, FLORIDA USING IRRADIATED MOSQUITOES

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

Aedes aegypti is the primary vector of dengue, chikungunya, yellow fever and Zika viruses and a significant nuisance to urban populations in temperate climates around the globe. It is a common domestic mosquito in urban and suburban areas in Florida including St. Augustine, St. Johns County, Florida. To date, efforts to control this mosquito have involved insecticides and reduction of breeding environment with limited success due to many reasons such as development of insecticide resistance, use of cryptic breeding and resting habitats to which insecticide penetration is difficult. Sterile Insect Technique (SIT) which includes the use of radiation to sterilize mosquitoes is a biological insect control method that was developed in the 1950s and has proven to be effective in the control with other insects. The purpose of this study is to evaluate the effectiveness of the use of irradiated mosquitoes on controlling wild populations of Ae. aegypti in downtown St. Augustine, Florida. Intervention and control study sites were identified in known Ae. aegypti

areas. Around 50,000 irradiated marked male Ae. aegypti (provided by the United States Department of Agriculture, Center for Medical, Agricultural and Veterinary Entomology- USDA-CMAVE) were released uniformly throughout the intervention site targeting over 5:1 irradiated wild males. Releases were initiated in early March 2020 when the population was at low density and the environmental temperatures were warm enough for mosquito activity. Multiple release points with ~100 m between every two points were used to ensure the even spread of released mosquitoes across the site. However, after five releases the USDA-CMAVE went on a COVID-19 lockdown, resulting in us missing the best period to target Ae. aegypti populations. Releases were resumed in July 2020, when the population was at its peak and continued until December 2020. Pre and post intervention population monitoring was conducted weekly using Biogents Sentinel traps and ovi-traps across the two sites to determine the male and female Ae. aegypti abundance and egg hatch rates. Current findings demonstrated no significant impact of the use of irradiated males in suppressing the wild population which could mainly be attributed to the missing out of the best release period. The study will be continued until June 2021 resuming releases in March to conduct multiple releases.

41. INVESTIGATING THE PRESENCE AND CO-INFECTIVITY RATE OF PATHOGENIC SPOTTED FEVER GROUP RICKETTSIOSES (SFGR) IN AMBLYOMMA AMERICANUM TICKS KNOWN TO BE POSITIVE WITH RICKETTSIA AMBLYOMMATIS WITHIN ALACHUA COUNTY, FLORIDA

Chanakya Bhosale - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Kristen Wilson** - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Carrie De Jesus** - 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; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Pathogenic Spotted Fever Group Rickettsioses (SFGR) are a recurrent and growing tick-borne bacterial public health threat within the Southeastern United States, especially in North Central Florida. Amblyomma americanum, the most common aggressive human-biting questing tick species is known to harbor both pathogenic and non-pathogenic SFGR. The high infection rate of the generally non-pathogenic Rickettsia amblyommatis in A. americanum, especially in Florida (30%), may cause other low-abundant pathogenic rickettsiae species to go undetected when utilizing commonly used PCR methods. This assay interference can create an underestimate of the prevalence and co-infectivity rate of rickettsiae species, especially that of pathogenic R. parkeri within A. americanum. Our investigation aims to test already known R. amblyommatis positive A. americanum ticks by utilizing a newly published cost-effective hemi-nested PCR assay which excludes R. amblyommatis and aims to more accurately find other rickettsiae species which may not be found with other traditional PCR methods. The ticks we aim to test were collected in a variety of greenspaces around the Gainesville

metropolitan area in Alachua County, FL, and previously tested positive for R. amblyommatis using a PCR assay targeting the ompA gene. Our preliminary data shows that our ticks may be co-infected with other SFGR rickettsiae species, most probably R. parkeri, a causative agent of R. parkeri rickettsiosis. Of 53 R. amblyommatis positive samples, 20 are likely also positive for a SFGR. Presence of pathogenic rickettsiae species in A. americanum collected in popular greenspaces could pose a public health problem and needs to be studied further. We aim to optimize this new PCR assay and use it for further testing of ticks we will be collecting in 2021.

42. JUVENILE HORMONE ANALOG ENHANCES ZIKA VIRUS INFECTION IN AN INVASIVE MOSQUITO

Abdullah A. Alomar - Department of Entomology and Nematology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Bradley H. Eastmond** - Department of Entomology and Nematology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Barry W. Alto** - Department of Entomology and Nematology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Zika virus (ZIKV) is an emerging infectious pathogen that causes a serious public health threat because of the associated neurologic complications including Guillain-Barré syndrome and microcephaly in adults and neonates, respectively. The development and clinical evaluations of ZIKV vaccines are still ongoing, and so reducing the number of mosquito vectors remains the primary preventative measure to mitigate ZIKV transmission. Juvenile hormone analog (JHA) pyriproxyfen is a chemical substance that prevents the pupal-adult transition of mosquitoes by mimicking their natural juvenile hormone. Here, we examined whether JHA interacts with environmental temperature during immature stages to influence life-history traits and ZIKV infection in the invasive mosquito Aedes aegypti. We exposed larvae to JHA at two temperature regimes (20°C and 30°C) to characterize the interactive effects on life-history traits and on adult infection with ZIKV. Female development time was

lengthened at 20°C and in the presence of JHA. Prevention of pupal-adult transition by JHA was higher at elevated temperature than low temperature. Size of females was larger at 20°C and smaller at 30°C. Infection of ZIKV in females was enhanced by JHA exposure during immature stages at both 20°C and 30°C, whereas disseminated infection was only enhanced at 30°C. Saliva infection (transmission) and loads of ZIKV were not affected by JHA or temperature. Our results show that the interactive effects of JHA and temperature during the immature stages can alter mosquito life-history traits and mosquito-ZIKV interactions. Enhancement of ZIKV infection in adults suggests that more caution should be made when using JHA in mosquito control programs.

43. MALARIA PARASITE MODULATES MOSQUITO METABOLIC PATHWAYS TO PROMOTE ITS SPOROGONIC DEVELOPMENT

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

Malaria remains a major threat to global health with 229 million cases and 409, 000 deaths reported in 2019 alone, the majority of which occur in sub-Sahara Africa. An obligatory step in the complex life cycle of the malaria parasite (Plasmodium species) is sporogony, whereby a single parasite undergoes multiple cellular divisions leading to the release of thousands of human-infective sporozoites. This rapid expansion of the parasite biomass requires nutrient resources, which it obtains from its adult female Anopheles mosquito vector. Among the key nutrients required by the replicating parasites are fatty acids, the precursors of phospholipids necessary for membrane formation. We hypothesized that Plasmodium falciparum exploits the adipokinetic hormone (AKH) signaling pathway in its Anopheles vector to mobilize lipid reserves necessary for its metabolic needs and sporozoite development. To investigate this, we

first monitored AKH titers in P. falciparum infected An. gambiae using competitive peptide ELISA. There was a significant upregulation of AKH peptides in infected mosquitoes compared to uninfected groups. Next, we manipulated AKH levels by dsRNA knockdown of AKH transcripts and synthetic AKH peptide injections and monitored how these changes effected the rate of parasite development and overall sporozoite yield. Depletion of AKH transcripts slowed P. falciparum development and reduced the sporozoite yield. On the other hand, treatment with synthetic peptide significantly increased the parasite growth rate and resulted in significantly higher sporozoite yields. We then sought to investigate if P. falciparum actively modulate the vector AKH signaling pathway to mobilize lipid resources for its development. To do this, we collected mosquito midgut supernatants from P. falciparum infected and uninfected mosquitoes and micro-injected them into healthy female mosquitoes. RNA-seq analyses of the injected mosquitoes revealed parasite-driven regulation of several pathways in the mosquitoes, key among them being lipid metabolic and immune pathways. We discuss the implications of these findings for parasite transmission potential and how these results could impact the design of novel malaria control strategies.

44. MECHANISMS OF RESISTANCE TO PYRETHROIDS IN THREE POPULATIONS OF LONE STAR TICKS (AMBLYOMMA AMERICANUM)

Caitlin E. Taylor - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Zachary D. Kaplan** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Emma NI Weeks** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Phillip Kaufman** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida

The number of captive deer farms has been increasing in the United States. The abundance of ectoparasites and their ability to transmit pathogens that cause cervid diseases necessitates the use of pesticides, especially pyrethroids, on deer farms mainly for the control of biting midges, mosquitoes, horse flies, deer flies, and ticks. Lone star tick (LST), Amblyomma americanum, exposure to pyrethroids is a secondary effect to biting midge control. There currently are no confirmed reports of pyrethroid resistance in LST populations. The goal of this study was to further our understanding of the potential for permethrin resistance in LST. The first objective was to quantify the activity of metabolic detoxification enzymes in permethrin susceptible and potentially resistant LST. This study compared the sensitivity of a laboratory-reared, pesticide-susceptible LST colony to two field-collected LST populations (Levy and Gadsden Co.), to permethrin with three synergists, piperonyl butoxide (PBO), triphenyl phosphate (TPP), and dimethyl maleate (DEM), representing three recognized detoxification pathways. Our results show LSTs already may utilize permethrin detoxification mechanisms as susceptibility was restored in field-collected ticks when permethrin was combined with TPP and PBO. Tolerance to permethrin in our field collected populations was too low to verify the potential mechanisms for resistance development. Therefore, a second objective included inducing permethrin resistance in the Gadsden Co. tick population. This population was colonized, and resistance is being induced by pressuring this strain with the generation-specific, permethrin lethal concentration to 80%

mortality (LC80) value for five successive generations. Understanding the potential for resistance development and the likely mechanisms involved enable appropriate resistance mitigation techniques to be incorporated into integrated pest management plans. The availability of a resistant LST population advances our understanding of pyrethroid resistance.

45. MODE OF ACTION OF NOOTKATONE, A BIORATIONAL INSECTICIDE AND REPELLENT

Edmund Norris - United States Department of Agriculture; **Werner Geldenhuys** - Department of Pharmacology, West Virginia University; **Jeffrey Bloomquist** - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Biorational vector control products are any chemistry that is inspired by or obtained from a natural plant compound, used for the purpose of controlling pest arthropods. As plants and insects have existed in a long evolutionary “arms race” with one-another, some of these compounds have potent bioactivities against various pest species. Moreover, we encounter these compounds every day in our food and perfumes. As such, they represent safer alternatives to synthetic insecticides and repellents, but they may be more labile in the environment. Nootkatone is a natural sesquiterpenoid found in grapefruit and one of the main constituents responsible for its characteristic aroma and flavor. It is an effective repellent and is toxic to mosquitoes and ticks, and its mode of action is not well understood. Experiments were therefore initiated to better understand the mode of action of nootkatone in a number of insect neurophysiological systems. Nootkatone was lethal to mosquitoes, synergized natural pyrethrins, and showed signs of neurotoxicity. It was inactive on a cockroach axonal preparation and showed a nerve blocking action on the larval Drosophila melanogaster central nervous system (CNS) preparation. It was successful in reversing the nerve blocking effect of applied GABA, an inhibitory neurotransmitter. Moreover, its GABA-blocking effect was reduced in a strain of D. melanogasterhaving a mutated GABA receptor (A302S) and this strain was resistant to the lethal effects of nootkatone. Molecular modeling studies indicated that the

mutation could account for reduced nootkatone binding within the chloride ion channel of the GABA receptor. These studies demonstrate that the lethal effects of nootkatone are mediated via GABAergic pathways in insects, but are unlikely to explain its repellent effects. The implications of these results for the commercialization of nootkatone for vector control are discussed.

46. NEGEVIRUS PIURA SUPPRESSES ZIKA VIRUS REPLICATION IN MOSQUITO CELLS

Valeria Carvalho - Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida; **Dhani Prakoso** - Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida; **Erika Schwarz** - Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida; **Sarah Beachboard** - Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida; **Maureen Long** - Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida

The Insect-specific virus (ISV) Piura (PIUV), belonging to the taxon Negevirus, was first isolated in the Piura city in Peru in 1996 from Culex sp. mosquitoes. In the subsequent years, PIUV was detected in countries such as Mexico, Colombia and United States. The ISVs in general may share the same mosquito vector with other group of viruses, the arboviruses; besides, some ISVs are phylogenetically related to certain arboviruses, while others, the negeviruses, are closer to plant viruses. Unlike arboviruses, the ISVs do not appear to replicate in vertebrate and their cells. Studies showed that certain ISVs suppress the replication of arboviruses in cells and mosquitoes. Little information is known about negeviruses and arboviruses interaction. Based on this, we are investigating the interaction between the negevirus PIUV and the arbovirus Zika (ZIKV) in Ae. albopictus cells. We performed coinfection experiments in C6/36 cells. The PIUV (Cor 33 strain from Colombia) and ZIKV (PRVABC58 strain from Puerto Rico) were concomitantly inoculated

in C6/36 cells (12-well plates), both at MOI 0.1. Four conditions were investigated: 1) PIUV and ZIKV coinfection, 2) ZIKV only, 3) PIUV only, 4) Negative control. The conditions 1, 2 and 3 were inoculated in triplicate. The cell suspension was collected daily through 7 days post-infection and frozen at −80 °C. Samples originated from the conditions ZIKV only and PIUV and ZIKV coinfection were titrated by TCID50 on Vero 76 cells aiming to compare ZIKV titers and detect PIUV interference in ZIKV replication. The conditions ZIKV only and PIUV and ZIKV coinfection were also tested by RT-qPCR for ZIKV using the CDC diagnostic one-step RT-PCR protocol for ZIKV. To investigate whether ZIKV interfered in the PIUV replication or not, we developed a RT-qPCR for PIUV (efficiency of 96%). Our preliminary results show that concurrent infection by PIUV suppresses the replication of ZIKV reaching 10,000-fold reduction in ZIKV titers with 3 days post-infection ($p=0.0001$). The RT-qPCR also showed reduction in ZIKV viral load in the coinfection samples. Otherwise, PIUV viral loads were not reduced in the co-infection wells compared to the PIUV only wells. We conclude that, whether concurrently infected, PIUV (Cor 33 strain) suppress ZIKV (PRV PRVABC58 strain) in C6/36 cells, nevertheless, ZIKV does not interfere in PIUV replication. Our results pointing to the possible future use of PIUV as an alternative tool for biological control of ZIKV, but further studies in mosquitoes are needed.

47. NEUROTOXICITY AND PHYSIOLOGICAL ACTIONS OF 1-METHYLPIPERAZINE, 1-METHYLPYRROLIDINE, AND TRIETHYLAMINE ON AEDES AEGYPTI MOSQUITOES

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

The mosquito, Aedes aegypti (Diptera: Culicidae), is a vector of dengue fever, Zika, chikungunya, and yellow fever. Basic amines have previously been shown to prevent mosquito feeding in human volunteer studies with caged mosquitoes. The mechanism of feeding protection was hypothesized to be due to anosmia, whereby the mosquitoes cannot use olfaction to orient to a host, although other mechanisms are possible. In this study, the behavioral responses and toxicity of three basic amines, 1-methylpiperazine, 1-methylpyrrolidine and triethylamine were investigated. The compounds showed some repellency to Aedes aegypti mosquitoes, followed by narcosis, knockdown and paralysis that increased with time and dose. The anesthetic triethylamine was an exception in that nearly all the mosquitoes recovered after 24 hr. Low topical toxicities were observed with all three basic amines, probably due to evaporation off of the cuticle. Electrophysiological experiments showed effects on central nervous system firing of Drosophila melanogaster as well as Kv2 channel blocking effects, with both systems having IC50 values of ca. 1 mM. The rank of order of effectiveness for these blocking effects was consistent with the relative vapor toxicity of the basic amines. Experiments on antennae showed that mosquitoes can smell these compounds and that an augmented response of clean air controls suggested an additional action on mechanoreceptors. The similarity of poison symptoms of triethylamine with the known chordotonal organ modulator, pymetrozine was of interest, and future study of these basic amines on chordotonal organs should be conducted.

48. PLANT ESSENTIAL OILS ENHANCE THE VAPOR TOXICITY AND REPELLENCY OF SPATIAL REPELLENT PYRETHROIDS

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

Effective control of vector-borne disease requires new types of insecticides and repellents, especially in light of widespread resistance to existing products. Screening of new repellents and insecticidal compounds was conducted in a glass tube assay to assess vapor repellency effectiveness in 1-hr exposures to Aedes aegypti mosquitoes and mortality assessments after a 24-hr exposure. As synergism of toxicity of topically applied pyrethroids by plant essential oils has been observed previously in our and other laboratories, we evaluated the potential of plant essential oils to synergize vapor toxicity of vapor active pyrethroids. For these studies, we screened a number of plant essential oils in combination with 3 vapor-active pyrethroids, metofluthrin, transfluthrin, and empenthrin. Synergism ratios were the largest and ranged from 2- to 380-fold for combinations of metofluthrin and fir needle and cedarwood-Virginian type oil, respectively. Synergism by plant essential oils was also observed in combinations with transfluthrin and empentrhin, with synergism ratios greater than 30 for transfluthrin and as high as 79 for empenthrin. Select essential oils also synergized the repellency produced by transfluthrin, with Amyris and cedarwood-Virginia being among the most potent enhancers. Interestingly, synergism ratios for plant essential oil/vapor active pyrethroid combinations were highly dependent on the plant essential oil in question, implicating specific bioactive constituents or mixtures thereof within the most synergistic plant essential oils. Future work will be performed to identify the specific constituents involved in this synergism and the potential mechanism(s) of action of these molecules.

49. RESISTANCE BREAKING INSECTICIDAL ACTIVITY OF NEW SPATIAL INSECTICIDES AGAINST AEDES AEGYPTI

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

Because the literature around N-aryl amide derivatives as spatially-acting insecticides remains relatively unexplored, we synthesized eighty-nine analogues and screened them for mortality against the insecticide susceptible Orlando (OR) strain of Aedes aegypti using a vapor exposure glass tube assay. Of the screened compounds, twenty-two produced > 92% mortality at twenty-four hours and warranted further investigation to determine LC50 values. Fifteen of these analogs had LC50 values within two orders of magnitude of transfluthrin, and of significant interest, N-(2,6-dichloro-4-(trifluoromethyl)phenyl)-2,2,3,3,3-pentafluoropropanamide was nearly as potent as transfluthrin and exhibited greater toxicity than metofluthrin when screened against OR Ae. aegypti. Select compounds were screened against the insecticide resistant, Puerto Rico (PR) strain of Ae. aegypti and it was discovered that not only did these N-arylamides typically show little resistance, some such as N-(2,6-dichloropyridin-4-yl)-2,2,3,3,4,4,4-heptafluorobutanamide and 2,2,3,3,4,4,4-heptafluoro-N-(3,4,5-trifluorophenyl)butanamide were actually more potent against the PR mosquitoes. Due to this promising insecticidal activity, five compounds were administered orally to mice to determine acute oral rodent toxicity. All five compounds were found to have mouse oral toxicity LD50 values well above the minimum level as set by the Innovative Vector Control Consortium (50 mg/kg).1 In addition to the promising biological activity documented here, we report the structure-activity relationship analysis used to guide the derivatization

approach taken and to further inform future efforts in the development of N-arylamides as potential resistance-breaking, spatially-acting insecticides.

50. SIMULATED IMPACTS ON VECTORIAL CAPACITY FROM RESISTANCE AND EXPOSURE TO INSECTICIDES AND REPELLENTS IN AEDES AEGYPTI

Cynthia Lord - College of Agricultural and Life Sciences, Florida Medical Entomology Laboratory, University of Florida; **Tse-Yu Chen** - College of Agricultural and Life Sciences, Florida Medical Entomology Laboratory, University of Florida; **Jebidiah Light** - College of Agricultural and Life Sciences, Florida Medical Entomology Laboratory, University of Florida; **Austin Henneberg** - College of Agricultural and Life Sciences, Florida Medical Entomology Laboratory, University of Florida; **Chelsea Smartt** - College of Agricultural and Life Sciences, Florida Medical Entomology Laboratory, University of Florida

Introduction: High levels of resistance to commonly used insecticides have been documented in many populations of Aedes aegypti, resulting in increased interest and use of alternative chemicals and spatial repellents. Mosquitoes carrying resistance genes to insecticides or repellents may have other changes in their physiology, affecting fitness and vector competence for arboviruses. Exposure to chemicals, whether sub-lethal doses of insecticides or spatial repellents, may also alter physiology. Vectorial capacity, the entomological components of the basic reproduction number of a vector-borne arbovirus, is a useful way to integrate these varied effects to estimate impact on pathogen transmission. Relatively little is known about the relationships between resistance and exposure and mosquito physiology traits (survival, biting rate) and virus interaction traits (infection and transmission rates, extrinsic incubation period).

Model: Using an individual based model, we are integrating net effects on vectorial capacity under different assumptions about relationships between traits and resistance or exposure (e.g. increasing, decreasing and no effect). Individual estimates are summed for population level vectorial capacity estimates. We compare population vectorial capacity across

traits, relationships and resistance levels to assess which are most influential and where better estimates will have the strongest effect on prediction of outcomes.

Results: These results will inform mosquito control strategies in an environment of increasing resistance and use of alternative chemicals, and identify regions of parameter space where risk of transmission may be affected by resistance.

51. SPATIAL ECOLOGY OF THE PROTOZOAN PARASITE, TRYPANOSOMA CRUZI, IN THE NATIVE MAMMALIAN RESERVOIR HOSTS OF NORTH CENTRAL FLORIDA: A PROPOSAL

Carson Torhorst - Department of Wildlife Ecology and Conservation, University of Florida; **Norman Beatty** - Department of Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Sarah Mays Maestas** - Department of Entomology and Nematology, University of Florida; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, Emerging Pathogens Institute, University of Florida

Chagas disease, which is caused by the protozoan parasite, Trypanosoma cruzi (T. cruzi), is endemic to both North, Central and South America, infecting both humans and native mammalian wildlife. This parasite is estimated to infect 6-8 million people worldwide, and 300,00-400,000 here in the United States. Autochthonous transmission in the US are low with 76 cases since 1955. Vector-borne transmission through the feces of a Triatoma spp. “Kissing bug” vector is the classically understood pathway of transmission for this disease; however, other nontraditional transmission pathways like oral and vertical transmission routes need further investigation in the wildlife reservoir hosts of endemic areas. These nontraditional pathways are likely leading to further transmission of T. cruzi in wildlife reservoir hosts. A previous study using serological based methods showed a 38 and 14 percent prevalence, respectively, in raccoons (Procyon lotor) and the Virginia opossum (Didelphis virginiana) in Florida. Opossums and raccoons are the most reported hosts across the endemic range of T. cruzi in the United States. A preliminary study conducted in the Wisely lab using qPCR-based methods showed a

prevalence across raccoons and opossums in Florida between 30 and 40 percent, as well as an association between DTU and host, which has public health impacts. Previous studies have also shown that T. cruzi is able to reproduce and sustain infection within the anal glands of the genus Didelphis (the Virginia opossum is the only species of Didelphis found in the US). This has significant public and veterinary health impacts if found to be true in Florida, which is likely. This study will be comprised of five study areas that include a paired sylvatic site and peridomestic site which has had Kissing bug (Triatoma sanguisuga) domiciliation. This will provide a spatial understanding of how T. cruzi is distributed within the reservoir hosts sampled across the sylvatic and peridomestic sites. The objectives of this study are to (1) understand the prevalence of T. cruzi under a paired sites study design to further comprehend the spatial distribution and differences among the known reservoir hosts of T. cruzi between sites, (2) to understand if consumption of T. sanguisuga is common among reservoir hosts which implicates an oral-transmission pathway of T. cruzi to the reservoir hosts and (3) to further understand the complex interaction between opossums and T. cruzi here in Florida, and this impact on public health.

52. THE EFFECTS OF HABITAT TYPE AND PATHOGEN INFECTION ON TICK HOST SEEKING BEHAVIOR

Elise A. Richardson - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **Caitlin E. Taylor** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Brittney Jabot** - Department of Biology, University of Florida; **Estelle Martin** - Department of Entomology and Nematology, University of Florida; **Carl N. Keiser** - Department of Biology, University of Florida

Tick borne pathogens pose a significant risk to public health, wildlife health, and livestock health. The lone star tick, Amblyomma americanum, is an aggressive tick that transmits a wide array of pathogens. This tick currently inhabits the southeastern Unites States, but its geographic range is predicted to expand as climatic change makes a variety of habitats more hospitable for the lone star tick. With this in mind, we set out to assess the relationship between habitat type, pathogen infection, and host seeking behavior (questing). Ticks were collected using a tick drag through two different habitat types: xeric hammock and successional hardwood forest in the Ordway-Swisher Biological Station. Standardized 10min behavioral assays were conducted with each tick to assess the average heights quested and total time spent questing. Finally, each tick was sequenced for Rickettsia amblyommatis by extracting DNA, PCR with Rickettsia-specific primers, and sequencing using Genewiz. R. amblyommatis is a member of the Rickettsia spotted fever group and can be highly prevalent in some lone star tick populations. We have found about 28.6% (infection prevalence of 0.295) of the ticks collected to be infected with R. amblyommatis. Specifically, the infection prevalence of R. amblyommatis in each habitat type was as follows, 28% of ticks collected in successional hardwood forest and 32% in xeric hammock habitats. Ticks infected with R. amblyommatis appeared to spend less time engaging in questing behavior than uninfected ticks. Additionally, we found that ticks collected from xeric hammock habitats spent over twice as long questing compared to ticks from successional hardwood forests. These results show that habitat type and infection status can influence

the total time a tick can spend engaging in questing behaviors, which can play a pivotal role in transmission rates and disease dynamics.

53. BACTERIAL PATHOGENS ISOLATED FROM FLORIDA FARMED WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) DURING 2017 - 2020

An-Chi Cheng - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Sydney L. Cottingham** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Angelique T. Fouché** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Austin L. Fajfer** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Juan M. Campos Krauer** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida

White-tailed deer (Odocoileus virginianus) farming is an emerging agricultural industry in Florida. Bacterial infections and viral hemorrhagic diseases cause high mortality in fawn and yearling deer and are a source of significant production loss among Florida deer farmers. Bacterial infection remained the most frequent cause of death among young Florida farmed white-tailed deer aged 0-3 months from 2017 to 2020. The University of Florida Cervidae Health Research Initiative (CHeRI) provides a diagnostic service to Florida deer farmers to determine and monitor the proportion of farmed white-tailed deer that have died from bacterial infections, hemorrhagic disease-causing viruses, or other causes of death. From 2017 to 2020, participating Florida ranches provided recently deceased farmed white-tailed deer for necropsy or shipped tissues for analysis by the CHeRI diagnostic program. Both necropsy and owner-sampled tissues were subjected to aerobically microbial culture. Escherichia coli remained the most frequently isolated bacterial pathogen in lung, kidney, heart, and liver tissue of farmed deer from 2017 to 2020 except lung tissue in 2020, heart tissue in 2017 and 2020. Pathogenic strains will colonize mucosal surfaces and produce disease. Predisposing

factors to infection include age, immune status, and stress levels. Trueperella pyogenes remained the second frequent isolated bacterial pathogen in lung, kidney, heart, and liver tissue of farmed deer from 2017 to 2020 except lung tissue in 2020, heart tissue in 2017 and 2020. Trueperella pyogenes is significantly associated with severe pneumonia in white-tailed deer. Traumatic events, such as sudden weather changes or introduction to a new environment and stress appear to increase infection susceptibility. Escherichia coli, Trueperella pyogenes, and Pseudomonas aeruginosa were identified as the most common bacterial pathogens isolated from deceased farmed white-tailed deer. However, it is clear that a range of bacteria are involved in severe infections in deer. These data provide valuable information to improve preventive measurements, and improve treatment in Florida farmed white-tailed deer, thereby improving herd health, and reducing mortalities.

54. C-DI-AMP IS ESSENTIAL FOR THE VIRULENCE OF ENTEROCOCCUS FAECALIS

Ling Ning Lam - Department of Oral Biology, College of Dentistry, University of Florida

The ability to sense and respond to environmental cues is essential for the survival of bacterial pathogens. Cyclic di-adenosine monophosphate (c-di-AMP), a nucleotide-based messenger, is important for central metabolism, stress tolerance, biofilm formation and virulence. Enterococcus faecalis, the casual agent for a variety of nosocomial infections, is remarkably tolerant of a variety of environmental stresses; yet, it is not known how c-di-AMP signaling impacts E. faecalis pathophysiology. In this study, we examined the importance of c-di-AMP in E. faecalis stress tolerance and virulence using isogenic deletion mutants for genes associated with c-di-AMP synthesis (diadenylate cyclase, CdaA) and degradation (phosphodiesterases, GdpP and Pde). We show that loss of CdaA resulted in no detectable intracellular c-di-AMP pools, and the loss of Pde and GdpP; collectively resulted in high intracellular c-di-AMP pools, confirming the predicted roles of these enzymes. We demonstrate that maintainence of c-di-AMP homeostatis is critical to survival in enriched media and biological fluids. In addition, we

show that CdaA modulates Ebp (endocarditis and biofilm associated pilus) which have been reported to impact E. faecalis virulence. Finally, we show that c-di-AMP is essential for virulence in G. mellonella infection, mouse model of peritonitis and catheter-associated urinary tract infection. Collectively, these findings offer conclusive evidence that c-di-AMP signaling is essential for virulence in E. faecalis.

55. CEFEPIME POPULATION PHARMACOKINETICS AND TARGET ATTAINMENT IN CRITICALLY ILL PATIENTS ON CONTINUOUS RENAL REPLACEMENT THERAPY

Mohammad Alshaer - Department of Pharmacotherapy and Translational Research, Emerging Pathogens Institute, College of Pharmacy, University of Florida; **Carolyn D. Philpott** - University of Cincinnati; **Christopher A. Droege** - University of Cincinnati; **Joshua D. Courter** - Cincinnati Children's Hospital Medical Center; **Daniel P. Healy** - University of Cincinnati; **Molly E. Droege -** University of Cincinnati; **Neil E. Ernst** - University of Cincinnati; **Eric W. Mueller** - University of Cincinnati; **Charles Peloquin** - Department of Pharmacotherapy and Translational Research, Emerging Pathogens Institute, College of Pharmacy, University of Florida

Introduction: Sepsis causes half of acute kidney injuries in the intensive care unit (ICU). ICU patients may need continuous renal replacement therapy (CRRT) which will affect their antimicrobial exposure. We aim to build a cefepime population pharmacokinetic (PK) model in CRRT ICU patients and perform simulations to assess target attainment.

Methods: Patients who were ≥18 years old, admitted to the ICU, and received cefepime 2 g every 8 hours as 4-hour infusion while on CRRT were enrolled prospectively. Samples were collected from the predialyzer, postdialyzer ports, and effluent fluid at times 1, 2, 3, 4, and 8 hours after the first dose and at steady state. Age, sex, weight, urine output, and CRRT parameters were recorded. Pmetrics was used for population PK and simulations. The target exposure was 100% fT>MIC and 60% fT>4xMIC.

Results: Ten patients were included and their mean age was 53 years and weight 119 kg. Seventy percent were males. Cefepime was described by a

five-compartment model. The downtime was applied to the CRRT flow rates which were used to describe the rates of transfer between the compartments. At MIC of ≤8 mg/L, cefepime 2 g intermittent infusion every 8 hours achieved good target attainment both early in therapy and at steady state. Only extended and continuous infusion regimens achieved good target attainment at MIC 16 mg/L.

Conclusions: Cefepime 2 g infused over 30 minutes followed by 2 g extended infusion every 8 hours achieved good target attainment at MIC ≤16 mg/L with different CRRT flow rates and may be considered in resistant bacterial infections.

56. CONVERGENT EVOLUTION OF DIVERSE BACILLUS ANTHRACIS OUTBREAK STRAINS TOWARDS ALTERED SURFACE OLIGOSACCHARIDES THAT MODULATE ANTHRAX PATHOGENESIS

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

Bacillus anthracis, a spore forming Gram-positive bacterium, causes anthrax. The external surface of the exosporium is coated with glycosylated proteins. The sugar additions are capped with the unique monosaccharide anthrose. West African Group (WAG) B. anthracis have mutations rendering them anthrose deficient. Through genome sequencing we identified two different large chromosomal deletions within the anthrose biosynthetic operon of B. anthracis strains from Chile

and Poland. In silico analysis identified an anthrose deficient strain in the anthrax outbreak among European heroin users. Anthrose deficient strains are no longer restricted to West Africa so the role of anthrose in physiology and pathogenesis was investigated in B. anthracis Sterne. Loss of anthrose delayed spore germination and enhanced sporulation. Spores without anthrose were phagocytized at higher rates than spores with anthrose, indicating anthrose may serve an antiphagocytic function on the spore surface. The anthrose mutant had half the LD50 and decreased time to death of wild type and complement B. anthracis Sterne in the A/J mouse model. Following infection, anthrose mutant bacteria were more abundant in the spleen indicating enhanced dissemination of Sterne anthrose mutant. At low sample sizes in the A/J mouse model, mortality of DantC infected mice challenged by intranasal or subcutaneous routes was 20% greater than wild type. Competitive index studies indicated spores without anthrose disseminated to organs more extensively than a complemented mutant. Death process modeling using mouse mortality dynamics suggested larger sample sizes would lead to significantly higher deaths in anthrose negative infected animals. The model was tested by infecting Galleria mellonella with spores and confirmed the anthrose mutant was significantly more lethal. Vaccination studies in the A/J mouse model showed the human vaccine protected against high dose challenges of the non-encapsulated Sterne-based anthrose mutant. This work begins to identify the physiologic and pathogenic consequences of convergent anthrose mutations in Bacillus anthracis.

57. GENOMIC AND PHYLOGENETIC ANALYSIS OF BACILLUS CEREUS BIOVAR ANTHRACIS ISOLATED FROM ARCHIVAL BONE SAMPLES REVEALS EARLIER NATURAL HISTORY OF THE PATHOGEN

Michael Norris - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Diansy Zincke** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **David Daegling** - Department of Anthropology, College of Liberal Arts and Sciences, University of Florida; **John Krigbaum** - Department of Anthropology, College of Liberal Arts and Sciences, University of Florida; **Ted Hadfield** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Jason Blackburn** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Bacillus cereus biovar anthracis (Bcbva) was discovered as the causative agent of anthrax-like fatal disease among wild chimpanzees in 2001 in Cote d'Ivoire. Before this, description of anthrax-like disease caused by typically avirulent Bacillus cereus had not been described. Genetic analysis found that B. cereus had acquired two anthrax-like plasmids, one a pXO1-like toxin producing plasmid and the other a pXO2-like encoding a protective capsule. Bcbva later caused fatalities in chimpanzees and a gorilla in Cameroon in 2004 and 2006. In 2012, Bcbva was isolated from livestock carcasses in the Democratic Republic of Congo. Also, in 2012 an elephant in the Central African Republic was found to be the victim of anthrax disease caused by Bcbva. Since this time, it was discovered the pathogen had acquired plasmids in the wild and only now had scientists started looking for this emergent pathogen that had caused widespread animal fatalities for an unknown amount of time. Primate bones had been shipped out of the endemic zone for anthropological studies prior to the realized danger of contamination with Bcbva and had been submitted to SEER Lab for analysis. Preliminary studies positively identified Bcbva was present in several of the bone fragments. The animals in question died between 1994 and 2010. Previously the earliest archival strains of Bcbva were identified in 1996. Our strains have the potential to unveil historical

genomic information not available elsewhere. This information could shed light on the evolution and emergence of a dangerous anthrax-causing pathogen.

58. IMMUNE-ASSOCIATED GENE EXPRESSIONS IN HOST RESPONSE TO BURKHOLDERIA PSEUDOMALLEI INFECTION IN RAW264.7 MURINE MACROPHAGE CELLS

Treenate Jiranantasak - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Apichai Tuanyok** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida

Melioidosis, a severe tropical disease, is endemic in Southeast Asia and northern Australia. It is caused by Burkholderia pseudomallei, a facultative intracellular Gram-negative bacterium that resides in water and soils. B. pseudomallei infection can cause a wide range of clinical signs including septic shock in acute infection to chronic forms with multiple abscesses. The elimination of this pathogen is difficult as it can survive intracellularly and replicate within many host-cell types, especially macrophages and lung epithelial cells. Macrophages play a critical role in bacterial clearance via controlling bacterial replication during infection. However, the information of how innate immune responses and signal transductions involving in B. pseudomallei infection in the macrophages at early and late stages are still limited. RNA-Seq has several advantages in terms of measuring dynamic changes of expression levels. The objective for this study is to identify host genes which are directly or indirectly responded to B. pseudomallei infection in RAW264.7 murine macrophage cells at early and late stages using RNA-Seq technique. :The current study revealed that genes known or predicted to be involved in signal transduction and immune-associated pathways were significantly upregulated at 2- and 24-hour post infection. This finding broadly agreed with previous reports that these pathways included TNF signaling, NF-kappa B signaling, MAPK signaling, Rap1 signaling, C-type lectin receptor signaling, Fc gamma R-mediated phagocytosis, Chemokine signaling, B cell receptor signaling, Toll-like receptor signaling, NOD-like receptor signaling, HIF-1 signaling, FoxO signaling, PI3K-Akt signaling, and JAK-STAT

signaling pathways are significant in B. pseudomallei infection. Interestingly, the RNA-seq data from the current study demonstrated two additional significant pathways, IL-17 signaling and PD-L1 & PD-1 immunological checkpoint, which are first discovered in B. pseudomallei infected macrophage in this study. Gene cd274 (Pd-l1) was significantly upregulated in the infected RAW264.7 at both time points. The cytokine and chemokine profiles assessed by ELISA was consistent with the RNA-Seq data showing increased cytokine levels at 2- and 24-hour post infection. Moreover, bacterial virulence genes including type 3 and type 6 secretion system were found in infected macrophages. In summary, B. pseudomallei can modulate host innate immune responses in RAW264.7 macrophages at early and late stage of infection. It is possible that B. pseudomallei evades from innate immune cells via upregulation of Pd-l1 in macrophages and cause T cell exhaustion. Therefore, understanding of host-pathogen interaction and pathogenesis of B. pseudomallei infection would lead to potential impact in novel host-directed target and improved therapies.

59. IMPACT OF ANTIBIOTIC SPRAY ON BACTERIAL COMMUNITIES IN CONVENTIONAL AND ORGANIC CITRUS GROVES IN FLORIDA

Marie de Gracia Coquerel - Department of Plant Pathology, Emerging Pathogens Institute, University of Florida; **Andrew McAuley** - Department of Plant Pathology, Emerging Pathogens Institute, University of Florida; **Nabil Chowdhury** - Department of Plant Pathology, Emerging Pathogens Institute, University of Florida; **Kwang Cheol Jeong** - Department of Animal Sciences, College of Agricultural and Life Sciences, University of Florida; **J. Glenn Morris, Jr.** - Department of Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Erica M. Goss** - Department of Plant Pathology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Marina S. Ascunce** - Department of Plant Pathology, Emerging Pathogens Institute, University of Florida

In the last 15 years, the citrus industry in Florida and worldwide have been seriously affected by citrus greening or Huanglongbing (HLB) disease. The causal agent, Candidatus Liberibacter asiaticus (CLas), is an

unculturable bacterium that it is transmitted by an invasive insect, the citrus psyllid, upon feeding on citrus leaves. As crop losses reach up to 90%, Florida growers are desperate, resulting in the spraying of antibiotics streptomycin (STR) and oxytetracycline (OTC) to control HLB. Because soil microbiota represents a natural source of antibiotic resistance genes, the ongoing antibiotic applications in citrus-growing areas are expected to select for antibiotic resistance bacteria (ARB) within the microbial community. We hypothesized that after antibiotic application on citrus trees, there will be an increase in the frequency of ARBs as measured by the proportion of ARBs among colony-forming units. In the summer of 2019, we visited three groves: two groves were subject to standard antibiotic spray application of both STR and OTC and one grove was organic (no antibiotic application). In each grove we sampled 6 trees by collecting leaves, soil, and roots leading to a total of 54 samples. Each sample was diluted and plated into three TSA agarose plates: one with no antibiotics (control), one with STR and one with OTC. Bacteria colonies were counted over one week and colonies-forming units (CFU) per gram of tissue were estimated. Data analysis was carried out using Kruskal-Wallis and Mann-Whitney-Wilcoxon procedures in R to test for significant effects of grove management on CFU. For leaf samples, CFU was not significantly affected by the origin of samples, although CFU values for leaves were overall low. For soil samples, contrary to our hypothesis, we found larger numbers of antibiotic-resistant CFU in the organic grove, which could reflect an overall larger microbial diversity in that environment. Interestingly, resistant CFU obtained from root samples were larger in both the organic and one of the antibiotic-treated groves than the other antibiotic-treated grove. The antibiotic-treated grove with the smaller number of root resistant CFU was also the youngest one. We hypothesize that the diversity of bacteria associated with young trees could reflect the natural bacteria recruitment process that occurs over time as trees mature. We are conducting 16S amplicon analysis to characterize the bacteria diversity, and we expect to include metagenomic work in the future.

60. IMPACT OF PHAGES ON THE DIVERSITY OF BURKHOLDERIA PSEUDOMALLEI

Iris Irby - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Apichai Tuanyok** - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida

The bacterium Burkholderia pseudomallei is the cause of Melioidosis, a tropical disease endemic to Asia. One factor that makes B. pseudomallei virulent is the widespread recombination of the genome. Genomic islands can be found throughout the genome in the form of prophage islands, containing temperate phages, viruses that insert themselves into the genome and can be replicated with the genome. These temperate prophages can be identified at the locations associated with tRNA site-specific recombination (SSR). Previous research has indicated that prophages of family Myoviridae recombine downstream of genes encoding tRNA-Phenylalanine (Phe) and Siphoviridae recombine downstream of genes encoding for tRNA-Proline (Pro). It has also been hypothesized that prophages of one family can block bacteriophages, viruses that kill bacteria, of the same family. Previously, we isolated the regions between all tRNA genes and their repeat sequences and found prophages at tRNA genes encoding for Phenylalanine, Arginine, Cysteine, Methionine of family Myoviridae, and Proline and Selenocysteine of family Siphoviridae. We plotted the geographic distribution of these prophages based on continent, and found that Australia-Oceana to have the highest diversity in prophages, and had prophages tRNA-Cysteine not found in strains from any other continents. We compared this diversity to the Internal Transcribed Spacer (ITS) types (G, C, E, or CE) of the strains in each continent. ITS is used as a genetic marker of B. pseudomallei, and it has been previously shown that ITS type G strains are more common in the Western Hemisphere. We hypothesize that these more isolated type G strains will have a lower prophage diversity. We were able to confirm this hypothesis, as 92% of prophages present in type G strains were of family Myoviridae, and by the most diverse continent having no type G

strains. By understanding this distribution of tRNA-SSR in B. pseudomallei, there will be a better understanding of the genetic diversity and causes of resistance in the bacteria.

61. NOCARDIA BRASILIENSIS-ASSOCIATED CALCANEUS OSTEOMYELITIS

Shoney Archana - Department of Medicine, College of Medicine, University of Florida

Introduction: Nocardiosis is an opportunistic disease in immune suppressed individuals. Approximately a third of infected patients are immunocompetent. It is a rare cause of osteomyelitis that is associated with skin trauma. Treatment consists of surgical debridement and antibiotic therapy.

Case Description: A 6-year-old healthy male child was admitted with pain, swelling and erythema of the heel with inability to bear weight two weeks after stepping on a nail with his right foot. No history of fever. MRI revealed the presence of osteomyelitis with associated sequestrum and intraosseous abscess of the right posteromedial calcaneus (Figure 1). CBC, ESR and CRP were within normal limits, and blood culture was negative. Gram and AFB stains were negative. Biopsy specimens were inoculated on conventional culture media. After 7 days, a partially acid-fast bacilli organism grew, later identified as Nocardia spp. Broad-range 16S DNA PCR and genetic sequencing confirmed N. brasiliensis. The patient was treated with trimethropim-sulfametoxazole (TMP-SMX) for 7 months with poor medication adherence. Two months after discontinuation of TMP-SMX, his symptoms returned. MRI showed recurrence of the intraosseous abscess within the calcaneus and along the peroneal tendons. Surgical debridement was performed, and cultures grew N. brasiliensis. He received IV followed by oral TMP-SMX for 6 and ½ months. Patient remained asymptomatic at follow-up visit 3 months after discontinuation of the antibiotic therapy.

Evaluations and Discussion: Nocardia is a genus of aerobic gram-positive bacteria that appears as branching, beaded, filamentous bacilli on gram stain. It exhibits weak acid fastness and slow growth in cultures usually requiring 5-21 days. It exists in the environment as a saprophyte, and

found on water, garden soil, house dust and beach sand (1). Inhalation is the most common route of infection, and the lungs are the most frequently involved organ. Direct inoculation in the skin is the second route of infection. Nocardia is a rare etiology of osteomyelitis. TMP-SMX is the first-line treatment. Optimal length of therapy is uncertain, but a minimal of 4-6 weeks of therapy is typical. In cases of bone infection, surgical debridement is essential (1).

Conclusions: We report a case of calcaneus chronic osteomyelitis due to Nocardia brasiliensis. Osteomyelitis due to this organism is uncommon, and in the immunocompetent host, more often follows a chronic course after direct inoculation of the microorganism. Poor adherence to antibiotic therapy contributed to infection recurrence following surgical debridement in this case.

62. NOT ALL SCAVENGERS: THE UNIQUE ROLE OF VULTURES IN ANTHRAX SURVEILLANCE AND THE LIMITED OVERLAP BETWEEN OBLIGATE SCAVENGERS AND ENDEMIC ANTHRAX ZONES IN THE UNITED STATES

Morgan A. Walker - Department of Geography, Emerging Pathogens Institute, University of Florida; **Maria Uribasterra** - Department of Geography, Emerging Pathogens Institute, University of Florida; **Valpa Asher** - Turner Enterprises Inc.; **Wayne M. Getz** - Department of Environmental Sciences, Policy, and Management, University of California, Berkeley; **Sadie J. Ryan** - Department of Geography, Emerging Pathogens Institute, University of Florida; **Jose Miguel Ponciano** - Department of Biology, University of Florida; **Jason Blackburn** - Department of Geography, Emerging Pathogens Institute, University of Florida

Anthrax is a zoonosis caused by the spore-forming bacterium Bacillus anthracis, with potential for high fatality rate, especially in herbivores. Upon host death, spores can enter the soil surrounding the carcass and be ingested by other animals feeding in the same location. Accordingly, surveillance to quickly identify and decontaminate anthrax carcasses is crucial to outbreak prevention. In endemic anthrax areas like Texas and

Africa, vultures are used as a surveillance tool for identifying presence and location of dead animals. However, many anthrax outbreaks in the United States have occurred in areas outside the ranges of both black and turkey vultures. Here, we used a longitudinal camera trap survey at carcass sites in southwestern Montana to investigate the utility of a small vulture population on disease and carcass surveillance in a re-emerging anthrax risk zone. From August 2016 to September 2018, camera traps at 13 carcass sites were triggered 2,019 times by avian scavengers. While the majority were facultative avian scavengers such as corvids and eagles, our results suggest facultative scavengers cannot replace vultures as a surveillance tool in this ecosystem due to their absence during the anthrax risk period (June-August), reduced search efficiency, or low flight patterns. We found that the conditions in Montana likely parallel systems elsewhere in the continental United States: using ecological niche models of B. anthracis distribution overlaid with relative abundance maps of turkey vultures, we found that much of North Dakota, South Dakota, Minnesota, Wyoming, Nebraska, and Iowa have areas of anthrax risk, but low or absent turkey vulture populations. Without vultures in these areas, surveillance capacity is reduced, and it becomes more difficult to identify anthrax cases, meaning fewer carcasses are decontaminated, and consequently, outbreaks could become more frequent or severe.

63. SIGNIFICANCE OF IRON ACQUISITION TO ENTEROCOCCUS FAECALIS PATHOPHYSIOLOGY

Debra N Brunson - Department of Oral Biology, College of Dentistry, University of Florida

Enterococcus faecalis is an opportunistic pathogen frequently associated with catheter-associated urinary tract infections, central line associated blood stream infections, and wound infections. The virulence of E. faecalis largely resides in its ability to survive under harsh conditions, form biofilms on indwelling devices, and evade the innate immune system. One obstacle that must be overcome by bacterial pathogens is the restricted access to essential metal ions, like iron, by an active host response known as nutritional immunity. To compete for access to iron during infection, bacteria utilize high affinity metal transporters while some can also secrete siderophores to scavenge iron from host tissues. Recently, we showed that manganese, another essential metal ion, is critical for the virulence of E. faecalis, however, the importance of iron and the mechanisms utilized by E. faecalis to acquire this biometal during infection are presently unknown. Through genomic and global transcriptional analyses, we identified five iron transporters in the core genome of E. faecalis. These include the highly conserved feoAB, fhuDCBG, and efaCBA, and two new putative metal ion transporters that we named eitABCD and emtABC. Characterization of single mutants showed only modest growth delays for the ΔeitAB and ΔemtB strains when grown in iron-depleted media despite all single mutants, except for ΔfeoB, showing ~ 40% decrease in intracellular iron content when grown in iron-replete media. A ΔΔΔΔΔ strain lacking all five iron transporters (Δ5) demonstrated a significant growth defect in iron-depleted media, which was partially restored by genetic complementation with any one of the five transporters. Virulence of Δ5 was significantly attenuated in an invertebrate model of systemic infection, but not in a mouse model of peritonitis. We suspect this may be due to the expression of additional, yet to be identified, iron or heme transporters. Current efforts focus on an uncharacterized ECF-type transporter showing ~75% similarity with a novel heme transporter recently identified in Streptococcus pyogenes, an

ABC-type transporter showing weak homology with an iron transporter from Streptococcus pneumoniae, and an NRAMP-type manganese transporter annotated as a dual Fe/Mn transporter.

64. A MULTI-METHODS ANALYSIS OF CHANGES IN HEALTH OUTCOMES AND MARIJUANA USE DUE TO THE COVID-19 PANDEMIC AMONG PERSONS LIVING WITH HIV

Christina Parisi - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Deepthi Varma** - Department of Epidemiology, College of Public Health and Health Professions, University of Florida; **Yan Wang** - Department of Epidemiology, College of Public Health and Health Professions, University of Florida; **Krishna Vaddiparti** - Department of Epidemiology, College of Public Health and Health Professions, University of Florida; **Gladys Ibañez** - Department of Epidemiology, Florida International University; **Robert Cook** - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

Background: Persons living with HIV (PLWH) are at enhanced risk for adverse substance use, physical health, and mental health outcomes, but no prior study has investigated changes in these outcomes in the context of COVID-19 or underlying reasons for changes. The objectives of this qualitative inquiry are to 1) describe changes in physical and mental health and marijuana use in this population due to the COVID-19 pandemic and 2) understand the reasons behind these changes.

Methods: This poster presents the analysis of open-ended questions administered during a 3-month follow-up call for a cohort study (MAPLE) aiming to examine the effects of marijuana on PLWH in Florida. Data from open-ended questions about experienced changes in mental health, physical health, and marijuana use were compiled in Microsoft Excel and manually coded a priori. Codes were categorized under broader themes to understand reasons for changes in health or marijuana use.

Results: Data were collected from 222 PLWH (mean age=50.2 [SD=11.3], 50.5% female, 68.0% Black/African American, 14.4% Hispanic/Latino). Compared with before the pandemic, 14% used marijuana less frequently while 9% used more frequently. Twelve percent reported improvement and 11% reported worsening of physical health. Thirty percent had worse mental health whereas 8% reported improvement. Among those with worsened mental health, 73% said marijuana was helpful to them. Responses to open-ended questions indicated alleviating boredom was a primary reason for increased marijuana use, while worries about procuring marijuana and COVID-19 exposure led to decreased use. Social isolation and anxiety related to acquiring COVID-19 worsened mental health; however, seeing the pandemic as an opportunity to focus on mental and physical wellness reportedly improved mental and physical health.

Conclusions: The COVID-19 pandemic has had a mixed impact on marijuana use as well as the physical and mental health of PLWH. These findings show many PLWH were impacted in some way by the COVID-19 pandemic; therefore, providers need to address these concerns in their routine care. These results can help inform health interventions aiming to improve physical and mental health among PLWH during the COVID-19 pandemic and beyond. These results also have potential to inform substance use interventions among PLWH.

65. A NEW FAMILY OF DNA VIRUSES CAUSING DISEASE IN CRUSTACEANS FROM DIVERSE AQUATIC BIOMES

Kuttichantran Subramaniam - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Donald C. Behringer** - Department of Forest Resources and Conservation, Emerging Pathogens Institute, University of Florida; **Jamie Bojko** - Department of Forest Resources and Conservation, Emerging Pathogens Institute, University of Florida; **Abigail S. Clark** - Department of Forest Resources and Conservation, Emerging Pathogens Institute, International Center for Coral Reef Research and Restoration; **Natalya Yutin** - National Center for Biotechnology Information; **Kelly S. Bateman -** International Centre of Excellence for Aquatic Animal Health; **Ronny van Aerle** - International Centre of Excellence for Aquatic Animal Health; **Eugene V. Koonin** - National Center for Biotechnology Information; **Grant D. Stentiford** - International Centre of Excellence for Aquatic Animal Health; **Thomas Waltzek** - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, University of Florida

Panulirus argus virus 1 (PaV1) is the only known virus infecting the Caribbean spiny lobster (Panulirus argus) from the Caribbean Sea. Recently, related viruses, Dikerogammarus haemobaphes virus 1 (DhV1) and Carcinus maenas virus 1 (CmV1), have been detected in the demon shrimp (Dikerogammarus haemobaphes) and the European shore crab (Carcinus maenas), respectively, from sites in the United Kingdom. The virion morphology of these crustacean viruses is similar to that of iridoviruses. However, unlike iridoviruses and other nucleocytoplasmic large DNA viruses (NCLDVs), these viruses complete their morphogenesis in the host cell nucleus rather than in the cytoplasm. To date, these crustacean viruses have remained unclassified due to a lack of genomic data. Using an Illumina MiSeq sequencer, we sequenced the complete genomes of PaV1, CmV1, and DhV1. Comparative genome analysis shows that these crustacean virus genomes encode the 10 hallmark proteins previously described for the NCLDVs of eukaryotes, strongly suggesting that they are members of this group. With a size range of 70 to 74 kb,

these are the smallest NCLDV genomes identified to date. Extensive gene loss, divergence of gene sequences, and the accumulation of low-complexity sequences reflect the extreme degradation of the genomes of these "minimal" NCLDVs rather than any direct relationship with the NCLDV ancestor. Phylogenomic analysis supports the classification of these crustacean viruses as a distinct family, "Mininucleoviridae," within the pitho-irido-Marseille branch of the NCLDVs.

66. ANALYTIC AND DIAGNOSTIC PERFORMANCE OF A TAQMAN REAL-TIME QUANTITATIVE PCR ASSAY FOR THE DETECTION OF TILAPIA LAKE VIRUS

Dorothea Megarani - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Lowia Al-Hussinee** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Kuttichantran Subramaniam** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Preeyanan Sriwanayos** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Kamonchai Imnoi** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Mohammad Shamim Ahasan** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Bill Keleher** - Kennebec River Biosciences, Richmond, Maine, USA; **Pamela Nicholson** - Next Generation Sequencing Platform, Institute of Genetics, Vetsuisse, University of Bern; **Win Surachetpong** - Department of Veterinary Microbiology and Immunology and Center for Advanced Studies for Agriculture and Food, Kasetsart University; **Thomas Waltzek** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida

Tilapia lake virus disease (TiLVD) is an emerging viral disease associated with high morbidity and mortality in cultured tilapia worldwide. Thus, the rapid identification and quantification of TiLV is crucial for the implementation of appropriate control measures to reduce the impact of TiLVD. In the current study, we developed and validated a TiLV TaqMan reverse transcription quantitative polymerase chain reaction (RT-qPCR)

assay, targeting a conserved region within the segment 10 sequence. The RT-qPCR assay is efficient (mean ± SD: 101.87 ± 2.43%), sensitive with a limit of detection of 10 TiLV genome copies per reaction, and specific for TiLV strains from different geographic regions including North America, South America, and Asia. The intra-assay (repeatability) and inter-assay (reproducibility) variability range from 0.15−3.16% and 0.63−3.74%, respectively. Analysis of 69 positive and 218 negative samples yielded a diagnostic sensitivity of 97% and a diagnostic specificity of 87%. Taken together, the newly developed TiLV TaqMan RT-qPCR assay is cost-effective and offers rapid results as compared to viral isolation in cell culture. This partially validated RT-qPCR assay can be integrated into surveillance programs aimed at mitigating the effects of TiLVD on global tilapia production.

67. ASSESSING SARS-COV-2 VACCINE INTEREST AMONG PERSONS WITH HIV IN FLORIDA: PRELIMINARY DATA FROM THE FLORIDA COHORT WAVE 3

Rebecca Fisk - Department of Epidemiology, College of Public Health and Health Professions, University of Florida; **Christina Parisi** - Department of Epidemiology, College of Public Health and Health Professions, University of Florida; **Preeti Manavalan** - Department of Medicine, College of Medicine, University of Florida; **Robert Cook** - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

Introduction: In December 2020, emergency use authorizations were granted to two vaccines to combat the ongoing SARS-CoV-2 pandemic. Historically, there have been disparities in vaccine uptake and current trends in vaccination intention mirror this trend. This is concerning given marginalized groups have been at higher risk for more severe outcomes of SARS-CoV-2 infection. As part of the ongoing Florida Cohort, we implemented a coronavirus questionnaire to assess prevention behaviors and vaccination intention among adults living with HIV in Florida.

Methods: The Florida Cohort is a longitudinal study that began enrollment in Fall 2020. Participants are eligible for the cohort if they

aged 18 or older, reside in Florida, and are living with HIV. Surveys may be completed online, on paper, or administered over the phone in English or Spanish. Questionnaires included items about the impacts of SARS-CoV-2 including masking behavior, vaccination intention in general and under various circumstances, and perceived susceptibility to coronavirus. Enrollment has just begun and 34 have completed the coronavirus items, with a planned enrollment of 1000 participants.

Results: The sample largely identified as female (41%) and non-Hispanic Black (82%), with a mean age of 48 years. No participants were aged 65 or older. Twenty-two (64.7%) thought they were at higher risk due to their HIV status. Most participants (85%) reported they always or almost always wore a mask while in indoor public spaces and 88% wore a mask on the last occasion. Half (50%) said they would get a vaccine once available to them, 7 (21%) were unsure, and 10 (29%) said they would not get one. Of 28 non-Hispanic Black participants, 46% said they would be vaccinated. The proportion of those who said they would get a vaccine increased to 62% when the vaccine was recommended by their HIV care provider. Similar increases in vaccination intention were not seen in the case where it was recommended by a community leader or family member or friend.

Conclusions: Our preliminary data suggest suboptimal intention to receive a vaccine against SARS-CoV-2 so greater efforts will be needed to increase interest. Relationships with HIV care providers could be leveraged to increase vaccine uptake among people living with HIV. The Florida Cohort will continue collecting prospective data on vaccination intention and uptake as eligibility for the vaccine expands across the state.

68. COMPLETE GENOME SEQUENCES OF INFECTIOUS SPLEEN AND KIDNEY NECROSIS VIRUS ISOLATED FROM FARMED ALBINO RAINBOW SHARKS EPALZEORHYNCHOS FRENATUS IN THE UNITED STATES

Samantha Ayumi Koda - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Kuttichantran Subramaniam** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Deborah Pouder** - Tropical Aquaculture Laboratory, Program in Fisheries and Aquatic Sciences, School of Forest Resources and Conservation, IFAS, University of Florida; **Roy Yanong** - Tropical Aquaculture Laboratory, Program in Fisheries and Aquatic Sciences, School of Forest Resources and Conservation, IFAS, University of Florida; **Salvatore Frasca Jr.** - Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida; **Vsevolod L. Popov -** Department of Pathology, University of Texas Medical Branch; **Thomas Waltzek** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida

The genus Megalocytivirus includes viruses known to cause significant disease in aquacultured fish stocks. Herein, we report the first complete genome sequences of two megalocytiviruses (MCVs) isolated from diseased albino rainbow sharks Epalzeorhynchos frenatus reared on farms in the United States in 2018 and 2019. Histopathological examination revealed typical megalocytivirus microscopic lesions (i.e., basophilic cytoplasmic inclusions) that were most commonly observed in the spleen and kidney. Transmission electron microscopic examination of spleen and kidney tissues from specimens of the 2018 case revealed hexagonally-shaped virus particles with a mean diameter of 153 ± 6 nm (n=20) from opposite vertices and 131 ± 5 nm (n=20) from opposite faces. Two MCV specific conventional PCR assays confirmed the presence of MCV DNA in the collected samples. Full genome sequencing of both 2018 and 2019 Epalzeorhynchos frenatus iridoviruses (EFIV) was accomplished using a next-generation sequencing approach. Phylogenomic analyses revealed that both EFIV isolates belong to the infectious spleen and

kidney necrosis virus (ISKNV) genotype within the genus Megalocytivirus. This study is the first report of ISKNV in albino rainbow sharks.

69. ENVIRONMENTAL ETIOLOGY OF GREEN TURTLE FIBROPAPILLOMATOSIS.

Costanza Manes - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Daniele Pinton** - Department of Engineering, University of Florida; **Raymond Carthy** - Department of Wildlife Ecology and Conservation, University of Florida; **Alberto Canestrelli** - Department of Engineering, University of Florida; **Ilaria Capua** - Department of Animal Sciences, University of Florida

Fibropapillomatosis (FP) is a neoplastic disease of sea turtles. It was first detected in Florida in 1938, and it is now spread globally and affects all seven species of marine turtles. The most affected species is the green turtle (Chelonia mydas), especially the juvenile band of the population. The disease is most likely caused by infection from the herpesvirus agent Chelonian Herpesvirus 5 (ChHV5). Infections lead to papilloma-like lesions concentrated on the mouth, eyes, and flippers areas. These debilitating lesions can impede basic survival activities such as feeding and swimming. Very often, turtles strand and die. Sometimes lesions also grow on internal organs such as lungs and kidneys, often with fatal results. The virus can be latent, and not all FP-positive individuals necessarily show symptoms and triggering of the cancerous conditions. Outbreaks have increased in the last few decades, and the epidemic is spreading across the oceans. The full etiology of the disease is still unknown, and it is uncertain why elevated incidence has been recently detected in multiple locations. In this study, we aimed at elucidating some aspects of FP epidemiology by investigating multiple environmental factors previously indicated as possibly responsible for triggering FP in wild sea turtle populations. We gathered a dataset of FP prevalence in green turtles across multiple US states, starting from the 1980s until the present. We also collected data from the same period on multiple environmental and habitat disturbance parameters. We analyzed our data series via linear regression to observe for statistical significance and correlation patterns.

Our results show that sea temperature variations, ocean current dynamics and human population density significantly affected FP prevalence. Occurrence of red tide events also significantly correlated with FP density. This study is one of the most integrative on FP environmental etiology to date. We shed light on the truly multifactorial nature of this devastating disease and encourage the inclusion of interdisciplinary efforts in future FP research.

70. GENOME SEQUENCE OF A NOVEL STRAIN OF YUNNAN ORBIVIRUS ISOLATED FROM A DEAD FLORIDA WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS)

Pedro H. O. Viadanna - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Thaís C.S. Rodrigues** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Kuttichantran Subramaniam** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Juan M. Campos Krauer** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **John Lednicky** - Department of Environmental and Global Health, Emerging Pathogens Institute, University of Florida; **Julia Loeb** - Department of Environmental and Global Health, Emerging Pathogens Institute, University of Florida; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, Emerging Pathogens Institute, University of Florida; **Thomas Waltzek** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida

Orbiviruses (family Reoviridae) possess genomes composed of 10-12 dsRNA segments that encode structural (VP) and nonstructural (NS) viral proteins. They are transmitted to mammals by haematophagous arthropods including Culicoides midges, mosquitoes, phlebotomine sand flies, and ticks. Pathogenic orbiviruses, as well as other orbiviruses of unknown pathogenicity have recently been isolated from farmed white-tailed deer in Florida. A farmed 2-year-old female white-tailed deer (OV1288) exhibited excessive salivation, lethargy, separation from the herd, and excessive recumbency four days prior to death. At necropsy, the main gross lesions were hepatic congestion and pulmonary

congestion/edema. The splenic tissue was processed for virus isolation in C6/36 cells and VeroE6 cells as previously described, and cytopathic effects were observed at seven days post inoculation only in C6/36 cells. Viral RNA was extracted from the clarified C6/36 cell culture medium and served as the template for the construction of a cDNA sequencing library and sequenced on an Illumina MiSeq. A total of 2,635,028 paired-reads with an average read length of 252 bp were obtained and de novo assembled in SPAdes with default parameters. BLASTX searches of the resulting contigs, in OmicsBox against the National Center for Biotechnology Information nonredundant protein database, recovered the complete coding sequences for all ten segments of a Yunnan orbivirus (YUOV). The total length of the complete coding sequences of the ten YUOV segments was 18,792 bp, with a GC content of 41.3%. BLASTP searches of all 11 proteins (VP1-VP7 and NS1-NS4) of the YUOV isolate OV1288 showed highest amino acid (aa) identity (97.18-99.68%) to other YUOV strains. Maximum Likelihood phylogenetic analyses, based on separate aa alignments of the major outer capsid protein and T2 protein sequences for 41 orbiviruses, supported the YUOV (OV1288) as a member of the serotype 1 YUOV clade. YUOV was first isolated from Culex tritaeniorhynchus mosquitoes collected in the Yunnan province of China. Similar to the present study, the Chinese YUOV was isolated in a mosquitoes cell line (C6/36), but not in mammalian cell lines. Similarly, two Indonesian YOUVs were isolated from Anopheles vagus mosquitoes in C6/36 cells and Mansonia uniformis mosquitoes in AP-61 cells, but both were refractory to growth in VeroE6 cells. Additional YUOVs have been isolated in C6/36 cells from mosquitoes (A. scapularis) and domesticated mammals experiencing neurological disease in Peru, and these same viruses did not grow in mammalian cell lines. Phylogenetic analysis of Middle Point orbivirus, isolated from an overtly healthy cow in Australia along with one the aforementioned YOUVs isolated from Anopheles vagus, represent a second YOUV serotype. The present study is the first detection of YUOV in North America and expands the host range to include white-tailed deer. Future research is needed to better define the mammalian host range of YUOV, and its potential role in

disease among wild and farmed mammal populations, including white-tailed deer.

71. GENOMIC CHARACTERIZATION OF A NOVEL SIADENOVIRUS FROM THE AFRICAN GREY PARROT (PSITTACUS ERITHACUS)

Austin C. Surphlis - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Jennifer Dill-Okubo** - Florida Department of Agriculture and Consumer Services; **Thomas Waltzek** - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Kuttichantran Subramaniam** - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida

The family Adenoviridae includes double-stranded DNA viruses organized into 5 genera (i.e., Mastadenovirus, Aviadenovirus, Atadenovirus, Siadenovirus, and Ichtadenovirus). Adenoviruses (AdVs) infect a wide range of vertebrate hosts such as fish, amphibians, reptiles, birds, and mammals including humans. Herein, we report the phylogenomic characterization of a novel AdV from an African grey parrot (Psittacus Erithacus), which was submitted for necropsy due to neurological symptoms and concern for Newcastle disease (Avian paramyxovirus – 1/APMV-1). Grossly the liver and spleen were enlarged and mottled marron and brown, while microscopically there was extensive pancreatitis, splenitis and hepatitis with no intranuclear or intracytoplasmic inclusion bodies. PCRs for APMV-1 were negative so brain tissue was used to build a DNA sequencing library and sequenced on an Illumina MiSeq. Scrutiny of the assembled data revealed the complete genome (25,386-base pair [bp]) of a novel AdV. Maximum Likelihood phylogenetic analysis using the amino acid (aa) sequence alignment of the DNA polymerase gene (Dpol) of this parrot AdV and 94 other AdVs supported the former as a unique member within the genus Siadenovirus. Genetic analysis of the aa sequence alignment of the Dpol gene of this parrot siadenovirus to 11 other siadenoviruses ranged from 52.6% to 62.8%, with the highest and lowest identities to the south polar skua adenovirus 1 and frog adenovirus 1, respectively. Proposed species

demarcation criteria for members of the genus Siadenovirus include >15% aa sequence divergence of the Dpol gene when compared to the closest relative. Given the unique phylogenetic position and sequence divergence of this siadenovirus, we propose the species name of Psittacine siadenovirus F pending submission of a formal proposal to and ratification by the International Committee on Taxonomy of Viruses. A siadenovirus has been reported as the potential cause of lethal disease in a plum-headed parakeet (Psittacula cyanocephala) and an umbrella cockatoo (Cacatua alba). Although only partial genome sequences (Dpol) have been determined for these siadenoviruses, they are identical to the African grey parrot siadenovirus. Importantly, the role of this parrot siadenovirus in disease of African grey parrots and other parrot species has not been determined. Future research including in situ hybridization or infectivity trials is needed to define the role (if any) of siadenoviruses in systemic disease in psittacines.

72. NO SARS-COV-2 DETECTED IN ENVIRONMENTAL SAMPLES COLLECTED AT A FITNESS CENTER THAT REOPENED FOLLOWING CDC GUIDELINES

Hongwan Li - Department of Environmental Engineering Sciences, College of Engineering, University of Florida; **Sripriya Nannu Shankar** - Department of Environmental Engineering Sciences, College of Engineering, University of Florida; **Chiran T. Witanachchi** - Department of Environmental Engineering Sciences, College of Engineering, University of Florida; **John Lednicky** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Julia Loeb** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Md Mahbubul Alam** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Z. Hugh Fan** - Department of Biomedical Engineering, College of Engineering, University of Florida; **Karim Mohamed** - Department of Biomedical Engineering, College of Engineering, University of Florida; **Arantzazu Eiguren-Fernandez** - Aerosol

Dynamics Inc.; **Chang-Yu Wu** - Department of Environmental Engineering Sciences, College of Engineering, University of Florida

Introduction: Airborne transmission is an important route for the spread of SARS-CoV-2 in indoor environments. Fitness centers provide a venue for maintaining the physical and mental health of their patrons, yet these indoor settings are understudied for SARS-CoV-2 transmissions.

Methods: In this study, air and surface samples were collected five times during peak hours (4-7pm) from August to November 2020 in a fitness center at Alachua county, Florida. Air was collected by four devices: VIVAS and BioSpot-VIVAS as stationary samplers at 8 L/min for 3-h, and a PTFE filter in an in-line holder and a NIOSH two-stage cyclone bioaerosol sampler (BC-251) as personal samplers at 3 L/min for 1-h. Moistened flocked nylon swabs were used to collect samples from high-touch surfaces. An infection risk model based on the Wells-Riley equation was developed to evaluate the effectiveness of ventilation systems in minimizing airborne SARS-CoV-2 transmission.

Results: The rRT-PCR tests for SARS-CoV-2 vRNA were negative for all air and surface samples. One possible reason may be the relatively low number of positive COVID-19 tests in Alachua County. The probability of infection risk due to airborne transmission indoors was 1.77%, assuming one contagious person was exercising in the fitness center. When optimal ventilation systems are used, the probability of infection due to airborne SARS-CoV-2 transmission at the fitness center may be reduced from 13.7 to 1.77%. If there are 500 people in the room, the probable number of infected people can be decreased by 87%, from 69 to 9, by adopting the optimal ventilation conditions.

Conclusions: To minimize airborne transmission, it is recommended for fitness centers to ventilate with high ACH, minimize air recirculation, use high efficiency filter, and install air disinfection devices. Amongst these measures, operating at high ventilation (10 ACH) with minimal air recirculation (0%) is the most critical, which can effectively reduce the probability of infection from 13.7 to 1.81%, even without any further treatment. For buildings that cannot modify their central air systems,

using filtration and air disinfection devices can also help reduce the probable infection risk from 13.7 to 8.47% (1 ACH), although higher than that can be achieved by increased ventilation with outdoor air. The reopening measures, including engineering controls, administrative controls, and face coverings, are significantly helpful in lowering the risks of airborne SARS-CoV-2 transmission in fitness centers, while appropriate adjustments should be made according to local circumstances.

73. PHYLOGENOMIC ANALYSES SUPPORTS INTERCLASS TRANSMISSION OF FROG VIRUS 3 BETWEEN A THREE-SPINE STICKLEBACK (GASTEROSTEUS ACULEATUS) AND A RED-LEGGED FROG (RANA AURORA)

Cody Conrad - Department of Infectious Disease, Archie Carr Center for Sea Turtle Research, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Kuttichantran Subramaniam** - Department of Infectious Disease, Emerging Pathogens Institute, University of Florida; **V. Gregory Chinchar** - University of Mississippi Medical Center; **Thomas Waltzek** - Department of Infectious Disease, Emerging Pathogens Institute, University of Florida

Ranaviruses are emerging pathogens that can cause rapid mortality events in species of the classes Actinopterygii, Reptilia, and Amphibia. Two isolates of ranavirus were recovered from tissue pools of threespine stickleback (Gasterosteus aculeatus) and northern red-legged frog (Rana aurora) from a wildlife epizootic in Redwood Creek (Redwood National Park, CA) in 1996. Preliminary analyses of the viruses isolated from threespine stickleback (stickleback virus, SBV) and northern red-legged frog tadpole (tadpole virus 2, TV2) showed the same protein profile in infected cells, identical electrophoretic patterns following restriction enzyme digestion of viral DNA, and the identical sequence within a highly conserved region of the iridovirus major capsid protein. In this study, the SBV and TV2 isolates were amplified in epithelioma papulosum cyprini cells until cytopathic effects were complete. The resulting supernatants were then clarified, and the total DNA was extracted using a DNeasy blood and tissue kit (Qiagen). DNA libraries were constructed using the Nextera XT DNA kit (Illumina), and sequencing was performed using a v3

chemistry 600-cycle kit on an Illumina MiSeq. The genomes of the two isolates were assembled de novo in in SPAdes v3.13.0 and annotated using Genome Annotation Transfer Utility (GATU) with Frog virus 3 (GenBank accession no. MG953518) as the reference genome. Additional putative open reading frames (ORFs) were identified using GenemarkS v4.28, and gene functions were predicted based on BLASTP searches against the NCBI GenBank non-redundant protein sequence database. A total of 97 putative ORFs were predicted in both SBV and TV2 genomes. An analysis of locally collinear blocks (LCB) in Mauve v2.4.0 revealed that the genomes of SBV and TV2 display the same genome arrangement as frog virus 3 (FV3). Some ranaviruses are known to exhibit low host specificity, which is demonstrated in this analysis, as two vertebrate animals of different classes were infected by the same FV3 strain. The potential impact of FV3 is noteworthy because it has the ability to jump between hosts of different vertebrate classes and has been shown to negatively impact commercially important species and those of conservation concern.

74. SARS-COV-2 RECEPTOR ACE2 INTERACTIONS WITH B0AT1 AMINO ACID TRANSPORTER IN MEMBRANE PATHOPHYSIOLOGY OF COVID-19

Bruce R. Stevens - Department of Physiology and Functional Genomics, College of Medicine, University of Florida

Introduction: The B0AT1 transporter was originally discovered and functionally characterized in our laboratory; then B0AT1 was subsequently utilized by Pfizer/BioNTech in an [ACE2-B0AT1]2 overexpression plasmid assay which they exploited as crucial to successfully screen and select their BNT162b2 mRNA for their FDA-approved COVID-19 vaccine. SARS-CoV-2 infection requires spike protein receptor binding domain (RBD) bound to the ectodomain of ACE2. The ACE2 can be thermodynamically stabilized on the surface of plasma membranes by complexing with its trafficking chaperone partner B0AT1 (literature aliases; NBB, B, B0, B0AT1), forming an [ACE2-B0AT1]2 dimer-of-heterodimers 4-mer complex.

Hypothesis: In the present study, we hypothesized that within this 4-mer complex, each [ACE2-B0AT1] heterodimer structural pairing behaves as a physiological 'functional unit' exhibiting Na+-dependent neutral amino acid transport, and further that B0AT1 sterically governs ACE2 interplay with COVID-19 inflammasome serine proteases TMPRSS2 and ADAM17.

Methods: [3H] radiotracer transport was measured in enterocyte purified apical membrane vesicles irradiated with an electron beam from a 16 MeV linear accelerator. Molecular docking modeling was conducted based on PDB ID 6M17 atomic coordinates.

Results: The radiation inactivation 'functional unit' target mw = 183.7 ± 16.8 kDa in situ in intact membranes, representing each of the thermodynamically stabilized [ACE2-B0AT1] heterodimer subsets that manifested amino acid transport activity housed within the [ACE2-B0AT1]2 parent complex mw = 345 kDa. Molecular docking indicated that active site catalytic pocket residues of TMPRSS2 and ADAM17 each formed bonds < 2 Å with monomer ACE2 specific residues within its neck region spanning Arg652 to Asp713; this region is known to involve serine proteases cleaving ACE2 soluble ectodomain release from membrane

surface. Without B0AT1, the ACE2 residues Lys657 and Asn699 dominated docking bonding with TMPRSS2 or ADAM17 active site residues. However, in the dimer-of-heterodimers arrangement replete with B0AT1, all ACE2 neck region residues were restricted to TMPRSS2 or ADAM17 approaches > 35 Å, with steric interference directly attributed to the presence of neighboring B0AT1 subunit; these modeling results were not significantly different ($p<0.05$) whether in the presence or absence of SARS-CoV-2 spike RBD putatively bound to ACE2.

Conclusion: Our Results collectively implicate the entanglement of ACE2 with B0AT1 and its neutral amino acid transport functional unit assembly as a major player in steering the landscape of COVID-19 pathophysiology engagement of TMPRSS2 and ADAM17. These findings enhance our understandings that can contribute to future translational developments leading to novel COVID-19 treatments and/or mitigation of SARS-CoV-2 mutant outbreaks.

75. VIABLE SARS-COV-2 AEROSOLS IN HOSPITAL ROOM WITH COVID-19 PATIENTS

John Lednicky - Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida; **Michael Lauzardo** - Department of Medicine, College of Medicine, University of Florida; **Z. Hugh Fan** - Department of Mechanical and Aerospace Engineering, College of Engineering, University of Florida; **Antarpreet Jutla** - Department of Environmental Engineering Sciences, College of Engineering, University of Florida; **Trevor B. Tilly** - Department of Environmental Engineering Sciences, College of Engineering, University of Florida; **Mayank Gangwar** - Department of Environmental Engineering Sciences, College of Engineering, University of Florida; **Moiz Usmani** - Department of Environmental Engineering Sciences, College of Engineering, University of Florida; **Sripriya Nannu Shankar** - Department of Environmental Engineering Sciences, College of Engineering, University of Florida; **Karim Mohamed** - Department of Biomedical Engineering, College of Engineering, University of Florida; **Arantzazu Eiguren-Fernandez** - Aerosol Dynamics Inc., Berkeley, CA, USA

Introduction: The uncertainty over airborne transmission of SARS-CoV-2 by coronavirus disease-2019 (COVID-19) patients stems from challenges in isolating infectious virus despite detection of the virus' RNA in material collected by air samplers. The aim of this study was to resolve the question whether viable ('live') SARS-CoV-2 can occur in aerosols produced by COVID-19 patients.

Methods: Air samples were collected in the hospital room of two COVID-19 patients, one who responded favorably to treatment of neurologic disease and was ready for discharge, and one newly admitted with respiratory illness. The air samplers - VIable Virus Aerosol Sampler (VIVAS) and BioSpot-VIVAS were positioned 2 to 4.8 m away from the patients and operated at 8 L/min for 3 h, resulting in a sampling volume of 1,440 L/air per sampler. Based on the principle of laminar-flow water vapor condensation, airborne particles in the air drawn through the samplers are first enlarged, then gently collected. The dimensions of the particles increase because water molecules get progressively layered around them through a condensation process, making them heavier and thus easier to remove from the sampled air stream. Moreover, the process is gentle and does not inactivate virions, making it possible to determine whether the virus in the air is infectious and thus poses an inhalation risk. The heavier 'droplets' fall out of suspension in the air, and in the process, gently deposit ('impact') onto specially formulated liquid collection medium in a Petri dish Aliquots of the samples collected for this work were subsequently analyzed by RT-qPCR and attempts were made to isolate the virus in cell culture to determine whether infectious virions had been collected. The genome of the SARS-CoV-2 collected from the air and isolated in cell culture, and that isolated from the patient with respiratory COVID-18, were sequenced.

Results: Virus-induced cytopathic effects were observed 4-6 days post inoculation of the samples onto LLC-MK2 and Vero E6 cells. From RT-qPCR analysis and the virus culture, it was evident that viable SARS-CoV-2 was isolated from the air samples. The genome sequence of the SARS-CoV-2 strain isolated from the material collected by the air samplers was identical to that isolated from the newly admitted patient. No other

respiratory virus was detected. Estimates of viable SARS-CoV-2 concentrations ranged from 6 to 74 TCID50 units/L of air.

Conclusions: Patients with respiratory manifestations of COVID-19 produce aerosols in the absence of aerosol-generating procedures that contain viable SARS-CoV-2, and these aerosols may serve as a source of transmission of the virus. The results also suggest environmental sampling could serve as a non-invasive route in tracking the spread of the virus in addition to deploying early mitigation measures. Further studies on transmission of SARS-CoV-2 in indoor settings can help modify guidance on infection control measures.

76. BEHAVIORAL OBSERVATION APPLICATIONS IN PUBLIC HEALTH RESEARCH: THE TABLET APP AND IN PRACTICE

Elizabeth Schieber - Department of Psychology, College of Liberal Arts and Sciences, University of Florida; **Crystal Slanzi** - Department of Psychology, College of Liberal Arts and Sciences, University of Florida; **Song Liang** - Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida; **Arie Havelaar** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Abdulmuen Ibrahim Mohammed** - Haramaya University; **Sarah L. McKune** - Department of Environmental and Global Health, Center for African Studies, College of Public Health and Health Professions, University of Florida

Behavior observations can be integral parts of public health research in order to determine what is happening in the natural environment, identify targets for behavior change, measure progress with public health programming, etc. This poster will describe an iOS and Android application, Countee©, which was designed with the express purpose of coding behavioral data. Countee allows users to customize templates for observations, record continuous data, and export data as .csv files for analysis in R© or Microsoft® Excel. We are using Countee to collect behavior observation data for a project, Exposure Assessment of Campylobacter Infections in Rural Ethiopia (EXCAM). We are tracking infants' locations, compartments, activities, and potential transfer events (i.e., mouthing, touching, pica) and comparing those data to biological samples to determine how infants may contract Campylobacter. We will display how we customized our Countee template and discuss the benefits of using this application.

77. CAUSE OF DEATH IN FLORIDA FARMED WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) DURING 2017 - 2020

An-Chi Cheng - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Sydney L. Cottingham** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Olivia Goodfriend** - Department of Wildlife Ecology and Conservation, 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; **John Lednicky** - Department of Environmental and Global Health, College of Public Health and Health Professions, 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; **Thomas Waltzek** - Department of Infectious Disease, 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) farming is an emerging agricultural industry in Florida. Bacterial infections and viral hemorrhagic diseases cause high mortality in fawn and yearling deer and are a source of significant production loss among Florida deer farms. Before management can be improved and properly implemented, the causality of death in the farmed herds must be determined. The University of Florida Cervidae Health Research Initiative (CHeRI) provides a diagnostic service to Florida deer farmers to determine and monitor the proportion of farmed white-tailed deer that have died from bacterial infections, hemorrhagic disease-causing viruses, or other causes of death. From 2017 to 2020, participating Florida ranches provided recently deceased farmed white-tailed deer for necropsy or shipped tissues for analysis by the CHeRI diagnostic program. Both necropsy and owner-sampled tissues were tested for hemorrhagic disease using qPCR, and were subjected to

additional microbial culture, histopathology analysis, and parasite identification as necessary to determine a probable cause of death. We distinguished a significant difference in the proportion of bacterial and viral diseases that affect captive-bred white-tailed deer based on the different categories and ages. Of the 174 deceased farmed white-tailed deer age 1 to 90 days sampled from 2017 to 2020, 57% of deaths were associated with bacterial infection, and only 17% were attributed to viral hemorrhagic disease. Of the 164 animals age 4 to 12 months sampled from 2017 to 2020, 69% of deaths were attributed to hemorrhagic disease virus, with 23% identified as bacterial infection. Lastly, of the 158 animals age 13 months or more sampled from 2017 to 2020, 41% of deaths were attributed to hemorrhagic disease viruses, and 43% were identified as bacterial infection. The overall average percentage for all categories analyzed together clearly confirms that hemorrhagic diseases and bacterial infections account for 80% of deaths. Hemorrhagic diseases are visibly seasonal, with pick cases during late summer through early fall, with significantly higher cases involving EHD serotypes 2 and 6. Conversely, bacterial infections significantly increase during fawn season from mate May to September. Viral hemorrhagic disease is a major source of mortality in deer aged 4-12 months, with bacterial infections being the major cause of death among young deer age 1 to 90 days. These data help better understand the prevalence and dynamic of pathogens affecting farmed white-tailed deer and provide insight to develop best management practices and treatment strategies.

78. CHARACTERIZING WOMEN'S EMPOWERMENT IN EASTERN ETHIOPIA AND ITS ASSOCIATIONS WITH DIETARY DIVERSITY, CHILD GROWTH AND CAMPYLOBACTERIOSIS IN YOUNG CHILDREN

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

Women's empowerment and animal source food (ASF) consumption have been associated with reduced rates of undernutrition in children. The Five Domains of Empowerment (5DE), as characterized in the Abbreviated Women's Empowerment in Agriculture Index (A-WEAI), are production, income, resources, leadership, and time. Empowerment of women across these domains has been linked to improved nutrition and health outcomes. As part of formative research associated with the CAGED (Campylobacter Genomics and Environmental Enteric Dysfunction) project, which aims to improve understanding about how livestock and gut health may be affecting child growth, we examined empowerment of mothers as a potential confounder. In the research presented here, we describe overall and domain-specific empowerment of these women and explore associations between empowerment indicators and dietary diversity, child growth, women's livestock ownership, and Campylobacter colonization in children. Results from 102 mother/child dyads indicate that 50% of women were empowered, based on the 5DE (overall empowerment). Domain-specific empowerment was more heterogeneous, with 84%, 93%, 94%, and 54% of women empowered in production, income, leadership, and time domains, respectively, and the resource domain reflecting 100% women empowered in the sub-domain

of ownership, and 6% in the sub-domain of credit. Preliminary bivariate analyses indicate that dietary diversity of the child was associated with women's empowerment in production (p=.043). Using child growth z-scores, wasting (weight for length z-score) was negatively associated with empowerment in leadership (p=.025), and both stunting and underweight (weight for age z-score) were negatively associated with women's empowerment in time (p=.002 and p=.027, respectively). Women's livestock ownership was positively associated with women's overall empowerment (p=.03), as well as with the empowerment domains of production (p=.047) and credit (p=.027). Finally, no significant associations were found between Campylobacter colonization in children and any indicator of women's empowerment. These findings characterize a group of women from Eastern Ethiopia as empowered by agricultural production activities, income, and leadership, but disempowered by a lack of access to credit and time poverty. While a positive association between production empowerment and dietary diversity supports a growing body of literature connecting empowerment and child diet, associations between poor child growth outcomes (lower z-scores) and women's empowerment in both leadership and time run counter to trends in global health and development to invest in women's traditional domains of women's empowerment. Further detailed multivariate analysis to explore the individual and joint effect of women's empowerment domains on various indicators of child growth and nutrition is ongoing.

79. COLONIZATION OF THE C. ELEGANS GUT WITH HUMAN ENTERIC BACTERIAL PATHOGENS LEADS TO PROTEOSTASIS DISRUPTION THAT IS RESCUED BY BUTYRATE

Alyssa Walker - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida; **Alfonso Vaziriyan-Sani** - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida; **Emily T. Donahue** - 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; **Autumn Dove** - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida; **Keelnatham T. Shanmugam** - 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

Human enteric bacteria influence the pathogenesis of protein conformational diseases; however, our current understanding of how individual microbes influence the pathology of such ailments has been limited by the complexity of the human microbiome. The ability to assess the effect of individual bacterial species on host proteostasis, rather than the influence of the microbiome community as a whole, would allow us to pinpoint which bacteria might be contributing to the onset and progression of neurodegenerative disease. As such, we investigated the effect of Gram-negative enteric bacteria on host proteostasis using the C. elegans gut as a test-tube. The intestine of this nematode can be colonized by individual bacterial species, which can help us determine bacterium-specific influence on the host. To assess bacteria-induced proteotoxicity, we used animals expressing tissue-specific polyglutamine (polyQ) reporters that detect changes in proteostasis. We found that colonization of the C. elegans gut with Gram-negative enteric pathogens disrupts proteostasis not only in the intestine, but in muscle, neurons, and gonads as well. Additionally, we found that such colonization also affected gross motor function in a polyQ-dependent manner. To

determine how the presence of commensal bacteria affect host proteostasis, we co-colonized the C. elegans intestine with bacteria that were generated to conditionally synthesize butyrate and observed significant suppression of bacteria-induced aggregation and associated toxicity. Moreover, supplementation of exogenous butyrate differentially influenced bacteria-mediated proteotoxicity at low concentrations, but uniformly, and almost completely, inhibited it at high levels, suggesting that the beneficial effect of this short-chain fatty acid depends on the bacteria present. Together, these results reveal that bacteria directly influence host proteostasis and illustrate how gut dysbiosis may contribute to the pathogenesis of protein conformational diseases. Further, our results demonstrate a potential prevention and treatment strategy utilizing butyrate-producing microbes to treat neurodegenerative disease.

80. DETERMINATION OF METABOLIC DETOXIFICATION MECHANISMS IN A RHIPICEPHALUS SANGUINEUS STRAIN HIGHLY RESISTANT TO PERMETHRIN

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

Rhipicephalus sanguineus Latreille is a three-host tick and an ectoparasite of dogs. It can establish residential infestations due to its ability to complete its lifecycle indoors. Rhipicephalus sanguineus are difficult to detect during the early infestation period due to low tick numbers, their small size (especially immature stages), and cryptic behavior. These factors can delay the initiation of tick population management. Rhipicephalus sanguineus are typically managed using acaricides applied to hosts, although space and surface treatment are utilized. Historically, permethrin and fipronil have been used widely, but efficacy has been

reduced due to resistance. Permethrin disrupts sodium channel function and can be used both on- and off-host, while fipronil blocks chloride ion flow by affecting the GABA-gated chloride channel and is used only on-host. Exceptionally high permethrin resistance has been reported in some R. sanguineus populations from the U.S., Grenada, and Mexico. Previous studies have demonstrated that high permethrin resistance in some R. sanguineus populations was due to metabolic detoxification. An R. sanguineus strain collected from Port St. Lucie, Florida exhibited high resistance to permethrin with an incalculable LC50 due to the low mortality at the highest concentration (30%) tested. Herein, the metabolic detoxification mechanisms were evaluated on this permethrin-resistant R. sanguineus strain using three synergists in combination with permethrin: triphenyl phosphate (TPP) as an esterase inhibitor, piperonyl butoxide (PBO) as a cytochrome P450 inhibitor, and diethyl maleate (DEM) as a glutathione-S-transferase inhibitor. The larval packet test was used to evaluate the tick mortality with permethrin (1.875–30%) and additions of synergists (2%). The addition of TPP increased tick mortality to 100% in almost every concentration. Tick mortalities were partially increased with the addition of PBO and the increased mortality exhibited a positive linear relationship with concentration. The addition of DEM slightly increased tick mortality in every concentration except 15%. Thus, increased esterase and cytochrome P450 activity play an important role in metabolic detoxification in this permethrin-resistant R. sanguineus strain.

81. DEVELOPMENT OF A VIRTUAL YOUTH OUTREACH PROGRAM IN CLIMATE CHANGE RESILIENCY TO EXPLORE CLIMATE CHANGE IMPACTS ON ECOSYSTEMS AND PUBLIC HEALTH

Cynthia Nazario-Leary - Center for Precollegiate Education and Training, University of Florida; **Dehlia Albrecht** - Center for Precollegiate Education and Training, University of Florida

The impacts from COVID-19 left a gap in summer educational opportunities for high school students as in-person programs were cancelled. To address this need, the University of Florida (UF) Center for Precollegiate Education and Training (CPET) offered an online summer youth program in Climate Change Resiliency (CCR) to over fifty 11th and 12th graders in July 2020. This project was funded by the Frances C. & William P. Smallwood Foundation. Two 2-week sessions were offered. The first session provided students with an introduction to the issues and scientific fields related to CCR including topics on the impact of climate change on public health and invasive species. The session culminated with a mock city council meeting debating CCR strategies needed to address sea level rise in Jacksonville, FL. Daily activities included a lecture and discussion, a virtual lab or field trip, followed by a guest presentation from university faculty. The second session explored CCR issues in more depth and utilized specific case studies to deliver content. Discussions included confronting climate change in human environments, and pathways to mitigate climate change impacts. Daily activities included participant-led group discussions on assigned case studies, followed by guest presentations from university faculty. Participants also designed, executed, and reported on experiments exploring simulated environmental effects on basil seedlings. Overall program satisfaction was positive, with 97% of Session 1 and 81% of Session 2 participants reporting they were somewhat or extremely satisfied with the CCR program. Approximately 83% and 85% of Session 1 and Session 2, respectively thought the program's approach to teaching and learning was very or extremely effective. Future recommendations to improve the program include increasing synchronous face-to-face instruction time and participant-led learning opportunities.

82. DRUG REPURPOSING SCREEN FOR ANTI-INFECTIVES IDENTIFIES QUINACRINE AS AN INTRACELLULAR HOST-TARGETED ANTIBIOTIC

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

The stagnation of antibiotic development and the dramatic rise in antibiotic resistance has created a concerning gap in the availability of effective antimicrobials. Antibiotic resistance claims nearly 35,000 lives in the United States each year, and it is estimated that by 2050 this number will increase tenfold. There is an urgent need to develop alternative therapies against antibiotic-resistant infections. Our work concentrates on repurposing current drugs for their ability to inhibit infection by targeting the host. Drug repurposing can potentially lower the cost and speed up the development of new therapeutics. Additionally, targeting the host, but not the pathogen, will circumvent antibiotic resistance. Host-targeting compounds could be used in combination with current antibiotics. We used a novel method of quantifying intracellular bacteria to explore modulation of bacterial entry into cells as a potential host-targeting mechanism against antibiotic-resistant bacteria. For extracellular bacteria, the enhancement of uptake should allow for an increased clearing of the infection, while inhibiting bacterial uptake should attenuate infections by intracellular bacteria. To this end, we used a library of 2,400 approved drugs to search for modulators of Escherichia coli uptake by RAW 264.7 macrophages. We identified 23 compounds that inhibited bacterial uptake at no cytotoxicity to bacteria or host cells. One of the inhibitors that we identified is quinacrine, an antimalarial drug that can intercalate DNA. Based on the set-up of our screen, we anticipated that quinacrine would inhibit macrophage-mediated bacterial

uptake, but instead we found that it rapidly concentrates inside host cells and facilitates killing of intracellular bacteria while having no toxicity to the host or the bacteria in axenic culture. We showed that quinacrine kills intracellular Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Salmonella Typhimurium. Quinacrine's effect is specific to gram-negative pathogens and as such has no effect on Staphylococcus aureus infection. This property of quinacrine could enhance bacterial killing and target persistent infections caused by bacteria that hide within and get disseminated by macrophages.

83. EFFECT OF VITAMIN D FORTIFICATION OF MILK ON GUT MICROBIOME

Massimiliano Tagliamonte - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Volker Mai** - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **H Oliveros** - Facultad de Medicina, Universidad de La Sabana, Chía, Colombia; **M Mora-Plazas** - Foundation for Research in Nutrition and Health, Bogotá, Colombia; **C Marín** - Facultad de Medicina, Universidad de La Sabana, Chía, Colombia; **K Flannagan -** Eunice Shriver Kennedy National Institute of Child Health and Development; **Eduardo Villamor** - Department of Epidemiology, School of Public Health, University of Michigan

Background: The vitamin D receptor is expressed ubiquitously in human tissues, which suggests that vitamin D exerts important functions beyond its well-known effects on calcium homeostasis and musculoskeletal health. Its effects on immunity suggest a possible role on the gut microbiome.

Objective: To determine the effect of vitamin D on the intestinal microbiota of adolescents and their adult mothers.

Methods: We conducted a randomized, masked, parallel trial among 80 families with one child aged 12–15 years and their mother in Bogotá, Colombia. Families were randomly assigned to receive one daily liter of either skim milk fortified with 600 IU vitamin D3 per 250 mL or unfortified

skim milk for a six-week period. Caregivers were instructed to provide the index child with two cups of milk per day (500 mL) and to distribute the remaining milk to the rest of the family. Children and mothers provided a stool sample prior to randomization and at the end of follow-up. Samples were transported in coolers on the same day of collection to a research laboratory where they were cryopreserved at -70C until shipping to the United States for analyses. Gut microbiota taxonomy was determined on the stool samples through sequencing of the V3-V4 regions from the 16S gene on NextSeq 500 using 2x150 reads, then trimmed down to 125+124. Raw forward reads were imported and analyzed with the Qiime2 pipeline. We estimated change in phylogenetics and amplicon sequence variant diversity between the baseline and end of follow-up samples within each randomized group, separately for children and mothers, with DESeq2.

Results: 68 children and 74 mothers had samples available at the two time-points. Mean ± SD age of children was 13.5 ± 0.6, 48.5% were female. Vitamin D fortification had no effect on gut microbiota composition or diversity in children. In mothers, those assigned to the vitamin D fortification arm experienced significant abundance increases in Clostridiales and Bacteroidales taxa, with decreases in C. clostridioforme species and Blautia genus. These changes were not observed in the control group.

Conclusions: Among adult women, milk fortification with vitamin D results in a microbiome shift towards bacterial taxa associated with gut health, lower body mass index and serum cholesterol, and lower risk of autoimmune diseases. These findings may contribute to explain potential extraskeletal effects of vitamin D.

84. ENVIRONMENTAL AND BEHAVIORAL RISK FACTORS FOR TRAUMATIC STINGRAY PUNCTURE INJURIES IN CEDAR KEY CLAM HARVESTERS

Rebecca Rash - Department of Environmental and Global Health, Florida Sea Grant College Program, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Andy Kane** - Department of Environmental and Global Health, Florida Sea Grant College Program, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

Commercial shellfish aquaculture in Cedar Key, Florida, has rapidly expanded over the past decade into a multimillion-dollar industry. Occupational health and safety challenges in this work sector are not well understood. 13/16 (81%) of clam harvester participants in an occupational health and safety survey considered stingray puncture injuries a top concern, and 6/16 clam harvesters (38%) reported experiencing these injuries. Stingray puncture injuries are associated with excruciating pain, secondary infection, and lost workdays. Risk factors for stingray injuries were examined using: (1) fisheries-independent monitoring data from 2000-2018 to discern the importance of environmental factors to Atlantic stingray (Hypanus sabinus) abundance, and (2) self-reported clam harvester behavior and stingray puncture injury experience while working in the water. Results demonstrated that H. sabinus were present inshore throughout the year, with the highest monthly average number of stingrays in March. Average depth and bottom type had highly significant effects on stingray abundance ($p<0.01$).Stingrays were found in average depths between 0.2m and 1.5m, and more stingrays were found in mud than sand. Although stingray abundance was related to these environmental factors, the number of stingray puncture injuries reported among clam harvesters did not always reflect these trends. The number of injuries experienced ranged from 0-9 per harvester, and injuries occurred in both muddy and sandy habitats throughout the year. Behaviors such as stepping instead of shuffling feet in the water, amount of time spent in the water, and disturbance of stingrays when manipulating clam bags were associated with stingray injuries. The differences between the environmental and

survey analysis suggests that clam harvester behavior can influence risk. This study empowers harvesters to better understand and mitigate stingray puncture injuries.

85. EPIDEMIOLOGY AND PALEOPATHOLOGY OF THE PLAGUE OF ATHENS

Sara Agnelli - One Health Center of Excellence; **Isa Koreniuk** - College of Liberal Arts and Sciences, Department of Classics

Introduction: In early May 430 BCE, an epidemic struck the city of Athens, which was then under siege by Sparta during the Peloponnesian War (431-404 BCE). Another wave broke in the summer of 428 BCE and in the winter of 427-426 BCE. Originating in Ethiopia, it spread throughout the Mediterranean. It spared no segment of the population, killing perhaps as many as 75,000 to 100,000 people, 25% of the city's population. The Greek historian Thucydides left an eye-witness account of this plague and a detailed description to allow future generations to identify the disease should it break out again. According to Thucydides, the highly contagious epidemic exhibited a pustular rash, high fever, and diarrhea. Other symptoms victims of the plague experienced were: redness and inflammation in the eyes, sore throat leading to bleeding and bad breath, loss of voice, sneezing, coughing, vomiting, extreme thirst, and insomnia. The epidemic was unknown with a high attack rate and an unvarying course in persons of different ages, sexes, and nationalities. In the past 100 years, scholars and physicians have disagreed about the identification of the disease. Based on clinical symptoms, three epidemiological interpretations have dominated: smallpox, typhus, and viral hemorrhagic fever. New methodologies, including forensic anthropology, demography, epidemiology, and paleopathology, including DNA analysis, have shed new light on the problem. Mathematical modeling has allowed the examination of the infection and attack rates and the determination of how long it takes a disease to spread in a city and how long it remains endemic.

Results: The epidemiological analysis excludes common source diseases and most respiratory diseases. The plague can be limited to either a reservoir disease (zoonotic or vector-borne) or one of the respiratory

diseases associated with an unusual means of persistence, either environmental/fomite persistence or adaptation to indolent transmission among dispersed rural populations. The first category includes typhus, arboviral diseases, and plague, and the second category includes smallpox (Littman 2009). Although in 2001 ancient microbial typhoid (Salmonella enterica serovar Typhi) DNA was extracted from 3 skeletons from a mass grave that belonged to the plague years (Papagrigorakis, et al. 2006), typhoid is not the likely cause of this sudden epidemic because it was endemic in the Greek world. Unfortunately, DNA sequence-based identification is limited by the lack of a durable signature by RNA viruses retrievable from archaeological remains after several millennia. Therefore, some etiologies are not testable hypotheses using currently available scientific techniques (Shapiro, et al. 2006).

86. EXPLORING FOOD SAFETY TRAINING AS A POTENTIAL RISK MITIGATION ACTIVITY: A PILOT CASE STUDY WITH 4-H VOLUNTEERS AND EXTENSION AGENTS IN FLORIDA

Virgilia D. Zabala - Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Amarat (Amy) Simonne** - Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Laurie Osgood** - Institute of Food and Agricultural Sciences, University of Florida; **Dale Pracht** - Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Kendra Zamojski** - Institute of Food and Agricultural Sciences, University of Florida

Background: Many educational programs and events (i.e. fund raising and concessions) associated with the 4-H in Florida involve foods, which are handled by volunteers. Limited information is available on food safety knowledge and practices among the 4-H volunteers, and this could be a potential liability to the organization.

Objectives: To assess retention of knowledge and adoption of key practices by the participants after the training.

Method: From January to August, 2019, we conducted nine one-day food safety training sessions at multiple locations in Florida. The training

materials was based on Safe Staff® program which is accepted by the Department of Business and Professional Regulations (DBPR) for professional food handlers. Three months after the training, participants (n=131) were asked to complete a 28 item survey instrument (selected and adapted from the FDA Consumer survey).

Results: Fifty three participants responded to the survey (40% rate of response). Ages of respondents (39/53) were between 24 to 65 years old with majority as female (41/53). Respondents (43/47[(91%]) reported correct use and cleaning of cutting boards; 97% (46/47) reported proper personal hygiene practices; 93% (44/47) reported proper glove uses; 87% (41/47) reported having thermometers at volunteer sites and 60% always use thermometers when cooking poultry. Majority (42/46; 91% know the right temperature of refrigerator. While some gaps in specific issues with cooking are identified, respondents exhibited equal or higher food safety knowledge and practices than the 2016 FDA Consumer Food Safety Survey.

Significance: This study revealed that volunteers need to be trained for food safety before handling foods to ensure the mitigation of foodborne illness.

87. IDENTIFICATION OF BACTERIAL GENES THAT DISRUPT HOST PROTEOSTASIS

Alfonso Vaziriyan-Sani - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Alyssa Walker** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Sagnik Ghosh** - Department of Engineering, College of Engineering, 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

The global prevalence of neurodegenerative diseases continues to rise, largely due to an increasing average lifespan and the absence of effective treatment. While the origin of these diseases is associated with host protein stability, the dearth of therapies is due to the paucity of information regarding the factors and conditions that contribute to disease progression. A growing body of evidence indicates that the presence of bacteria influences aggregation-prone proteins associated with specific neurodegenerative diseases. Our preliminary work has demonstrated significant effects on host proteostasis that vary according to the enteric bacteria that colonize the Caenorhabditis elegans intestinal tract. We used C. elegans expressing intestine-specific fluorescent polyglutamine (PolyQ) reporter to detect disruptions in host proteostasis that manifest as fluorescent and quantifiable puncta. Our work revealed that Pseudomonas aeruginosa is one of the most robust inducers of protein aggregation and associated polyQ-dependent toxicity. In a manner similar to that used in our preliminary studies, we are assessing the effects of mutant strains of P. aeruginosa on proteostasis using C. elegans that express the intestine-specific polyQ reporter. To accomplish this, we began screening a genome-wide knockout library of 9,437 P. aeruginosa non-essential mutations for genes that affect polyQ aggregation. We have developed a data analysis pipeline in CellProfiler image analysis software to automate quantification of aggregates in individual worm intestines. This approach, which relies on the creation of

multiple neural networks for identification, permits consistent and accurate quantification. To ensure the precise timing of aggregate formation and to eliminate background fluorescence, we collect images of intestinal polyQ following a freezing period. Mutants that display significant differences in aggregation with respect to the wild-type Pseudomonas strain are selected for further bioinformatics analysis. This approach allows us to identify specific bacterial genes and pathways involved in the disruption of host proteostasis. Identification of genes that affect host proteostasis can provide targets for bacterial pathways responsible for such proteomic perturbations. Furthermore, these specific genes that are shared amongst different bacterial species can be used as predictors of human microbiome residents that affect host proteostasis.

88. ISOLATION OF NOVEL BACTERIOPHAGES AGAINST A LIBRARY OF CLINICAL ANTIMICROBIAL-RESISTANT ISOLATES OF PSEUDOMONAS AERUGINOSA: A CELL-BASED APPROACH TO IMPROVED PHAGE THERAPY

Garrett 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; **Naim Montazeri** - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, 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

Every year, an estimated 35,000 individuals in the U.S. alone are killed by antibiotic resistant bacteria. This number is expected to increase ten-fold by the year 2050 if no effective alternative to antibiotic therapy is found. Bacteriophages (phages), viruses that target and infect bacteria, provide a promising tool for the treatment of antibiotic-resistant bacterial infections. Despite recent advancements, selecting phages that exhibit specificity for Pseudomonas aeruginosa (P. aeruginosa)—a multidrug resistant, opportunistic pathogen that commonly infects ill, hospitalized

patients—has been challenging mainly because of the knowledge gaps in understanding virus-host interactions. Currently, we have isolated a bacteriophage that targets 32 of 55 clinical isolates of Pseudomonas aeruginosa, each with a unique antibiotic resistance profile and a sequenced genome. Furthermore, we have now begun isolating bacteriophages against P. aeruginosa strains from the library that have previously shown phage resistance. Our goal is to generate a cocktail of phages against all clinical strains of P. aeruginosa, and ultimately employ functional genomics to identify bacterial determinants that affect phage specificity. Finally, we will investigate the effect of phage-bacteria interaction on antibiotic sensitivity. Ultimately our approach has a high potential to advance phage therapy into clinical settings.

89. SEEKING AND SHARING SCIENCE: A NATIONAL SURVEY ASSESSING AMERICANS INFORMATION SEEKING AND SHARING CHANNELS IN THE EARLY STAGES OF THE COVID-19 PANDEMIC

Lauri Baker - Department of Agricultural Education and Communication, College of Agricultural and Life Sciences, UF/IFAS Center for Public Issues Education in Agriculture and Natural Resources; **Meredith Oglesby** - Department of Agricultural Education and Communication, College of Agricultural and Life Sciences, University of Florida; **Shelli Rampold** - College of Agricultural and Life Sciences, UF/IFAS Center for Public Issues Education in Agriculture and Natural Resources; **Ashley McLeod-Morin** - Department of Agricultural Education and Communication, College of Agricultural and Life Sciences, UF/IFAS Center for Public Issues Education in Agriculture and Natural Resources; **Angela B. Lindsey** - College of Agricultural and Life Sciences, Family, Youth, & Community Sciences, UF/IFAS Center for Public Issues Education in Agriculture and Natural Resources; **Ricky Telg** - Department of Agricultural Education and Communication, College of Agricultural and Life Sciences, UF/IFAS Center for Public Issues Education in Agriculture and Natural Resources

Introduction: Zoonotic disease outbreaks have been on the rise in recent years with the COVID-19 pandemic being the worst in recent history. The spread of both accurate and inaccurate information happened quickly in the early stages of the disease, and understanding how this occurred is

important to prepare for communication of future disease outbreaks. Information sources and channels used to communicate during a crisis warrant consideration as these can impact an individual's perception of the crisis.

Purpose and Objectives: The purpose of this study was to understand the American public's information seeking and sharing behaviors during the early stages of the COVID-19 global pandemic. The study was guided by the following research objectives: (1) identify passive sources/channels of information; (2) identify active sources/channels of information; and (3) describe how frequently and across which channels/sources the U.S. public shared information about COVID-19 in the early stages of the pandemic.

Methods: An online survey was used to collect responses from U.S. residents during the second and third weeks of March, 2020. Useable responses were obtained from 1,512 residents. Post-stratification weighting methods were executed post hoc. Specifically, demographics were used to balance the results based on the 2010 Census data to ensure the sample reflected the adult U.S. population and to produce results intended to approximate the population of interest.

Findings: Results indicated people first found information about COVID-19 from personal communication, but turned to national and international organizations if they were to actively seek information. Scientists and universities were some of the least sought after and shared sources of information. Although scientists could have been featured on other sources like mainstream media and newspapers. The vast majority of people had actively searched for information related to COVID-19 ($f = 1431$, 96.4%) to some extent, with the largest number of people searching very often for information ($f = 626$, 41.4%). The sources shared most were from the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO).

Implications and Recommendations: Implications from this research are a need for communicators and scientists to use grassroots communication efforts during a crisis, to actively share information early

during a crisis. A multi-tiered approach is recommended in pandemic communication that includes passive and active sources of information and embedding science communication strategies within mainstream communication channels.

90. THE INTERPLAY OF MOVEMENT AND SPATIOTEMPORAL VARIATION IN TRANSMISSION DEGRADES PANDEMIC CONTROL

Nicholas Kortessis - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **Margaret W. Simon** - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **Michael Barfield** - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **Gregory Glass** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Burton Singer** - Emerging Pathogens Institute, University of Florida; **Robert D. Holt** - Department of Biology, College of Liberal Arts and Sciences, University of Florida

Any successful public health regime for an infectious disease must push the long-term effective reproductive number (Rt) – the average number of secondary cases caused by an infectious individual – below one. We show that temporal variation in transmission rate that is partly asynchronous between populations, coupled with movement of disease carriers, can elevate long-term average Rt. Consequently, two localities with local controls on a disease (i.e., local average Rt < 1) can still fail to control an infectious disease. Similarly, an infectious disease spreads more rapidly when populations are asynchronous in their control efforts.

91. THE THE OF TWO AGENCIES: COMPARING AMERICANS' ATTITUDES AND BEHAVIORS TOWARD THE CDC AND LOCAL HEALTH DEPARTMENTS DURING COVID-19

Ashley McLeod-Morin - Department of Agricultural Education and Communication, College of Agricultural and Life Sciences, University of Florida; **Lauri Baker** - Department of Agricultural Education and Communication, College of Agricultural and Life Sciences, University of Florida; **Angela B. Lindsey** - Department of Family, Youth and Community Sciences, University of Florida; **Lisa K. Lundy** - Department of Agricultural Education and Communication, College of Agricultural and Life Sciences, University of Florida; **Ricky Telg** - Department of Agricultural Education and Communication, College of Agricultural and Life Sciences, University of Florida

Introduction: The Centers for Disease Control and Prevention (CDC) provided recommendations throughout the COVID-19 pandemic about topics such as travel, social gatherings, and business closures (Jernigan, 2020). During the COVID-19 pandemic, most Americans reported that they felt the federal government should be responsible for response efforts to the pandemic. However, more people reported the local elected officials as doing an excellent job responding to the pandemic than public health officials on the federal level (Pew Research Center, 2020). The purpose of this study was to explore Americans' attitudes and behaviors toward the CDC and local health departments (LHDs) during the COVID-19 pandemic.

Method: To address the purpose of this study, a quantitative survey was conducted between April 23 and May 7, 2020. Qualtrics was used to recruit respondents through non-probability opt-in sampling techniques. Respondents included 1,538 adults in the United States. Attitude toward the CDC and LHDs were measured using a three-item, five-point semantic differential scale, including items such as trust, helpfulness, and knowledge. Behavior toward the CDC and LHDs were assessed using a three-item, five-point Likert-type scale and included statements related to following instructions from the agency. Data were analyzed using SPSS and measurements were compared using a paired samples t-test.

Results: There was no significant difference in respondents' attitude toward the CDC (M = 3.67, SD = 1.27) and attitude toward LHDs (M = 3.65, SD = 1.65; t(1,537) = .741, p = .459) during the COVID-19 pandemic. There was a significant difference in respondents' behavior toward the CDC (M = 3.52, SD = 1.29) and behavior toward LHDs (M = 3.63, SD = 1.23; t(1,537) = -4.44, p = .000) during the COVID-19 pandemic.

Conclusions: Despite there being no significant differences between respondents' attitudes toward the CDC and LHDs, there was a significant difference in respondents' behavior toward the CDC and LHDs. Respondents indicated more positive behaviors toward their LHDs compared to the CDC. This finding aligns previous research that suggests Americans have more positive perceptions and posits more supportive behaviors but not a difference in attitudes (Pew Research Center, 2020). Federal public health practitioners should work with LHDs to respond to risk situations and make recommendations. Future research should further investigate the public's attitude toward the CDC and LHDs to determine how attitude can be improved and identify segments of the population that have unfavorable attitudes and behaviors toward these agencies.

Index of Author by Page

A

Abid, Nabil - 32

Abraham, George E. - 36

Aerle, Ronny van - 97

Agnelli, Sara - 127

Ahasan, Mohammad Shamim - 98

Alam, Md Mahbubul - 34, 106

Alam, Meer Taifur - 8

Albrecht, Dehlia - 122

Al-Hussinee, Lowia - 98

Ali, Afsar - 8

Alomar, Abdullah A. - 65

Alshaer, Mohammad - 82

Alto, Barry W. - 65

Archana, Shoney - 90

Aryaprema, Vindhya - 62

Ascunce, Marina S. - 87

Asher, Valpa - 91

Ayers, Jasmine B. - 61

B

Baker, Lauri - 132, 135

Balakrishnan, Meenakshi - 24

Barfield, Michael - 134

Barker, Hailey - 19

Bateman, Kelly S. - 97

Beachboard, Sarah - 70

Beatty, Norman - 41, 76

Becker, Torben - 16

Begum, Yasmin Ara - 10

Behringer, Donald C. - 97

Bellot, Julia - 46

Bernier, U. - 72

Beshears, Elizabeth M - 13

Beshearse, Elizabeth - 15

Bhargava, Rohan - 119

Bhosale, Chanakya - 64

Bisesi Jr., Joseph H. - 38

Bisesi, S. - 28

Blackburn, Jason - 15, 83, 85, 91

Blanton, Jason L. - 13, 15

Bloomquist, Jeffrey - 50, 57, 69, 73, 74

Bluhm, Andrew - 23, 83

Bojko, Jamie - 97

Borocci, Stefano - 32

Boucher, Christina - 18

Boughton, Raoul K. - 21

Breard, D. - 50

Brinkac, Lauren - 27

Brunson, Debra N - 93

Bucher, Michael - 131

Burchfield, David J - 26

C

Cacho, Nicole - 26

Campbell, Lindsay - 56

Canestrelli, Alberto - 102

Capua, Ilaria - 102

Carthy, Raymond - 102

Carvalho, Valeria - 58, 70

Chen, Dehao - 7, 117

Chen, Tse-Yu - 75

Cheng, An-Chi - 80, 115

Cherabuddi, Kartikeya - 11

Chillemi, Giovanni - 32

Chinchar, V. Gregory - 108

Chowdhury, Nabil - 87

Clark, Abigail S. - 97

Cocquerel, Q. - 50

Conrad, Cody - 108

Cook, Robert - 95, 99

Coquerel, Marie de Gracia - 87

Cottingham, Sydney L. - 43, 80, 115

Courter, Joshua D. - 82

Crisp, Amy M. - 60

Cueto, Robert - 24

Cummings, B.S. - 28

Cummings, Derek - 24, 52

Czyz, Daniel - 119, 123, 130, 131

D

Daegling, David - 85

Dean, Natalie - 60

Demares, F. - 50

DeMent, Jamie - 13, 15

Diepold, Sheila - 27

Dill-Okubo, Jennifer - 105

Dinglasan, Rhoel - 61, 66

Donahue, Emily T. - 119

Dove, Autumn - 119, 123

Driver, Joseph - 18

Droege, Christopher A. - 82

Droege, Molly E. - 82

E

Eastmond, Bradley H. - 65

Edelmann, Mariola J. - 5, 19

Eiguren-Fernandez, Arantzazu - 106, 111

Ellward, Garrett - 131

Elzo, Mauricio - 18

Emerson, Lisa E. - 5, 19

Ernst, Neil E. - 82

F

Fajfer, Austin L. - 80

Fan, Peixin - 18, 21

Fan, Z. Hugh - 34, 106, 111

Finnerty, M. - 28

Fisk, Rebecca - 99

Flaherty, Katelyn - 16

Flannagan, K - 124

Fouché, Angelique T. - 80

Frasca Jr., Salvatore - 101

G

Gangwar, Mayank - 111

Garcia-Carreras, Bernardo - 52

Gauthier, Josee - 36

Geldenhuys, Werner - 50, 69

Getz, Wayne M. - 91

Gharaibeh, Raad Z. - 36

Ghosh, Sagnik - 130

Glass, Gregory - 134

Glover, Sarah C. - 36

Goodfriend, Olivia - 115

Gordon, Emily - 51

Goss, Erica M. - 44, 87

Grembi, Jessica A. - 16

H

Hadfield, Ted - 83, 85

Hamed, Mohamed F. - 40

Hamerlinck, Gabriela - 51

Hamerly, Timothy - 66

Haque, Farhana - 16

Harman, Madison - 54

Havelaar, Arie - 7, 13, 15, 114, 117

Healy, Daniel P. - 82

Henneberg, Austin - 75

Hernandez, Maria C. - 36

Hilliard, Nicole B. - 11

Hoffman, Carol - 5, 19

Holcomb, Kevin - 44

Holt, Robert D. - 134

Hossain, Mohammad Shahnoor - 8

Hryckowian, Andrew J. - 16

Huang, Angkana T. - 52

Humes, S.T. - 28

I

Ibañez, Gladys - 95

Imnoi, Kamonchai - 98

Ingram, L. - 28

Iovine, Nicole M. - 11

Irby, Iris - 89

Isaza, Ramiro - 43

J

Jabot, Brittney - 78

Jalali, Neda - 33

Jara, Manuel - 44

Jeffrey, Bloomquist - 72

Jennings, Katharine - 27

Jeong, Kwang Cheol - 11, 18, 21, 87

Jesus, Carrie De - 54, 64

Jiang, Shiyao - 74

Jiranantasak, Treenate - 86

Jobin, Christian - 36

Jutla, Antarpreet - 111

K

Kane, Andy - 126

Kaplan, Zachary D. - 68

Kaufman, Phillip - 56, 68, 120

Keiser, Carl N. - 78

Keleher, Bill - 98

Khan, Ashraful Islam - 10

Khatun, Selina - 16

Kim, Justin - 30

Kirpich, Alexander - 83

Koda, Samantha Ayumi - 101

Kolakowski, Lee F. - 27

Koonin, Eugene V. - 97

Koreniuk, Isa - 127

Kortessis, Nicholas - 134

Krauer, Juan M. Campos - 43, 80, 103, 115

Krigbaum, John - 85

L

Lam, Ling Ning - 81

Larkin III, Joseph - 5, 26

Lauzardo, Michael - 46, 47, 111

Ledger, Kimberly - 55

Lednicky, John - 24, 28, 34, 103, 106, 111, 115

Lee, Shinyoung - 11, 18, 21

Legros, C. - 50

Li, Hongwan - 106

Li, Nan - 26

Li, Xiaolong - 13, 15

Liang, Song - 114

Light, Jebidiah - 75

Lindsey, Angela B. - 132, 135

Linthicum, Kenneth - 57, 72, 74

Liu, Ting - 21

Lo, Ming - 58

Loeb, Julia - 24, 34, 103, 106

Long, Maureen - 24, 58, 70

López-Gutiérrez, Borja - 66

Lord, Cynthia - 75, 120

Luaces, Vivian Valcarce - 26

Lundy, Lisa K. - 135

M

Ma, Zhengxin - 11, 18

Machado, Gustavo - 44

Maegawa, Gustavo - 5, 123

Maestas, Sarah Mays - 56, 76

Mai, Volker - 46, 124

Manavalan, Preeti - 99

Manes, Costanza - 102

Manzanas, Carlos - 34

Maple, Stacey - 16

Marín, C - 124

Martin, Estelle - 78

Maurelli, Anthony - 38

Mavian, Carla - 32

McAuley, Andrew - 87

McKune, Sarah L. - 7, 114, 117

McLeod-Morin, Ashley - 132, 135

Megarani, Dorothea - 98

Merck, Lisa - 24

Mitchell, Shane - 27

Mohamed, Karim - 106, 111

Mohammed, Abdulmuen Ibrahim - 114

Montazeri, Naim - 131

Montero, Cindy - 24

Mora-Plazas, M - 124

Morris, Jr., J. Glenn - 2, 8, 11, 24, 87

Mueller, Eric W. - 82

N

Nazario-Leary, Cynthia - 122

Nelson, Corwin - 18

Nelson, Eric J. - 10, 16

Nelson, William M. - 27

Neu, Josef - 26

Newsome, Rachel - 36

Nicholson, Pamela - 98

Norris, Edmund - 57, 69, 73, 74

Norris, Michael - 23, 83, 85

Nyasembe, Vincent - 66

O

O'Connor, A. - 28

Oglesby, Meredith - 132

Oliveros, H - 124

Osgood, Laurie - 128

Ostrov, David A. - 32

Ou, Mark - 19

P

Pape, Jean William - 8

Parisi, Christina - 95, 99

Parker, Leslie - 26

Pascual, David W. - 5, 19

Peloquin, Charles - 46, 82

Philpott, Carolyn D. - 82

Pinton, Daniele - 102

Ponciano, Jose Miguel - 83, 91

Pond, Sergei L. Kosakovsky - 32

Popov, Vsevolod L. - 101

Pouder, Deborah - 101

Pracht, Dale - 128

Prakoso, Dhani - 70

Pu, Ruiyu - 49

Q

Qadri, Firdausi - 10

Qualls, Whitney - 62

R

Rabil, Anna - 117

Rainey, Andrew - 38

Ramachandran, Vasavi V. - 16

Ramirez, Darlin - 47

Rampold, Shelli - 132

Rand, Kenneth H. - 11

Rash, Rebecca - 126

Rashid, Mohammed - 8

Ray, A.-M. Le - 50

Reynolds-Bigby, Stephanie - 47

Richardson, Elise A. - 78

Richomme, P - 50

Richoux, Gary M. - 57, 74

Robinson, Tanya O. - 36

Rochars, Valery Madsen beau de - 8

Rodrigues, Thaís C.S. - 103

Romagosa, Christina M. - 54

Rouzier, Vanessa - 8

Ryan, Sadie J. - 91

S

Sabo-Attwood, Tara - 28, 38

Sakib, Sk Nazmus - 8

Salemi, Marco - 32

Sangiovanni, Elisa - 32

Sayeed, Md. Abu - 10

Schieber, Elizabeth - 114

Schoolnik, Gary K. - 16

Schwarz, Erika - 70

Seraphin, Marie Nancy - 46, 47

Shankar, Sripriya Nannu - 106, 111

Shanmugam, Keelnatham T. - 119

Sharma, Jatin - 5

Sheppe, Austin - 5

Simon, Margaret W. - 134

Simonne, Amarat (Amy) - 128

Singer, Burton - 134

Singh, Nitya - 7, 13, 15, 117

Slanzi, Crystal - 114

Sloan, Meredith - 36

Small, Coulter N. - 41

Smartt, Chelsea - 75

Southwick, Frederick - 30

Sriwanayos, Preeyanan - 98

Stafford, Lauren Stewart - 26

Stentiford, Grant D. - 97

Stevens, Bruce R. - 110

Subramaniam, Kuttichantran - 97, 98, 101, 103, 105, 108, 115

Surachetpong, Win - 98

Surphlis, Austin C. - 105

Sypes, Olivia - 62

T

Tagliamonte, Massimiliano - 32, 46, 124

Tal-mason, Aya - 49

Taylor, Caitlin E. - 68, 78, 120

Telg, Ricky - 132, 135

Teng, Lin - 11, 18

Threadcraft, Marcus - 30

Tian, Yuexun - 120

Tilly, Trevor B. - 111

Torhorst, Carson - 76

Tuanyok, Apichai - 86, 89

Tyndall, Adrian - 24

U

Ukhanova, Maria - 46

Uribasterra, Maria - 91

Usmani, Moiz - 111

V

Vaddiparti, Krishna - 95

Varma, Deepthi - 95

Vaziriyan-Sani, Alfonso - 119, 130

Viadanna, Pedro H. O. - 103

Villamor, Eduardo - 124

Vittor, Amy - 49

W

Walden, Heather D. S. - 43

Walker, Alyssa - 119, 123, 130

Walker, Morgan A. - 91

Waltzek, Thomas - 97, 98, 101, 103, 105, 108, 115

Wang, Xuechun - 44

Wang, Yan - 95

Weeks, Emma NI - 68

Wenzel, Richard - 30

White, Zoe - 64

Williams, Haley B. - 36

Wilson, Kristen - 64, 115

Wisely, Samantha - 43, 54, 55, 56, 64, 76, 80, 103, 115

Witanachchi, Chiran T. - 106

Wright, Patricia - 47

Wu, Chang-Yu - 34, 107

Y

Yang, Liu - 57, 72, 74

Yang, Yang - 33

Yanong, Roy - 101

Yutin, Natalya - 97

Z

Zabala, Virgilia D. - 128

Zamojski, Kendra - 128

Zhai, Yuting - 11

Zincke, Diansy - 83, 85

Notes

Notes