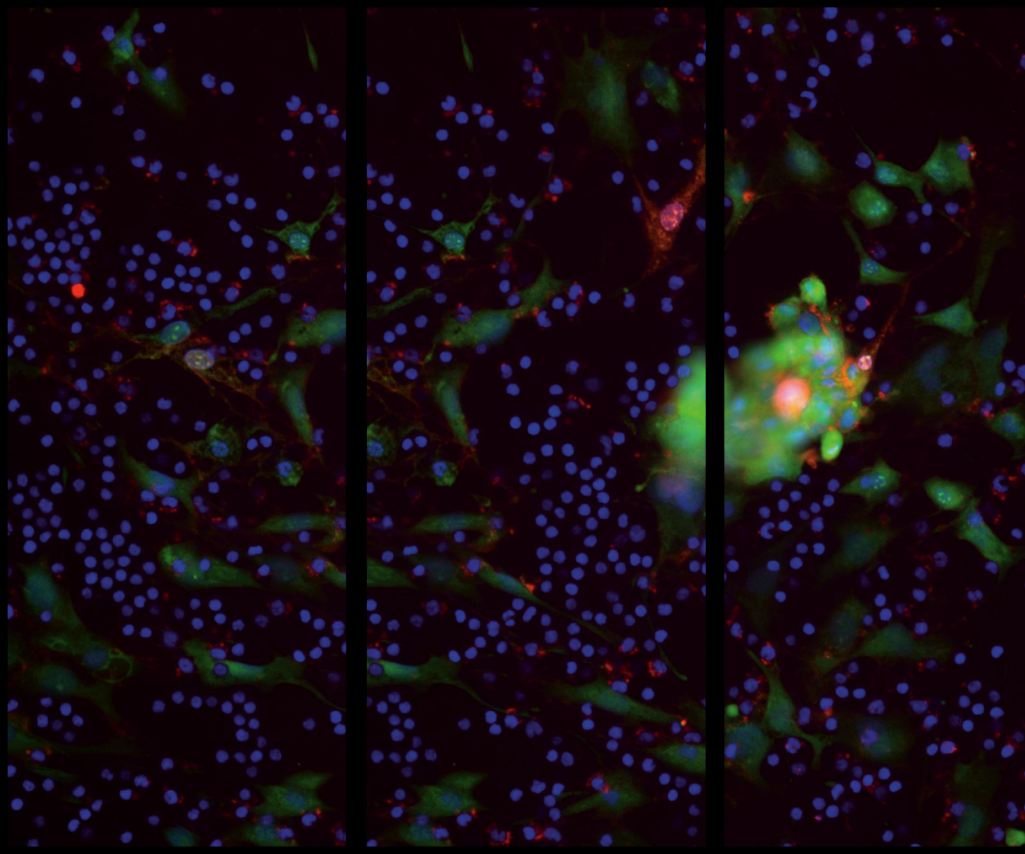
"In the Vittor lab, cross-reactive immunity is explored as a factor in zoonotic viral emergence. In Darien, Panama, the encephalitic Venezuelan equine encephalitis virus has been circulating for decades. When the antigenically similar Madariaga virus caused a first-ever human outbreak in 2010, field studies in collaboration with Panama's Gorgas Commemorative Institute of Health Studies showed that the affected populations were primarily migrant communities with lower levels of exposure to Venezuelan equine encephalitis virus. An antibody-dependent cell mediated cytotoxicity assay is pictured here, demonstrating heterologous killing of virus-infected cells. Human dermal fibroblasts (green) were infected with Madariaga virus, and subsequently exposed to serum from a Venezuelan equine encephalitis virus-recovered study participant. The serum antibodies bind to the cell surface where viral antigens are presented. The cells were then incubated with healthy donor peripheral blood monocytes (blue), which include natural killer cells. The natural killer cells bind to the Fc portion of the bound antibodies, triggering the secretion of cytotoxic granules. The human dermal fibroblasts thus killed over the course of the assay stain progressively yellow, then red, as their membranes become permeable and take up viability dye."

EMERGING PATHOGENS INSTITUTE | UNIVERSITY OF FLORIDA

EPI RESEARCH DAY BOOK OF ABSTRACTS 2022

EMERGING PATHOGENS INSTUTUTE



RESEARCH DAY

BOOK OF ABSTRACTS

FEBRUARY 2022

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Letter from the Director

Welcome to the 15th annual EPI Research Day! Once again, we're going virtual, given that Omicron numbers are not dropping as quickly as we had hoped. While we will miss the close contact of previous years, we're hopeful you will like the virtual format and will 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 seven different UF Colleges. We are also pleased to welcome investigators from outside of UF, including collaborators from other U.S. and international Universities, as well as from state and federal agencies.

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

Grant D. Stentiford, FRC Path, FLS is the Healthy Seafood Theme lead at Cefas, Head of the OIE Collaborating Centre for Emerging Aquatic Animal Diseases and co-Director of the Sustainable Aquaculture Futures Centre at the University of Exeter, UK. His work focuses on the identification and impact of aquatic animal diseases in farmed and wild environments.

Marco Salemi, PhD is the Holloway Professor of Experimental Pathology in the Department of Pathology, Immunology & Laboratory Medicine at the UF College of Medicine. Based at EPI, he leads an internationally recognized program in the genetic epidemiology of viral and bacterial pathogens, including NIH-funded studies of HIV, Vibrio cholerae, arboviruses, and, most recently, SARS-CoV-2.

Please visit our website, www.epi.ufl.edu, to join our mailing lists 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

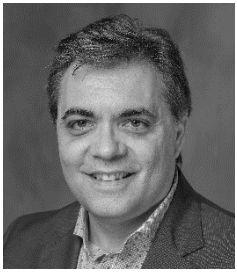
9:00 AM – 10:30 AM	Poster Session 1 (odd poster numbers) *Presenters: Start a meeting in your designated Poster Room*
10:30 AM – 12:00 PM	Poster Session 2 (even poster numbers) *Presenters: Start a meeting in your designated Poster Room*
12:00 PM – 1:00 PM	Lunch Break
1:00 PM – 1:10 PM	Introductions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

1:10 – 2:10pm

Grant D. Stentiford, FRC Path, FLS

Center for Environment Fisheries and Aquaculture Science
University of Exeter
United Kingdom

"*Sustainable aquaculture through the One Health lens*"



2:10 – 3:10pm

Marco Salemi, Ph.D.

Holloway Professor of Experimental Pathology
Department of Pathology, Immunology and Laboratory Medicine
University of Florida College of Medicine

"*Phylogenomic tracking of SARS-CoV-2 variants and emerging coronavirus zoonoses*"

01. A NOVEL COMBINATION THERAPY FOR MULTIDRUG RESISTANT PATHOGENS USING CHITOSAN NANOPARTICLES LOADED WITH B-LACTAM ANTIBIOTICS AND B-LACTAMASE INHIBITORS

Arianna Partow - Department of Agricultural and Biological Engineering, Emerging Pathogens Institute, College of Engineering, University of Florida; **Kwangcheol Casey Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Antimicrobial resistance is one of the greatest global threats. Particularly, multidrug resistant extended-spectrum β-lactamase (ESBL) producing pathogens confer resistance to many commonly used medically important antibiotics, especially beta-lactam antibiotics. Here, we developed an innovative combination approach to therapy for multidrug resistant pathogens by encapsulating cephalosporin antibiotics and β-lactamase inhibitors with chitosan nanoparticles (CNAIs). The four combinations of CNAIs including two cephalosporin antibiotics (cefotaxime and ceftiofur) with two β-lactamase inhibitors (tazobactam and clavulanate) were engineered as water-oil-water emulsions. Four combinations of CNAIs showed efficient antimicrobial activity against multidrug resistant ESBL-producing Enterobacteriaceae. The CNAIs showed enhanced antimicrobial activity compared to naïve chitosan nanoparticles and to the combination of cephalosporin antibiotics and β-lactamase inhibitors. Furthermore, CNAIs attached on the bacterial surface changed the permeability to the outer membrane, resulting in cell damage that leads to cell death. Taken together, CNAIs have provided promising potential for treatment of diseases caused by critically important ESBL-producing multidrug resistant pathogens.

02. BENEFITS AND RISKS OF SMALLHOLDER LIVESTOCK PRODUCTION ON CHILD NUTRITION IN LOW- AND MIDDLE-INCOME COUNTRIES

Dehao Chen - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Karah Mechlowitz** - College of Public Health and Health Professions, University of Florida; **Xiaolong Li** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Nancy Schaefer** - University of Florida; **Arie H. Havelaar** - Department of Animal Sciences, Emerging Pathogens Institute, 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

Livestock production can improve nutritional outcomes of pregnant women and children by increasing household income, availability of nutrient-dense foods, and women's empowerment. Nevertheless, the relationship is complex, and the nutritional status of children may be impaired by presence of or proximity to livestock and their pathogens. We review the benefits and risks of livestock production on child nutrition. Evidence supports the nutritional benefits of livestock farming through income, production, and women's empowerment. Increasing animal source food consumption requires a combination of efforts, including improved animal management so that herd size is adequate to meet household income needs and consumption and addressing sociocultural and gendered norms. The inclusion of behavior change communication strategies into livestock production interventions could facilitate the sustainability of nutritional benefits over time, particularly interventions that engage women and foster dimensions of women's empowerment. In evaluating the risks of livestock production, evidence indicates that a broad range of enteric pathogens may chronically infect the intestines of children and, in combination with dietary deficits, may cause environmental enteric dysfunction (EED), a chronic inflammation of the gut. Some of the most important pathogens associated with EED are zoonotic in nature with livestock as their main reservoir. Very few studies

have aimed to understand which livestock species contribute most to colonization with these pathogens, or how to reduce transmission. Control at the point of exposure has been investigated in a few studies, but much less effort has been spent on improving animal husbandry practices, which may have additional benefits. There is an urgent need for dedicated and long-term research to understand which livestock species contribute most to exposure of young children to zoonotic enteric pathogens, to test the potential of a wide range of intervention methods, to assess their effectiveness in randomized trials, and to assure their broad adaptation and sustainability. This review highlights the benefits and risks of livestock production on child nutrition. In addition to identifying research gaps, findings support inclusion of poor gut health as an immediate determinant of child undernutrition, expanding the established UNICEF framework which includes only inadequate diet and disease.

03. ISOLATION OF BACTERIOPHAGES AGAINST PATHOGENIC POLYKETIDE SYNTHASE-POSITIVE KLEBSIELLA PNEUMONIAE

Jesus Mendoza - Department of Medicine, College of Medicine, University of Florida; **Fadia Fakhre** - Department of Medicine, College of Medicine, University of Florida; **Lynn El Haddad** - Department of Medicine, College of Medicine, University of Florida

Introduction: Klebsiella pneumoniae is one of the main causes of infections and inflammation leading to disease. In particular, an intestinal colonization with K. pneumoniae carrying a pathogenic polyketide synthase (pks) island was shown to singly induce intestinal inflammation and carcinogenesis in a pre-clinical model. Unfortunately, the current strategy to eliminate infectious microorganisms rely almost exclusively on antibiotic strategy, which significantly disrupts microbiota balance and often causes adverse effects. Bacteriophages (i.e., phages) are ubiquitous and naturally occurring viruses that eradicate specific bacteria without disrupting the individual's microbiota. Phages have been shown to be effective and safe in fighting difficult to treat bacterial infections. This study aims to isolate and characterize phages against pks+ K. pneumoniae.

Method: Sewage samples were collected from the city of Gainesville. Isolation of phages against pks+K. pneumoniae 51-5 was conducted through a series of amplifications, centrifugations and filtrations steps. To assess the host range of the newly isolated phages, phage filtrates were tested against several K. pneumoniae strains. Phages were purified by collecting plaques (Figure 1). Additionally, phages were observed under the electron microscope (FEI Tecnai G2 Spirit Twin TEM) for morphology identification after treatment with ammonium acetate and staining with 2% phosphotungstic acid. The DNA of each isolated phage was extracted for downstream genome sequencing.

Results: Three phages against pks+K. pneumoniae were isolated from sewage samples (phi51-5, phi2, and phi3). All phages were lytic and were able to specifically inhibit pks+K. pneumoniae 51-5. Phages 51-5 and phi3 produce very small plaques on the bacterial lawn whereas phage phi2 produces large plaques with a halo (Figure 1). Using electron transmission microscopy, we were able to visualize phage properties such as a long contractile tail, presence of a “neck”, and an icosahedral head. Having all these characteristics, phage 51-5 belongs to the Caudovirales order and the Myoviridae family (Figure 2).

Conclusions: As naturally occurring viruses that can eradicate specific bacteria, phages are a valuable tool in clinical applications minimizing the range of bacterial infections while circumventing damaging effects to the microbiota. Our investigation has shown that pks+K. pneumoniae-specific phages isolated from waste sewage samples have lytic activity against pks+K. pneumoniae in vitro. Future projects involve in vivo testing of phage cocktail phi51-5-phi2-phi3 in a germ-free wild type mouse colonized with pks+K. pneumoniae 51-5 as well as in a mouse model of colitis and colitis-associated tumorigenesis.

04. DETERMINING THE QUALITATIVE CHARACTERIZATION OF ANTIBIOTIC RESISTANCE IN V. CHOLERAE

Lindsey Brinkley - Department of Pediatrics, Undergraduate Research, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

It is estimated by the CDC that there are 1.3 to 4.0 million Vibrio cholerae cases each year. Cholerae infections are characterized by their severe acute watery diarrhea often caused by contamination of food or water. Cholera continuously is a strong threat to public health as it remains endemic in countries such as Haiti and Bangladesh. In 2018, the collection of stool samples began in Bangladesh. In this collection, four patients were enrolled each day with samples being analyzed for acute watery diarrhea at the International Centre for Diarrhoeal Disease Research, Bangladesh. These samples were plated for bacterial growth and the colonies suspected to be V. cholerae were serotyped with a monoclonal antibody specific to V. cholerae O1. These are the samples that were tested for antibiotic resistance. Based on a prior study, the rates of V. cholerae resistance to ciprofloxacin and azithromycin were predicted to be higher in anaerobic conditions compared to aerobic conditions. Growth curves were used to determine if each strain was resistant or sensitive to antibiotics under anaerobic and aerobic conditions. In groups of eight, each strain was plated onto LB and LB + 100M of streptomycin. After 16-24 hours, colonies were selected from the plate to inoculate 5mL of LB broth. Following a period of growth, these samples were back diluted to 1 OD, and each was used to inoculate eleven wells of a 96 well plate. Within the 96 well plate, there were three antibiotics each with three dilutions based on the breakpoint concentrations as well as two controls and one blank. The 96 well plate was placed on a plate reader where the optical density for each well was measured for 8 hours to plot a growth curve for each sample in each condition. Out of 120 samples, 47 of them were resistant to ciprofloxacin and 120 of them were resistant to azithromycin. Ultimately it was found that the likelihood of resistance is higher in anaerobic conditions than aerobic conditions as ciprofloxacin and azithromycin had odds ratios of 37.5 and 136.2 respectively.

05. SURVIVAL OF SALMONELLA ON TOMATOES BY VARIOUS LEVELS OF DAMAGE SOLD AT INFORMAL MARKETS IN LOW- AND MIDDLE-INCOME COUNTRIES IN AFRICA

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

Introduction: Salmonella on tomatoes has been a source of several foodborne outbreaks in low- and middle-income countries in Africa and contributes to the public health burden. Previous studies have shown that Salmonella is better able to survive on damaged tomatoes compared to undamaged tomatoes but have yet to determine if the degree of damage affects the survival. Tomatoes with various degrees of damage are sold at informal African markets where the consumers include elderly, children under five and those with underlying health problems.

Purpose: The purpose of this study was to assess if the degree of damage on a tomato impacted the survival of Salmonella.

Method: Culled tomatoes were categorized by degree of damage on a one to five scale with five having the highest degree of damage. Tomatoes with category three and four damage were used as the treatment groups while tomatoes without damage were used as the control (n= 18). Tomatoes with category five damage were discarded as they were typically not consumed raw. All of the tomatoes were spot inoculated with a rifampicin resistant Salmonella cocktail, left to dry for two hours and held at 29□C and sampled over five days. To recover the Salmonella, 25 mL of 0.1% peptone was added to each tomato in a Whirlpak bag and subjected to a shake-rub-shake, before a 1 mL sample was diluted and the pathogen populations were enumerated on Tryptic Soy Agar supplemented with rifampicin. A two-way ANOVA followed by a Tukey's HSD test was used to analyze statistical significance between Salmonella survival over the five days.

Results: The survival of Salmonella was significantly different ($p > 0.05$) between undamaged and category four damaged tomatoes. The survival

of Salmonella was not significantly different between undamaged and category three damaged tomatoes, or between tomatoes with category three and four damage. Across the three groups of tomatoes there was a significant decline ($p>0.05$) in Salmonella populations between days: zero and five; one and three; one and four; and one and five. The interaction between damaged tomatoes and time was not significant.

Conclusion: Food safety risks associated with the contamination of tomatoes vary depending on the degree of damage. The survival of Salmonella on tomatoes with category four damage was significantly higher compared to the undamaged tomatoes.

06. COMPARATIVE ASSESSMENT OF BIOFILM FORMATION AND PHAGE SENSITIVITY AMONG VARIOUS SEROTYPES OF VIBRIO CHOLERAE ISOLATED IN HAITI

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

Cholera caused by toxigenic strains of Vibrio cholerae remains a major public health concern in mostly poor and developing countries globally lacking safe drinking water, sanitation and hygiene. V. cholerae is ubiquitous in aquatic environments wherein it faces numerous stressors. However, survival and persistence of V. cholerae in aquatic reservoirs are key to cholera epidemics and largely depend on its ability to form biofilm in association with biotic and/or abiotic surfaces in these reservoirs. Moreover, resistance against lytic bacteriophage infection promotes the survival and persistence of V. cholerae resulting in co-evolution and co-persistence of both bacteria and phage. Recently, we have isolated diverse serotypes (toxigenic Ogawa and Inaba and non-toxigenic Ogawa, Inaba and poly positive serotypes) of V. cholerae O1 strains from aquatic reservoirs in Haiti. In the present study, we aim to evaluate the comparative biofilm forming-ability and phage sensitivity between clinical and diverse environmental serotypes of V. cholerae isolated in Haiti. Our results indicate that non-toxigenic Ogawa and poly positive serotypes of V. cholerae strains produced significantly higher biofilm (~6 and ~8 fold higher respectively) compared to toxigenic or non-toxigenic Inaba serotype. We also found that non-toxigenic strains are better biofilm producers (~2-fold higher) than the toxigenic strains, and the environmental strains exhibited comparatively higher biofilm formation (~3 and ~5 fold higher for environmental Ogawa and poly positive respectively) compared to the clinical isolates. Our phage sensitivity data indicate that, environmental non-toxigenic Ogawa, Inaba and poly-positive serotypes were resistance (100%) to MAA01, ICP2 and ICP1 phage. In contrast, environmental toxigenic Inaba strains were sensitive (100%) to all three types of phages examined. Collectively, the non-toxigenic and environmental strains provided strong resistance against phage infection while clinical toxigenic strains were severely attenuated in their ability to confer resistance to phage infection. In summary, our preliminary findings suggest that V. cholerae strains show diverse biofilm-forming ability and phage sensitivity; these traits depend on V. cholerae's serotypes, isolation sources, and presence of toxigenic genes. We conclude that different serotypes might have responded to distinct (vps-dependent and vps-independent) biofilm-forming and phage resistance

mechanism. We suggest that higher biofilm former strains/serotypes and strains showing higher resistance to phages' infection 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 and phage sensitivity as exhibited by the strains/serotypes examined in this study.

07. A MACHINE LEARNING BASED APPROACH TO UNDERSTAND THE BACTERIAL GENOMIC SIGNATURES CONTRIBUTING TO HOST ADAPTATION OF EXTENDED SPECTRUM B-LACTAMASE-PRODUCING ESCHERICHIA COLI

Ting Liu - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Yuting Zhai** - 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; **An-Chi Cheng** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Juan M Campos Krauer** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Raoul K. Boughton**; **Laxmi Rathor** - Department of Aging and Geriatric Research, College of Medicine, University of Florida; **Sung Min Han** - Department of Aging and Geriatric Research, College of Medicine, University of Florida; **Kwangcheol Casey Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Introduction: Extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae are "critical priority" antimicrobial resistance pathogens proposed by the World Health Organization. ESBL-producing E. coli (ESBLs) have caused numerous infections in humans worldwide and have resulted in enormous economic costs. It is increasingly postulated that the interface between wildlife and livestock may play an important role in the transmission of ESBLs. However, bacterial genetic features

involved in colonization and dissemination of ESBLs at the wildlife-livestock interface remain elusive.

Methods: Here, we identified unique genes contributing to the colonization of ESBLs in different hosts using a machine learning (ML) approach in conjunction with comparative genome analysis.

Results: A total of 392 genomes of ESBLs from wildlife (n=142) and cattle (n=250) were used to develop a supervised ML model. We found that most genomes were classified into the same host from which they were isolated, while approximately 5% and 15% of ESBLs from cattle and wildlife, respectively, did not cluster into the source. Consistently, the phylogenetic tree consisting of 392 genomes revealed that most ESBLs were mainly grouped in line by source, whereas four clades of isolates from different hosts were clustered together, indicating that some ESBLs colonize different hosts. Through pan-genome comparative analysis, fliD and pglA were identified exclusively in ESBLs isolated from both hosts. The fliD and pglA mutants were defective in adhesion on Hep-2 cells and suppressed the decreased longevity in the nematode Caenorhabditis elegans, a well-characterized aging animal model. These data indicate that these two genes are associated with host adaptation and pathogenicity.

Conclusions: Unique genes present in ESBL-producing E. coli that associated with bacterial adhesion ability and pathogenicity facilitating their colonization and adaptation in the hosts. Importantly, the interface of wildlife and livestock should be controlled to prevent potential transmission of antimicrobial resistance

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

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

Methicillin resistant Staphylococcus aureus (MRSA) has been identified in hospitalized patients, animals, including wildlife, companion, and food-producing animals, and the environments. Due to its high transmission and evolution rates, MRSA infection poses a significant threat to public health and veterinary medicine. However, the genetic features enabling host adaptation and specificity of MRSA are still poorly understood. Here, we aimed to identify genetic and molecular functions associated with host specificity in MRSA infection by applying a machine learning approach. We included a total of 2,512 MRSA genomes from human (n=2,195) and non-human hosts (n=317) to develop a source-attribution considered machine learning (ML) model. The Support Vector Machine (SVM) model accurately predicted the origin of MRSA based on genomic contents and provided the genetic features that might be important for host specificity. The SVM model showed a clear distinction between

human and non-human MRSA isolates, except for strains from companion animals. Several genes have been identified as important genes for human-specific adaptation, including hsdM, cdaR, xerC, clpP, arlS, arlR, degV, ctpA, and mdtH. Identified genes have been reported as determinants for virulence, genetic recombination, bacterial signaling, lipid transportation, and antimicrobial resistance. Furthermore, correlation and permutation importance analysis suggested that adaptation to environmental stress, acquisition for new virulence factors, and resistance to antimicrobials could cooperate and facilitate MRSA colonization and adaptation in humans. Overall, we report specific genetic features using ML that are important for the host specificity of MRSA.

09. SEROSURVEILLANCE OF A FLORIDA ISLAND POPULATION OVER THE COURSE OF THE SARS-COV-2 PANDEMIC

Adriana Gallagher - Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida; **Andrew Kane** - College of Public Health and Health Professions, University of Florida; **Eric Nelson** - College of Medicine, University of Florida; **Maha El Badry** - Procter & Gamble; **Ruiyu Pu** - Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida; **John Lednicky** - College of Medicine, University of Florida; **Tracey Logan** - Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida; **Derek Cummings** - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **J. Glenn Morris, Jr.** - Emerging Pathogens Institute, University of Florida; **Maureen Long** - Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) pandemic has affected millions of people and as vaccines became available it is important to monitor vaccine induced antibody responses. An island population in Florida severely restricted access to the island for several weeks beginning in April 2020. During the shutdown, >90% of the population was tested using an in-house ELISA to a receptor binding domain (rbdELISA) and only 1% of the population tested positive. The rbdELISA is specific to the SARS-CoV-2. One year later 183 samples were collected from the same population. For this collection, needle sticks were used to collect dried blood spot (DBS) samples. In addition to the rbdELISA, a second ELSA was used that tested for antibody to the SARS-CoV-2 nuclear protein (nELISA) in order to possibly differentiate infection from vaccination. The nELISA cross-reacts with the N proteins of other coronaviruses. At the time of collection 141/183 participants had been vaccinated with one of the three available vaccines. A total of 128/141

(91%) of the vaccinated participants tested positive using the rbdELISA and all recipients of the mRNA vaccines demonstrated seroconversion while only 50% of recipients testing positive to the Johnson&Johnson vaccine. Six percent of the vaccinated participants and 43% of the nonvaccinated participants tested positive using the nELISA protein indicating past exposure to SARS-CoV-2 or another coronavirus. This study indicates that mRNA vaccines are more reliable inducers of SARS-CoV-2 antibody and the dual testing could be used to differential vaccine response from historical exposure.

10. ASSESSMENT OF A MASS BALANCE EQUATION FOR ESTIMATING COMMUNITY-LEVEL PREVALENCE OF COVID-19 USING WASTEWATER-BASED EPIDEMIOLOGY

Andrew Rainey - 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; **Julia Loeb** - Department of Environmental and Global Health, Emerging Pathogens Institute, University of Florida; **Sarah Robinson** - 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; **John Lednicky** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Eric Coker** - Department of Environmental and Global Health, 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; **Joseph H. Bisesi** - 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 T. Maurelli** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

Background: Wastewater-Based epidemiology (WBE) is a novel epidemiologic tool that analyzes wastewater to detect the presence of pathogens and track disease trends within a community. WBE has the potential to estimate the true number of infections within a community by using a mass balance equation, however this application has yet to be rigorously assessed. The objective of this study is to conduct weekly wastewater surveillance for the presence of SARS-CoV-2 to estimate community-level prevalence of COVID-19, and to compare it with a COVID-19 prevalence estimate derived from clinical surveillance data.

Methods: We utilized WBE over a 53-week period in Gainesville, FL. We processed influent wastewater samples to extract SARS-CoV-2 RNA and analyzed the samples for the CDC N2 genetic target using qPCR. We used the SARS-CoV-2 wastewater concentrations in three mass balance equation models (Model A-C) that utilized different parameter estimates for the individual SARS-CoV-2 fecal shedding rate to generate the estimated weekly COVID-19 prevalence. We assessed the wastewater-derived COVID-19 prevalence estimates with the clinical prevalence in Gainesville to determine if the mass balance equation generated accurate prevalence estimates.

Results: The median wastewater-derived COVID-19 prevalence was 4.51% (Model A), 5.90% (Model B), and 11.81% (Model C). There was a significant difference in the prevalence estimate generated between each model using the mass balance equation (Chi-Square, 75.03; P-Value, <0.0001). The median difference between the weekly wastewater-derived COVID-19 prevalence estimate and clinically-derived COVID-19 prevalence estimate was -0.90% (Model A) (P-Value, 0.27) 1.18% (Model B) (-260.5; P-Value, 0.02), and 2.35% (Model C) (P-Value, <0.0001).

Conclusions: The mass balance equation is an effective approach for generating accurate community-level prevalence estimates, demonstrating how wastewater-based epidemiology improves community surveillance of COVID-19.

11. VALVE-ENABLED SEQUENTIAL REAGENT DELIVERY AND PAPER-BASED ENRICHMENT FOR SIMULTANEOUS DETECTION OF SARS-COV-2 AND INFLUENZA VIRUSES

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With expected co-circulation of SARS-CoV-2 and other respiratory viruses such as influenza, early and accurate detection of these viruses at POC is crucial for reducing their transmission. Adequate access and availability of POC devices for virus detection will significantly reduce the number of people who get infected and are desperately needed. To address this need, we have developed a duplex valve-enabled lysis, paper-based RNA enrichment, and RNA amplification device (VLEAD) for simultaneous detection of influenza A virus and SARS-CoV-2. It is very important to detect these two viruses simultaneously since they can cause contagious respiratory illnesses with similar symptoms. Each side of the 2-plex VLEAD consists of 4 buffer wells at the top for a lysis buffer, binding buffer, and two wash buffers needed for sample preparation, 1 mixing unit in the middle, and 1 detection unit, which is an assembly of a polycarbonate well layer and a laminated chromatography paper. The mixing unit can slide against the buffer unit using slots on the sides and in the middle to enable the valve mechanism to sequentially discharge the sample preparation reagents from the buffer unit into the mixing unit. The

detection units are integrated with the mixing unit by inserted them in the protrusions at the bottom of each mixing well. After RNA purification, the detection units are detached from the mixing unit and prepared for reverse transcription loop-mediated isothermal amplification (RT-LAMP) before placing them in a commercially available smart coffee mug set at 62.5oC for 25 min. Finally, the detection units are taken out of the mug for addition of SYBR Green dye for detection of amplicons by color change that can be visualized by the naked eye or recorded using a smartphone camera. We have demonstrated the specificity of the duplex VLEAD for SARS-CoV-2 and influenza A H1N1 pdm2009. We tested each RT-LAMP assay for both viruses and CoV-OC43, and the results confirmed the specific detection of the target virus with no cross-reaction with any of the other two viruses tested. The RT-LAMP assays also showed great sensitivity with limit of detection at 10 genome equivalents (GEs) for SARS-CoV-2 and 0.06 TCID50 for Influenza A H1N1 pdm2009. The feasibility of the device was tested using heat-inactivated SARS-CoV-2 samples obtained from BEI Resources (NR-52286), and influenza A H1N1 pdm2009 samples. This device has potential to help reduce the transmission of COVID-19 and other respiratory viral infections.

12. SOCIO-ECONOMIC FACTORS RELATED TO COVID-19 AMONG PERSONS WHO COLLECT RECYCLABLE MATERIAL IN CENTRAL BRAZIL

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Introduction: Persons who collect recyclable material (PCRM) are at enhanced risk for contracting several infectious diseases including COVID-19. In addition to occupation-related risks, we hypothesize that SARS-CoV2 will be more common among those with other factors such as – (a) limited access to personal protective equipment like masks and access to handwashing, (b) living in poverty, (c) living in crowded households with limited or no opportunities for social isolation, and (d) presence of flu-like symptoms or contact with people exhibiting such symptoms.

Objective: To compare sociodemographic and other risk factors in PRCM with and without COVID-19 in Goiania, Goiás, Brazil.

Method: Data for these analyses come from a cross-sectional study conducted between July 2020 and October 2020, a period when Brazil was the epicenter of the COVID-19 pandemic. A non-probabilistic sample of 153 PCRM recruited from companies engaged in recyclable material

collection participated in the study. Data collection was conducted in the Universidade Federal de Goiás (Federal University of Goiás), in a place designated to ensure biosafety. Informed consent was obtained from all participants before administering a face-to-face semi-structured questionnaire developed for the study. SARS-COV-2 infection was established based on a positive result of a total anti-SARS-COV-2 antibodies test (ELISA) or a Real-Time PCR from the oropharynx and nasopharynx collected by a combined swab (nasal/oral).

Results: About one-third (30.7%) of participants were positive for SARS-CoV2. The median age of the sample was 37 years (IQR=27-48), and the median household income was $231 (IQR=185.18 – 387.03). PCRM positive for COVID-19 compared to those who tested negative were less likely to engage in preventive practices such as wearing masks and handwashing (81% vs. 88%, p. 0,263); more likely to live in a crowded household (28% vs. 26%, p.0,873) and have flu-like symptoms or in contact with symptomatic individuals (64% vs, 61%, p. 0,768); and more likely to have 1 or more chronic disease (17% vs. 15%, p. 0,762).

Conclusion: As a group, PCRM showed a higher prevalence of SARS-CoV2 than the general population of Brazil (30.5% vs. 2.4%), which highlights the need to increase access to testing and vaccination among this at-risk population. The lack of association between well-established SARS-CoV-2 related factors and SARS-CoV-2 infection status should be further studied with larger samples of PCRM and their networks.

13. A THERMOSTABLE CAS12B FROM BREVIBACILLUS LEVERAGES ONE-POT DISCRIMINATION OF SARS-COV-2 VARIANTS OF CONCERN

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Introduction: Current SARS-CoV-2 detection platforms lack the ability to differentiate among variants of concern (VOCs) in an efficient manner. CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated) based detection systems have the potential to transform the landscape of COVID-19 diagnostics due to their programmability; however, most of these methods are reliant on either a multi-step process involving amplification or elaborate guide RNA designs. Here, for the first time, we describe a complete one-pot detection reaction using a thermostable Cas12b effector endonuclease from Brevibacillus sp. to overcome these challenges detecting and discriminating SARS-CoV-2 VOCs in clinical samples.

Methods: Three Cas12b proteins from Alicyclobacillus acidoterrestris (AacCas12b), Alicyclobacillus acidiphilus (AapCas12b), and Brevibacillus sp. SYP-B805 (BrCas12b) were expressed and purified, and their thermostability was characterized by differential scanning fluorimetry, cis-, and trans-cleavage activities over a range of temperatures. The BrCas12b was then incorporated into a reverse transcription loop-mediated isothermal amplification (RT-LAMP)-based one-pot reaction system, coined CRISPR-SPADE (CRISPR Single Pot Assay for Detecting Emerging VOCs).

Results: CRISPR-SPADE was then applied for discriminating SARS-CoV-2 VOCs, including Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529) and validated in 206 clinical samples. CRISPR-SPADE achieved 92·7% sensitivity, 99·4% specificity, and 96·7% accuracy within 10-30 minutes for discriminating the SARS-CoV-2 VOCs, in agreement with S gene sequencing, achieving a positive and negative predictive value of 99·1% and 95·1%, respectively. Interestingly, for samples with high viral load (Ct value ≤ 30), 100% accuracy and sensitivity

were attained. To facilitate dissemination and global implementation of the assay, a lyophilized version of one-pot CRISPR-SPADE reagents was developed and combined with an in-house portable multiplexing device capable of interpreting two orthogonal fluorescence signals. This technology enables real-time monitoring of RT-LAMP-mediated amplification and CRISPR-based reaction at a fraction of the cost of a qPCR system.

Conclusion: The ultra-thermostable Brevibacillus sp. Cas12b offers relaxed primer design for accurately detecting SARS-CoV-2 VOCs in a simple and robust one-pot assay. The lyophilized reagents and simple instrumentation further enable rapid deployable point-of-care diagnostics that can be easily expanded beyond COVID-19.

14. GENOME-WIDE CRISPR SCREENS IDENTIFY HOST FACTORS THAT PROMOTE HUMAN CORONAVIRUS INFECTION

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Background: The COVID-19 pandemic has resulted in 275 million infections and 5.4 million deaths as of December 2021. While effective vaccines are being administered globally, there is still a great need for

antiviral therapies as antigenically novel SARS-CoV-2 variants continue to emerge across the globe. Viruses require host factors at every step in their life cycle, representing a rich pool of candidate targets for antiviral drug design.

Methods: To identify host factors that promote SARS-CoV-2 infection with potential for broad-spectrum activity across the coronavirus family, we performed genome-scale CRISPR knockout screens in two cell lines (Vero E6 and HEK293T ectopically expressing ACE2) with SARS-CoV-2 and the common cold-causing human coronavirus OC43. Gene knockdown, CRISPR knockout, and small molecule testing in Vero, HEK293, and human small airway epithelial cells were used to verify our findings.

Results: While we identified multiple genes and functional pathways that have been previously reported to promote human coronavirus replication, we also identified a substantial number of novel genes and pathways. The website https://sarscrisprscreens.epi.ufl.edu/ was created to allow visualization and comparison of SARS-CoV2 CRISPR screens in a uniformly analyzed way. Of note, host factors involved in cell cycle regulation were enriched in our screens as were several key components of the programmed mRNA decay pathway The role of EDC4 and XRN1 in coronavirus replication in human small airway epithelial cells was verified. Finally, we identified novel candidate antiviral compounds targeting a number of factors revealed by our screens.

Conclusions: Overall, our studies substantiate and expand the growing body of literature focused on understanding key human coronavirus-host cell interactions and exploit that knowledge for rational antiviral drug development.

15. A MIXTURE MODEL TO ESTIMATE SARS COV 2 SEROPREVALENCE IN CHENNAI, INDIA

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Introduction: Serological assays used to estimate SARS-CoV-2 seroprevalence rely on manufacturer cut-offs established based on more severe early cases who tended to be older.

Method: We conducted a household-based serosurvey of 4,677 individuals from 2,619 households in Chennai, India from January to May, 2021. Samples were tested for SARS-CoV-2 IgG antibodies to the spike (S) and nucelocapsid (N) proteins. We calculated seroprevalence using manufacturer cut-offs and using a mixture model in which individuals were assigned a probability of being seropositive based on their measured IgG, accounting for heterogeneous antibody response across individuals.

Results: The SARS-CoV-2 seroprevalence to anti-S and anti-N IgG was 62.0% (95% confidence interval [CI], 60.6 to 63.4) and 13.5% (95% CI, 12.6 to 14.5), respectively applying the manufacturer's cut-offs, with low inter-assay agreement (Cohen's kappa 0.15). With the mixture model, estimated anti-S IgG and anti-N IgG seroprevalence was 64.9% (95% Credible Interval [CrI], 63.8 to 66.0) and 51.5% (95% CrI, 50.2 to 52.9) respectively, with high inter-assay agreement (Cohen's kappa 0.66). Age and socioeconomic factors showed inconsistent relationships with anti-S IgG and anti-N IgG seropositivity using manufacturer's cut-offs, but the mixture model reconciled these differences. In the mixture model, age was not associated with seropositivity, and improved household ventilation was associated with lower seropositivity odds.

Conclusions: With global vaccine scale-up, the utility of the more stable anti-S IgG assay may be limited due to the inclusion of the S protein in several vaccines. SARS-CoV-2 seroprevalence estimates using alternative targets must consider heterogeneity in seroresponse to ensure seroprevalence is not underestimated and correlates not misinterpreted.

16. PREVENTING COVID-19 IN CLASSROOM SETTINGS AT A LARGE PUBLIC INSTITUTION OF HIGHER LEARNING – OCTOBER 2020 – OCTOBER 2021

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Background: The pandemic of Coronavirus Disease 2019 (COVID-19) has led to widespread disruption of daily life, including in education settings. In response to the need to control on campus and in classroom spread of SARS-CoV-2, causative agent of COVID-19, at a large Public Institution of Higher Learning, we created a passive surveillance program to identify classrooms of concern and implement targeted prevention.

Methods: The classroom monitoring team detected class sections where a high proportion of students were "withheld" as cases, contacts, or suspect cases, assessed whether spread in the classroom was possible, and identified mitigation strategies. Course sections were designated as face-to-face if any components were in-person and were confirmed by a registered room number. The Classroom Team reviewed reports on the number of students "withheld" by course section and sections were flagged for further investigation if: 1) ≥2 students were withheld, and 2) the proportion of the section that was withheld was 10% or greater. For sections with >50 students, a cutoff of 5% was used. Potential classroom transmission clusters were identified if there were multiple cases with similar onset dates and no alternative contact or high-risk exposures were reported or if cases or contacts reported contact occurring in class during their interviews.

Results: The number of sections identified each semester ranged from 149 in the Fall of 2020 to 0 during Summer A 2021. In Fall 2020, classroom monitoring identified 5 sections from 2 classes that met the threshold of concern and where no alternative explanation could be identified in the case records. It was recommended that targeted testing be initiated for all sections, including the 5 flagged during monitoring, and this was initiated for all 1573 students. Within 2 days of notification, 136 students were tested, identifying an additional 6 cases and 13 suspect cases. After testing, these sections fell below the cutoffs for investigation. Classroom-based targeted testing was initiated in 2 other instances: in Spring 2021 for 18 students, of whom 3 were tested; and in Fall 2021 for a section of 36, of whom 4 were tested. No additional cases were identified in either class.

Conclusions: Classroom monitoring was able to identify several classrooms of concern that were not identified through normal contact tracing. These activities have been carried out successfully under voluntary COVID-19 testing and routine testing policies by leveraging data being collected through contact tracing.

17. SARS-COV-2 INFECTIONS IN INFANTS IN HAITI 2020-2021; EVIDENCE FROM A SEROLOGICAL COHORT

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Little information is available regarding COVID-19 infection rates in children < 2 years of age. This study examined the seroprevalence of antibodies to the SARS-CoV-2 receptor binding domain (RBD) protein using dried blood spots (DBS) obtained from infants up to 2 years of age, collected both before and during the pandemic. An in-house rbdELISA was used to detect exposure to SARS-CoV-2. A total of 388 samples from 257 children were collected at one or more time points including birth, approximately 4, 12, and 18-24 months of age from February 2019 to March 2021. A spline analysis investigating the relationship between time of sampling and OD indicated that average ODs increased ~0.200 in value at 400 days past January 1, 2019 or approximately March 2020. Mixture

models identified an OD = 0.21 as a cut-off with 95% specificity in identifying positive samples. Prior to March 2020 none of 131 samples (117 children) had an OD >0.21 while 43 (16.7%) of 257 unique children became seropositive at some point during our study. Eighteen (19%) of 95 children with longitudinal samples became seropositive. Overall, 21% of children tested were seropositive between March 2020 and April 2021. Because of DBS variations at collection, we confirmed the average protein concentration for all samples was 5,194 μg/mL. There were no differences in protein levels for age or year. This data indicates that children less than two years are susceptible to infection and are likely play a role in transmission of SARS-CoV-2 within households.

18. SARS-COV-2 IN FARMED FLORIDA WHITE-TAILED DEER: A PROPOSAL

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COVID-19, which is caused by the SARS-Cov-2 virus, is a continuing global health emergency. Multiple animal species including white-tailed deer have been shown to sustain infection with SARS-CoV-2. Susceptibility of white-tailed deer specifically is likely due to their ACE2/S protein binding motif as it shares high homology with that of human ACE2. Human to deer, and deer to deer transmission of SARS-CoV-2 has been demonstrated; however, it is not fully understood whether white-tailed deer are capable of transmitting the virus back to humans. Our work will aim to further understand the persistence and transmission of the SARS-CoV-2 virus within farmed Florida white-tailed deer, as well as the history and evolution of other coronaviruses in these farmed species.

19. ASSESSMENT OF RESIDENTIAL ENVIRONMENTS FOR SARS-COV-2 AEROSOLS FROM SELF-ISOLATING COVID-19 PATIENTS

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Introduction: Individuals who test positive for SARS-CoV-2 are advised to self-isolate at their residences unless they require hospitalization. Persons sharing a dwelling with someone who has COVID-19 are at risk of being exposed to the virus. However, environmental monitoring data for the detection of the virus in such settings are limited. We present a pilot study on environmental sampling for SARS-CoV-2 virions in the residential rooms of three volunteers with COVID-19.

Method: This work involved surface sampling with sterile swabs and the employment different air samplers such as a Viable Virus Aerosol Sampler (VIVAS), BioSpot-VIVAS, an inline air sampler that traps particles on polytetrafluoroethylene (PTFE) filters, a NIOSH 2-stage cyclone sampler (BC-251), and a Sioutas personal cascade impactor sampler (PCIS).

Results: SARS-CoV-2 RNA was detected by real-time Reverse-Transcription quantitative Polymerase Chain Reaction (rRT-qPCR) analyses of particles in one air sample from the room of volunteer 1 and in various air and surface samples from that of volunteers 2 and 3. The one positive sample collected by the NIOSH sampler from volunteer 1's room had a quantitation cycle (Cq) of 38.21, indicating a low amount of airborne virus. In contrast, air samples and surface samples collected off the mobile phone in volunteer 2's room yielded Cq values ranging from 14.58 to 24.73 and 21.01 to 24.74, respectively, on the first day of sampling, indicating that this volunteer was actively shedding relatively high amounts of SARS-CoV-2 at that time. The SARS-CoV-2 GE/cm3 of air for the air samples collected by the PCIS was in the range 6.84E+04 to 3.04E+05, the highest being from the stage 4 filter, and similarly, ranged from 2.54E+03 to 1.68E+05 GE/cm3 in air collected by the NIOSH sampler. Attempts to isolate the virus in cell culture from the samples from volunteer 2's room with the aforementioned Cq values were unsuccessful due to out-competition by a co-infecting Human adenovirus B3 (HAdVB3) that killed the Vero E6 cell cultures within 4 days of their inoculation, although Cq values of 34.56–37.32 were measured upon rRT-qPCR analyses of vRNA purified from the cell culture medium. Samples collected from volunteer 3's residence by both NIOSH and BioSpot-VIVAS air samplers were positive by RT-PCR. We recorded viable SARS-CoV-2 in samples collected by BioSpot. Genome sequencing of these samples showed the virus to be a Delta variant. The size distribution of SARS-CoV-2-laden aerosol particles collected from the air of volunteer 2 and 3's room were <4.4 μm, suggesting a risk of aerosol transmission since these particles can remain suspended in air for an extended time.

Conclusions: This work shows that particles containing virus can travel over long distances, are capable of passing ciliated airways, and potentially could infect cells within the lower respiratory tract. We saw improved ability to culture SARS-CoV-2 with condensational growth tube samplers and suggest that environmental sampling could serve as a non-invasive route in tracking the spread of the virus. Results further indicate that measures such as physical distancing by 6 feet may not be helpful

indoors, potentially providing a false-sense of security and leading to more exposures and outbreaks.

20. SARS-COV-2 SEROSURVEILLANCE IN FLORIDA ANIMALS

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Since the inception of the SARS-CoV-2 pandemic, most surveillance has focused on how the virus affects humans. Animal specific ELISAs were developed to the receptor binding domain (RBD) (rbdELISA) of SARS-CoV-2 virus for dogs and cats. The rbdELISA results were compared to a surrogate neutralizing antibody assay (snELISA) which had previously been validated against the neutralization test according to several publications. This study included two discrete data sets, the first consisted of dogs and cats with a history of proximity to COVID-positive humans or tested positive to SARS-CoV-2 via rtPCR. The second group was a convenience sample of animals with no history of SARS-CoV-2 human exposure. A total of 393 animals were sampled consisting of 165 cats (155 domestic and 10 exotic) and 228 dogs. Serum from four experimentally

infected cats was used as positive assay controls. Negative control samples consisted of 101 canine and 10 feline samples obtained before 2019. Samples were tested from 507 individuals, including 39 clinical samples, 115 control samples, and 353 convenience samples. Using the rbdELISA,18 of 165 (10.9%) cats tested positive and 10 of 228 (4.4%) dogs tested positive. There was agreement between the two tests in 501/507 (98.8%) of the samples. This study demonstrates that species-specific indirect ELISAs can be used as a serological assay for surveillance of SARS-CoV-2 exposure in dogs and cats.

21. EVALUATING THE EFFECT OF RUSTY CRAYFISH INVASION HISTORY ON CRAYFISH PATHOGEN COMMUNITY COMPOSITION

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Invasive species alter freshwater ecosystems and threaten native biodiversity. However, we know little about the impacts of invasion on pathogen communities and how these impacts may affect invasion success. Our study focused on crayfish pathogen communities in northern Wisconsin, a region where the invasive rusty crayfish (Faxonius rusticus) replaces resident congeners. Our goal was to determine whether pathogen prevalence and composition differ among lakes that vary in rusty crayfish abundance and impacts and to determine if differences in behavior are associated with differences in pathogen prevalence, density, or infection intensity. We dissected and histologically prepared over 450 crayfish to evaluate pathogen prevalence and composition, with ad hoc use of molecular tools. We also conducted behavioral assays to assess crayfish anti-predator behavior and condition prior to dissection. So far, we have identified pathogens from nine taxonomic groups. Rusty crayfish share several of these pathogens with native virile crayfish (Faxonius virilis) and the pathogen community composition in these two species can overlap. In addition, our preliminary results suggest rusty crayfish behavior differs across lakes with high and low rusty crayfish densities. We discovered a microsporidian (Nosema sp.) outbreak in rusty crayfish in Trout Lake, WI. Rusty crayfish infected by the microsporidian had an altered anti-predator behavior and decrease in physiological condition. Future research will assess whether impacted communities are able to recover as pathogens accumulate in invasive populations.

22. TRANSCONTINENTAL DISPERSAL OF NON-ENDEMIC FUNGAL PATHOGENS THROUGH WOODEN HANDICRAFT IMPORTS

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This study examined the viability and diversity of fungi harbored in imported wooden handicraft products sold in six retail stores in Florida, United States. Despite being subjected to trade regulations that require various sterilization/fumigation protocols, our study demonstrates high survival and diversity of fungi in wood products originating from at least 7 countries on 3 continents. Among these fungi were non-endemic plant and human pathogens as well as mycotoxin-producers. Several products that are sold for use in food preparation and consumption and harbored a novel (to North America) crossover human x plant pathogen, Paecilomyces formosus. In addition, a high number of species isolated were thermophilic and included halophilic species, suggesting adaptability and selection through current wood treatment protocols that utilize heat and/or fumigation with methyl-bromide. This research suggests that current federal guidelines for imports of wooden goods are not sufficient to avoid transit of potential live pathogens and demonstrates the need to increase safeguards at both points of origin and entry for biosecurity against introduction from invasive fungal species in wood products.
"fungal pathogens" "invasive organisms" "imports"

23. EFFICACY OF HOPS ESSENTIAL OIL AND MYRCENE AGAINST CRYPTOSPORIDIUM PARVUM INFECTION IN HCT-8 CELLS

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Introduction: Cryptosporidiosis is one of the leading causes of diarrhea among young children and life-threatening disease among immunocompromised individuals. Hops essential oil (HEO) is a byproduct of the brewing industry with proven antibacterial and antifungal activities. Its anti-cryptosporidial activity, however, has not been explored extensively. In this work, we hypothesized that HEO and myrcene, its main monoterpene, will show a dose-dependent anti-cryptosporidial activity as evaluated in a cell model of invasion and growth in vitro.

Methods: Cytotoxicity (IC50) was determined in HCT-8 intestinal cell monolayers using flow cytometry and staining for non-viable cells with propidium iodide after treatment with controls and different concentrations (0-1000 µg/mL) of HEO and myrcene for 24 and 48 h (37oC and 5% CO2). To determine the anti-Cryptosporidium effect of bioactives, varying concentrations of HEO or myrcene alone below their IC50 values (0-100 µg/mL) in HCT-8 cells at 48 h were evaluated. For invasion inhibition tests, confluent HCT-8 cells were infected with C. parvum sporozoites (1x104 sporozoites/mL) and immediately treated with bioactives dissolved in DMSO followed by incubation for 48 h (37 oC and 5% CO2). For growth inhibition tests, confluent HCT-8 cells were infected with C. parvum sporozoites, and 2 h later were treated with fresh medium containing bioactives following incubation for 46 h (37 oC and 5% CO2). DMSO was used as the diluent and control. Paromomycin was used as a positive control. The presence of parasites was evaluated by immunofluorescence using a fluorescence-conjugated anti-Cryptosporidium antibody (Sporo-GloTM), which broadly recognizes sporozoites and life-cycle stages.

Results: HEO showed low cytotoxicity to HCT-8 cells (IC50= 237.5 µg/mL). Myrcene showed higher cytotoxicity in comparison to HEO (IC50=176.5

µg/mL; P<0.05). In the invasion and growth inhibition tests, HEO elicited a dose-dependent reduction in C. parvum growth with IC50 of 60.38 and 57.96 µg/mL, respectively. In similar tests, myrcene alone dose-dependently reduced C. parvum growth showing IC50= 43.4 ± 2.06 µg/mL and 46.1±2.60 µg/mL, respectively. Overall, HEO and myrcene showed moderate to high anti-cryptosporidial activity compared to other drugs used such as Paromomycin (IC50= 277 µg/mL) and the FDA-approved drug nitazoxanide (IC50= 10 µg/mL) as shown in the literature.

Conclusions: HEO and myrcene showed in vitro anti-Cryptosporidium efficacy. Due to its relatively high activity against C. parvum, further studies should explore the potential efficacy and mechanisms of action of these volatile bioactives in animal models.

24. PHARMACOKINETIC ANALYSIS OF IMPACT-TB TRIAL DATA

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The purpose of this study was to compare pharmacokinetic (PK) parameters in adults taking imatinib (IMAT) with and without isoniazid (INH) and rifabutin (RBN). Data was from the Imatinib Mesylate Per Oral as a Clinical Therapeutic for TB (IMPACT-TB) trial, a phase I trial investigating effects of imatinib with and without INH and RBN on myelopoiesis in healthy adults. The goal is to use imatinib as TB therapy, as it may improve clearance of M. tuberculosis by disrupting host cell entry. Patients received IMAT 50/100 mg daily for two weeks, then INH 300 mg and RBN 300 mg daily added for two weeks. Plasma samples for quantification were drawn at 0, 0.5, 2, 4, 8, and 24 hours after drug administration while subjects were on IMAT alone and after adding INH+RBN. PK parameters were compared using t-test, non-compartmental analysis was performed on Phoenix WinNonlin v8.3, and statistical analysis was performed on JMP Pro v16. 14 subjects and 184 plasma samples were included. After 50mg IMAT, mean (SD) area under the concentration-time curve (AUC(hr*mcg/ml)) was 9.57(±7.77) with and 7.47(±7.51) without RBN+INH. The max IMAT concentration (Cmax(mcg/ml)) was 0.87(±0.67) with and 0.58(±0.51) without. After 100mg IMAT, mean AUC was 7.48(±0.70) with and 9.47(±3.15) without, and Cmax 0.69(±0.04) with and 0.63(±0.17) without RBN+INH. There were no significant differences with and without RBN+INH after 50mg IMAT for AUC (9.57 vs. 7.74[p=0.49]) or Cmax (0.87 vs 0.58, [p=0.23]) and 100mg IMAT for AUC (7.48 vs 9.47[p=0.30]) or Cmax (0.69 vs 0.63 [p=0.55]). Des-

imatinib mean concentrations were higher with RBN+INH after 50mg (0.005 vs. 0.02, p=0.004) and 100mg (0.01 vs. 0.14, p=0.01). In conclusion, IMAT PK parameters were similar in subjects receiving IMAT 50mg or 100mg with and without INH and RBN. Des-IMAT concentrations were significantly higher following the addition of INH and RBN. Increased des-IMAT levels could potentially be due to CYP enzyme induction from rifabutin administration, resulting in increased IMAT metabolism. Additional research will be needed to determine if the addition of INH and RBN contributes to myelopoiesis in patients taking IMAT.

25. TEMPERATURE EFFECTS ON MAYARO VIRUS VECTOR COMPETENCY OF FLORIDIAN AEDES AEGYPTI MOSQUITOES

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Mayaro virus (MAYV) is a positive-sense single-stranded Alphavirus that spreads among humans by the bite of infected mosquitoes. In most cases, human infection may cause influenza-like illness (3-5 days duration) or may lead to severe debilitating arthralgia that persists for months. There has been an increase in the number of MAYV isolations and detection of antibodies in several countries, such as Brazil, Ecuador, and Colombia. Despite MAYV limited distribution in South America, it has potential to rapidly expand its geographical distribution to emerge in new regions in North America due to human movements and vector invasion, leading to increased risk of local transmission as observed with other viruses, e.g., chikungunya and Zika. Temperature variation is an important driver that shapes mosquito life history, virus infection, and likelihood of transmission events by bite. Here we characterize the influence of temperature on susceptibility to infection, replication, and transmission of MAYV in invasive vector Aedes aegypti.

26. EFFECTS OF LANDSCAPE ON MOSQUITO COMMUNITY COMPOSITION AND ABUNDANCE IN MANATEE COUNTY, FLORIDA

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Introduction: Subtropical and year-round humid climate and the availability of expansive suitable breeding habitats make Florida home to over 90 species of mosquitoes, the most important vectors of arthropod-borne disease globally. As vector-borne disease transmission requires vector, host and pathogen to be present and overlap in space and time, abiotic and biotic factors can impact the distributions and abundances of mosquito species, altering transmission risk. Previous studies investigating environmental drivers of mosquito abundances and distributions often focus on individual vector species, despite the potential for interspecific variations in vector competency within mosquito communities to affect transmission outcomes.

Method: We utilized joint species distribution models to quantify effects of landscape composition on individual species and mosquito community

diversity, and we used enhanced vegetation index values to predict proportions of West Nile virus (WNV) competent vector species across 56 sites in Manatee County Florida during the 2020 sampling season. Mean number of mosquitoes per trap night for each species served as the response variables in our model, and percent land cover for water, developed, cropland, herbaceous wetland and woody wetland within a 5 km buffer of trap sites served as environmental variables. A binary trait matrix of WNV vector competency was added to predict proportions of WNV vector species across the study area.

Results: Results indicated that proportions of variance explained by percent land cover for individual species coincided with known habitat associations for the majority of species. Species richness values decreased with increasing percentages of developed land cover and increased with increasing percentage of all other investigated land cover classes. Maps visualizing spatial predictions of the proportion of WNV competent vector species across Manatee County were generated, predicting the highest proportion of competent vector species in urbanized areas.

Conclusions: Quantifying impacts of landscape on individual mosquito species and communities contributes to understanding how landscape impacts disease transmission systems, while community-weighted mean traits can provide insights relevant to public health, vector control management, and research investigating disease systems and ecology.

27. HETEROLOGOUS IMMUNITY AND HOST SUSCEPTIBILITY TO EMERGENT ALPHAVIRUSES: WHY IS THERE SO LITTLE MAYARO VIRUS IN PANAMA?

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In the Darien province in Panama, Venezuelan equine encephalitis virus (VEEV, a zoonotic mosquito-borne virus in the genus Alphavirus) infections in humans is common. Mayaro virus (MAYV), also an Alphavirus, has caused numerous outbreaks in neighboring countries, but has limited circulation in Panama despite known presence of the vector. In this study, we examine the hypothesis that heterologous immunity due to prior VEEV exposure provides protection against MAYV infection via Fcγ receptor functions such as antibody-dependent cell mediated cytotoxicity (ADCC). Using sera from our Panamanian alphaviral cohort (N=65), we established alphaviral exposure status by virus-specific plaque reduction neutralization tests. All sera were negative for MAYV neutralizing Abs (PRNT80). ADCC assays of MAYV-infected target cells (K562s) demonstrated significantly more lysis of infected cells in the VEEV-exposed group compared to the Alphavirus-negative group (normalized lysis of MAYV-infected cells 34.04 % (95% CI 7.69 – 68.82%) vs 12.37% (95% CI -17.84 - 40.54%)). Similarly, ADCC assays of MAYV-infected human dermal fibroblasts resulted in a significantly higher rate of killed cells in the VEEV-exposed group compared to the Alphavirus-

negative group (normalized killing rate 2.86 (95% CI 0.88 – 5.84) vs. 1.78 (95% CI 0.63 – 3.12)). This relationship was absent for those exposed to a virus similar to VEEV, Madariaga virus (MADV). The MADV exposed group showed no enhanced lysis or killing of MAYV-infected target cells. Blocking CD16 abrogated the killing of MAYV-infected fibroblasts, revealing the importance of the Fcγ III receptor (CD16) in cross-reactive ADCC. These results provide evidence for heterologous immunity in alphaviral infections. This heterologous immunity, in turn, may help explain the observed geographic constraints on alphaviral transmission.

28. IDENTIFICATION OF THE PARASITE, TRYPANOSOMA CRUZI, IN MULTIPLE TISSUES OF EPIDEMIOLOGICAL SIGNIFICANCE IN THE VIRGINIA OPOSSUM (DIDELPHIS VIRGINIANA): IMPLICATIONS FOR ENVIRONMENTAL AND VERTICAL TRANSMISSION

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Trypanosoma cruzi is a parasitic protozoan and the causative agent of Chagas disease in humans. The parasite is vectored by triatomine bugs and transmission is maintained through the infection of mammalian reservoir hosts. In South America, opossums are suspected to facilitate transmission via infected anal gland secretions and feces, in addition to transmission via the arthropod vector. The Virginia opossum is a known reservoir host for T. cruzi across the parasite's endemic range in the US, including Florida. The objectives of this study were to identify the pathogen prevalence among Virginia opossums and to investigate potential routes of transmission that are facilitated by Virginia opossums, including vertical and environmental transmission. Virginia opossums were sampled at 5 trapping sites in a 5-county region over a 10-month

period in North Central Florida. Peripheral blood, anal gland secretion, and fecal swabs, were collected from each adult individual, and peripheral blood was collected from joey opossums. In total 198 opossums were sampled: 112 were adults and 86 were joeys. Following sample collection nuclear DNA was extracted and using real time PCR methods, T. cruzi infected individuals and the T. cruzi Discrete Typing Unit (DTU) was identified. In total, 57 adults (50.9%), and 4 joeys were infected, with T. cruzi, and each infected, across the three sample types collected and each joey had an infected mother. One pair of joeys had yet to emerge from the marsupium, and it was concluded vertical transmission took place between this mother-joey pair. Based on real time PCR data it is likely that Virginia opossums are maintaining transmission among the species through vertical transmission. The only DTU found in the sampled population was TcI which supports the known DTU-host association seen in other locations around the US. Opossums were observed expressing their anal gland secretions which were also observed contaminating their perianal region and feces. No fecal swabs were positive for T. cruzi where the individual, both, expressed their anal glands and had T. cruzi infected anal gland secretions. However, the detection of T. cruzi DNA in the anal glands and the observation that fecal matter was contaminated with anal gland secretion does suggests that opossums play a role in the horizontal transmission of the parasite through the environmental contamination of infected feces and spraint. It is proposed that at latrines opossums may be transmitting the parasite to other mammalian hosts as they contact concentrated environmental T. cruzi.

29. A SURVEY OF TICKS AND TICK-BORNE MICROORGANISMS WITHIN RECREATIONAL GREEN SPACES ACROSS THE GAINESVILLE, FLORIDA METROPOLITAN AREA AND SURROUNDING LANDSCAPE

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Tick-borne infections are of increasing medical and veterinary concern within the southeastern United States. Florida, USA, has multiple tick species that can vector microorganisms that are relevant to human and companion animal health. Within north central Florida, various cases of tick-borne diseases are reported each year, with some still likely underreported. The purpose of this study was to describe and compare the presence, abundance, and biodiversity of medically important tick species, their life stages, and their associated microorganisms in recreational greenspaces in Gainesville, Florida, that span across an ecological gradient in the Gulf Coast Forest ecosystem that dominates the southeastern US. From January 1st to June 30th, 2021, we collected ticks bimonthly via dragging and flagging across trails or designated recreational areas in 18 publicly accessible greenspaces within a manicured or natural gradient. Across the six tick species collected as adults, nymphs, or pools of larvae, we detected bacteria or protozoa within the Babesia, Borrelia, Cryptoplasma, Cytauxzoon, Ehrlichia,

Hepatozoon, Rickettsia, and Theileria genres. The abundance of the aggressive human biting tick, A. americanum (Lone star tick) in both management types is important, as it is a vector of various pathogens like Ehrlichiosis and Rocky Mountain Spotted Fever and can cause Alpha gal-syndrome and STARI rash. Finding ticks within city or county limits might indicate that favorable ecological conditions for tick presence may persist across urban and forested parklands. Overall, due to the high visitor rates and the detection of pathogenic microorganisms in ticks at recreational areas during the time of year sampled, public health educational efforts regarding ticks and tick-borne diseases will be beneficial to conduct.

30. NS1, DENGUE VIRUS' SECRETED WEAPON FOR EVADING INNATE IMMUNE DETECTION IN MOSQUITOES?

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Every year, an estimated 400 million people are infected with dengue virus (DENV), and about 80% develop mild dengue fever but for others the more severe illnesses, dengue hemorrhagic fever or dengue shock syndrome occur. Approximately 40% of the world's population (3 billion) live in at-risk areas for dengue. Dengue virus is a mosquito-vectored flavivirus with a positive sense single-stranded RNA genome that encodes 3 structural and 7 nonstructural proteins. Nonstructural protein-1 (NS1) is a glycoprotein that anchors the virus replication complex and plays a dynamic role during DENV infection in mammals and mosquitoes. In humans, NS1 is used as a biomarker of infection, and has been shown to enhance DENV infection by binding to a mannose binding lectin to block part of the complement system, an innate immune response in humans, thereby leading to increased DENV infection. In mosquitoes, NS1 not only increases DENV uptake from an infected blood meal, but through inhibition of the mosquito's innate immune response will enhance DENV titers in the mosquito leading to increased virus replication and increased

transmission of the virus to humans. Despite the important role NS1 plays in DENV infection, the receptor for NS1 binding in the mosquito midgut has not been identified. We hypothesize that secreted NS1 binds to C-type lectins (CTLs) in the mosquito midgut to interfere with and reduce innate immune response, resulting in increased DENV titers in the midgut. To identify NS1 binding partners, recombinant NS1 will be added to mosquito midgut preparations and coimmunoprecipitation for bound NS1 will be performed; NS1 binding partners will be identified through mass spectrometry and proteomic analysis. To confirm identified binging partners, targeted CTLs will be knocked down in mosquitoes using RNAi, levels will be validated using qPCR. C-type lectin-knocked down mosquitoes will be fed a DENV infectious blood meal and plaque assays will be conducted to determine virus titers in the mosquitoes. Additionally, these same CTLs will be expressed and incubated with recombinant NS1, followed by gel shift assay to confirm binding of expressed CTLs and NS1. Understanding how NS1 impacts the uptake and establishment of DENV in the mosquito midgut is necessary in developing new methods of DENV prevention. This study will identify CTL-NS1 binding partners that lead to enhanced DENV infection in the mosquito.

31. CAUSE OF DEATH IN FLORIDA FARMED WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) DURING 2017 – 2021

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White-tailed deer (Odocoileus virginianus) farming is a major economic industry in Florida. Bacterial infections and viral hemorrhagic diseases result in high mortalities for fawns and yearling deer in Florida deer farms. To aid deer owners with herd management strategies and solutions, the causes of death of farmed deer must first be determined. The University of Florida Cervidae Health Research Initiative (CHeRI) provides a free diagnostic service to Florida deer farmers to determine the causes of death of deer in their herds. From 2017 to 2021, diagnostic testing was performed to determine the causes of death for 583 farmed White-tailed deer. Tissues samples were tested for hemorrhagic certain viruses using RT-qPCR. Samples were also tested through microbial culture, histopathology analysis, and parasite identification to determine a probable cause of death. Through data analysis in the form of percentage calculations, we determined substantial differences in the proportions of bacterial and viral diseases affecting deer within different age categories. Of the 190 deceased farmed white-tailed deer aged 1-90 days sampled from 2017 to 2021, 48% of deaths were caused by bacterial

infection, and only 9% were due to viral hemorrhagic disease. Of the 175 animals aged 4-12 months sampled from 2017 to 2021, 30% of deaths were attributed to hemorrhagic disease viruses, and 18% to bacterial infection. Lastly, of the 199 animals aged 13 months and older sampled from 2017 to 2021, hemorrhagic disease viruses caused 26% of deaths and 34% were due to bacterial infection. When analyzing the causes of death for farmed white-tailed deer aged 1 day to 13 months and older, viral hemorrhagic diseases and bacterial infections account for 76% of deaths. Our results show that viral hemorrhagic diseases are seasonal, with the peak death season for farmed white-tailed deer occurring late summer through early fall. Among the epizootic hemorrhagic disease virus (EHDV)-positive cases in 2021, all infections were attributed to EHDV serotypes 2 and 6. Additionally, bacterial infections significantly increase during fawn season from late May to September. These data help better understand the prevalence and seasonal dynamic of pathogens affecting farmed white-tailed deer and provide insight to develop best management practices and treatment strategies.

32. BLACK AND SICHUAN PEPPER CONSTITUENTS SIGNIFICANTLY ENHANCE THE TOXICITY AND NEUROPHYSIOLOGICAL EFFECTS OF NATURAL PYRETHRINS IN AEDES AEGYPTI

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With insecticide-resistant mosquito populations becoming an ever-growing concern, new vector control technologies are needed. With the lack of available new chemical classes of insecticides to control mosquito populations, the development of novel synergists may improve the performance of already utilized insecticides. We have identified two plant extracts, black pepper and Sichuan pepper, that significantly synergize (increase the effect beyond additivity) the effects of natural pyrethrins when applied topically to Aedes aegypti females. Black pepper extract (SR = 17) and Sichuan pepper (SR = 15.3) synergized the toxicity of natural pyrethrins on Aedes aegypti when applied topically in combination with piperonyl butoxide, a commercially available synergist. This synergism of natural pyrethrins was also observed directly on the mosquito central nervous system (CNS), indicating that it is mediated via neurophysiological action, which is unique from PBO, a monooxygenase inhibitor. Piperine, the primary active constituent of black pepper extract, and □-hydroxysanshool, the primary active constituent of Sichuan pepper extract, were screened individually as synergists of natural pyrethrins. Both piperine and α-hydroxysanshool were shown to synergize natural pyrethrins both topically (SR = 13.9 and 8.5, respectively) and in vitro. Interestingly, only piperine was capable of synergizing natural pyrethrins on the CNS of a pyrethroid-resistant strain of mosquitoes, indicating that both molecules possess a slightly different mechanism of action. To probe this question further, we evaluated the effects of both compounds on American cockroach (Periplaneta americana) giant axons. Both piperine and α-hydroxysanshool applied at 10 μM progressively diminished action potential amplitudes when axons were stimulated at 10 Hz. However, α-

hydroxysanshool also produced marked depolarization in axon resting membrane potential, whereas piperine did not. This activity-dependent block of action potentials caused by both compounds, but the differences in their ability to depolarize nerve membranes, may help us better understand how these two pepper constituents synergize pyrethroids on both a pyrethroid-susceptible and pyrethroid-resistant strains. We anticipate that the identification and characterization of the effects of target-site synergists may lead to the development of more selective and potent pest control chemistries in the future.

33. FIR NEEDLE OIL SYNERGIZES THE KNOCKDOWN ACTIVITY OF DIVERSE INSECTICIDES VIA A NON-NEUROACTIVE MECHANISM

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The generally pleasant aroma and perceived salubrious qualities of plant essential oils (PEO) have given rise to a shift in consumer preference from synthetic insecticides to these natural formulations for both home pest control and personal bite protection. In addition to their overall safety to mammals and non-target organisms, they are effective at controlling pest insects, making them ideal candidates in future insecticidal and repellent formulations. This study characterized the ability of fir needle oil to synergize the knockdown of 7 different synthetic insecticides. Thus far, fir needle oil strongly synergized the 1 hr knockdown potency of the neonicotinoids, clothiandin and thiamethoxam (between 16 and 24-fold), as well as natural pyrethrins (12-fold). Furthermore, we have analyzed the individual chemical constituents within this sample to identify those most responsible for its activity. Thus far, delta-3-carene has proved to be the most bioactive constituent, producing synergism similar to that of the whole oil. In fact, it synergized the 24-hr mortality of clothianidin to a higher degree than fir needle oil itself (4.9-fold vs. 2.4-fold). These effects are not mediated by synergism on the nervous system, as fir needle oil produces no excitation or block of mosquito central nervous system firing

at a concentration (100 ppm) which produces block or excitation by other plant oils. Moreover, fir needle oil at high concentrations (100 ppm) does not synergize the nerve blocking potential of an inactive concentration of natural pyrethrins (10 nM). To better understand why fir needle oil increases the knockdown of select insecticides, but not toxicity, we evaluated the ability of this oil to increase or decrease the ability of Aedes aegypti cytochrome P450 monooxygenases to degrade a model substrate, 7-ethoxycoumarin. Interestingly, fir needle oil pretreatment caused a significant increase in metabolic degradation of 7-ethoxycoumarin. This finding suggests that fir needle oil upregulates metabolic processes that allow for degradation of certain insecticides and provides a good working hypothesis to explain why fir needle oil enhances knockdown but not 24-hr mortality.

34. A MOLECULAR SURVEILLANCE-GUIDED VECTOR CONTROL RESPONSE TO CONCURRENT DENGUE AND WEST NILE VIRUS OUTBREAKS IN A COVID-19 HOTSPOT OF FLORIDA

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Simultaneous vector-borne disease outbreaks of dengue virus (DENV) and West Nile virus (WNV) in Florida, USA, in 2020 resulted in 71 dengue virus serotype 1 and 86 WNV human cases. We collected and screened the DENV (Aedes aegypti and Ae. albopictus) and WNV (Anopheles crucians, Culex coronator, Cx. nigripalpus, and Cx. quinquefasciatus) vectors from Miami-Dade County (Florida) for DENV and WNV by rRT-qPCR. Spatial statistical analyses were performed to capture positive mosquito pool distribution in relation to land use, human demography, environmental variables, mosquito trap placement, and reported human travel associated DENV cases to guide future mosquito control outbreak responses. A rapid screen of 7,668 mosquitoes detected four DENV serotype 2 (DENV-2), nine DENV-4 and nine WNV-positive mosquito pools, which enabled swift and targeted abatement of trap sites by mosquito control. As expected, DENV-positive pools were in urban areas; however, we found WNV-positive mosquito pools in agricultural and recreational areas with no historical reports of WNV transmission. These findings demonstrate the importance of proactive arbovirus surveillance in mosquito populations to prevent and control outbreaks, particularly when other illnesses (e.g., COVID-19), which present with similar symptoms, are circulating concurrently. Growing evidence for substantial infection prevalence of dengue in mosquitoes in the absence of local index cases suggests a higher level of dengue endemicity in Florida than previously thought.

35. MANGROVE CRABS ARE CARRIERS OF 'CASSAVA MOSAIC VIRUS' (ALPHAFLEXIVIRIDAE)

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Our view of the global virome is constantly expanding, with over 300,000 new RNA viruses discovered in the last decade. The arthropod virome is a lucrative source of viral discovery, leading to huge changes in our view of viral taxonomy, prevalence and impact. Of greatest concern are those viruses that cause significant pathological impacts in cultured plants and animals, leading to potentially high economic damage. In our poster, we present the discovery of a new strain of 'Cassava mosaic virus' (CsCMV) (6,403bp), a member of the Alphaflexiviridae, which are a viral family persistent in plants, invertebrates, and vertebrates. CsCMV causes 30-40% of cassava crop loss in South America, meaning that the discovery of new vectors is vital for management and protection of the agricultural process. Our new strain was identified from the hepatopancreas of a mangrove crab (Aratus pisonii) sampled from the Florida Keys (Jan, 2018) and showed replicase (87%-98%) and capsid protein (96%-99%) similarity to other CsCMV strains. Our mitochondrial haplotype mapping, based on the complete mitogenome of the crab highlights high similarity across Central and South American populations, suggesting that the same species is present and could act as vectors of the disease. Alternatively, it is possible that this crab species is a natural host or "dead end" for this virus, consuming white fly (known vector) and possibly protecting the local cassava agriculture by consuming the insect vector.

36. ASSESSING THE FITNESS OF ZIKA AND DENGUE VIRUS INFECTIOUS CLONES PRODUCED IN INSECT AND MAMMALIAN CELLS

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Virus infectious clones are commonly utilized as a tool for investigating the relationship between viral genotypes and phenotypes such as virulence and infectivity. They can also be useful when isolating live virus is not possible. However, an infectious clone produced from a single genome sequence does not necessarily duplicate every feature of the virus it is designed from. Arthropod transmitted RNA viruses such as dengue (DENV) and Zika viruses (ZIKV), introduce approximately one mutation every time they replicate their genome, and as such, infections are caused by a “mutant swarm” of diverse individual viruses. Some of these mutations which are advantageous in one host type (e.g., human) can be deleterious in the other (e.g., mosquito), as has been demonstrated in an array of arthropod-borne RNA viruses that switch between hosts. Handling of these viruses in a lab setting often necessitates expansion or serial passage in a single tissue type, which may unwittingly apply different selective pressure than the virus would face in nature. The goal of this project is to investigate whether production in insect or mammalian cell cultures alters the fitness of ZIKV and DENV infectious clones during mosquito infection. We hypothesize that the production of infectious clones in mammalian cells may artificially reduce the infectiousness and transmissibility of the virus of interest in mosquitoes. To investigate this, a dengue virus infectious clone using a

genome sequence isolated from mosquitoes was produced in a mammalian cell line (Vero E6 cells). We characterized the infectious clone by examining infection rate and intensity in mosquito midguts and ovaries, the presence of virus in mosquito saliva, and its replication rate in insect and mammalian cell culture. Our preliminary data supported this hypothesis, as the infectious clone was outperformed by a genetically similar wild-type virus in mosquito and insect cell culture infections, although insect cell adaptation was possible by serial passage. Our present work aims to compare the fitness of genetically identical infectious ZIKV clones (produced in insect or mammalian cell lines) during mosquito and cell culture infection. These clones will also be compared against their wild-type parental virus strain. We hope that this study will help define best practices for using infectious clones to accurately characterize medically important dual-host RNA viruses, as well as expand upon the methodology for creating these clones.

37. INTERSPECIFIC VARIATION IN DIEL PATTERNS OF SUPPLEMENTAL FEEDER VISITATION ON A BIG-GAME DEER FARM IN NW FLORIDA

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The deer farming industry encompasses a spectrum from small breeding farms to high-fenced hunting preserves thousands of hectares in size. As the deer farming industry, so too has the range of species available for harvest expanded to include numerous non-native species. The presence of exotics on preserves, and unnaturally high animal densities, inevitably changes the ecological interactions and disease transmission dynamics between natives, exotics, and the surrounding environment. One site of

disease transmission within farms is at supplemental feeders. Farmed animals visit feeders often and encounter other individuals which exposes them to direct and indirect disease transmission. Thus, the objective of this study was to better understand the diel patterns of feeder visitation of 9 target species on a private big-game ranch in northern Florida. Further, as a case study, we focus on one virus which causes serious impacts to the white-tailed deer farming industry. Epizootic hemorrhagic disease virus (EHDV) is a virus affecting white-tailed deer (WTD; Odocoileus virginianus) and related ruminants. The study area for this case study has been the focus of a long-term research effort to better understand EHDV transmission on big-game farms in Florida. This 6-year study provides a unique opportunity to better understand farmed animal behaviors that may impact EHDV transmission. While there was much similarity in the patterns of feeder visitation with many species visiting feeders at dawn and dusk, some exotic species appeared to visit feeders at different times as compared to native WTD. We postulate that observed differences in diel patterns of feeder visitation may directly influence host exposure to vectoring midges and thus may explain observed interspecific variations in epizootic hemorrhagic disease virus (EHDV) seroprevalence and Culicoides midge host preference within the study area.

38. UNDERSTANDING FARMED DEER FEEDER VISITATION RATES: INSIGHTS INTO FARMED ANIMAL BEHAVIOR AT DISEASE TRANSMISSION HOTSPOTS

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Cervid game farms are a common fixture throughout North America, with over 8,000 farms in the U.S.A. Game farms are economically important to many rural communities, but disease risks exist within and beyond (spillover risk) farm boundaries as animals are often stocked at densities greater than in surrounding wildlands. These conditions necessitate a thorough understanding of animal behaviors to promote reductions in disease risks. Supplemental feed sources, such as bait piles or feeders, are sites of high potential disease transmission as animals may come into direct or indirect contact with saliva, feces, nasal secretions, and urine when feeding. The objective of this study was to determine how long and how often three farmed cervid species (white-tailed deer [Odocoileus virginianus], elk [Cervus canadensis], and Père David's Deer [Elaphurus davidianus]) visited feeders. We established ear-tag readers at 4 of 12 supplemental feeders within a high-fenced hunting pre-serve in northern Florida and monitored them for 18 months. Animals were present at feeders often (~2 times a day). On average, they stayed for over five minutes at each presence event per visit, and they were present primarily during crepuscular hours (dawn and dusk). Supplemental feeders should be treated as areas of high animal use when developing disease intervention strategies.

39. EVALUATION OF A NANOENCAPSULATED FORMULATION OF THE ESSENTIAL OIL INSECTICIDE: BIGSHOT MAXIM

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A bottle bioassay and spatial spray bioassay were conducted to assess the efficacy of a non-commercial, formulation of BigShot Maxim distributed by PreVasive USA, LLC and evaluated against Aedes aegypti. The formulation is a glycerin-based polymer encapsulated cedar oil (15.2% AI by weight). For the bottle bioassay, four concentrations of the test material (1:50, 1:100, 1:150 & 1:200) were prepared and applied to bottles with 1mL of solution. A negative control of acetone and a positive control of permethrin (CDC diagnostic dose) were included. For the spatial spray bioassay five solutions of the BigShot Maxim nanoformulation were prepared with pyrethrin and PBO corresponding to 0, 0.12, 0.24, 0.45, & 0.95 and 0, 0.76, 1.44, 2.72 & 5.96 11.2 µg/cm2 respectively. For the bottle bioassay, mortality at 1h post-treatment ranged between 27.3-93.0% with LC50= 4.9 µg/cm2 (4.7 – 5.2 µg/cm2 95% CI; LC90=10.0µg (9.1 – 11.2 µg/cm2 95% CI). For the spatial spray bioassay, mortality ranged between 19.6-28.0% with no positive correlation with increasing pyrethrin and PBO concentration.

40. MOLECULAR SURVEILLANCE OF POTENTIAL MAMMALIAN HOSTS FOR BORRELIA AND BARTONELLA IN FLORIDA: A PROPOSAL

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The bacterium, Borrelia burgdorferi, is endemic to the northeast and Midwestern U.S., with nationwide infections nearly doubling from 1996 to 2019. The disease is spread through the bite of the black-legged tick (Ixodes scapularis), primarily the nymph form. The ticks often feed on small mammals and ground-dwelling birds where they pick up and transmit B. burgdorferi. The primary reservoir host, the white-footed mouse, is found commonly in the eastern half of the U.S., excluding Florida. This leads to the question of how the bacterium is surviving and maintaining itself in Florida's ecosystem. Previous studies have shown other animals besides the white-footed mouse to be carrier hosts of the bacteria but information is limited and focused in areas such as California. Studies have shown rodents such as the Norway rat and the gray squirrel to have B. burgdorferi infections and cotton mice to be carriers of the bacterium but this has not been shown in Florida. Additionally, Bartonella spp. causes a variety of diseases and is carried by rodents and fleas causing infections in large and small mammals including cats and humans, with Florida having some of the highest infection rates. This leads to the question of what hosts are responsible for the high infection rates. To test this, we trapped and sampled 305 rodents from 9 species across 5 counties in Northcentral Florida. The blood samples were extracted and will be screened for B. burgdorgeri and Bartonella to gain an understanding of rodent infection prevalence in Florida. For B.

burgdorferi, we amplified a partial sequence of the flab gene which encoding the periplasmic flagellin filamentous core. This gene segment has been used to characterize multiple genospecies of Borrelia and is often instrumental in creating Borrelia phylogenic trees. For Bartonella, the 16S-23S rRNA intergenic transcribed spacer (ITS) region was amplified. The objectives of this study are to gain a better understanding of B. burgdorferi and Bartonella reservoirs in northcentral Florida and gain a better understanding of the impact of B. burgdorferi and Bartonella in Florida ecosystems and possible impacts this may hold on human cases.

41. PRIMARY CELL CULTURES FROM ASIAN CITRUS PSYLLID (DIAPHORINA CITRI KUWAYAMA)

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The Asian citrus psyllid (ACP), Diaphorina citri Kuwayama (Hemiptera: Psyllidae), is a global pest of citrus of significant importance in Florida and elsewhere in the US. In addition to causing feeding damage, ACP vectors a phloem-limited bacterium Candidatus Liberibacter asiaticus (CLas) that causes the fatal citrus disease termed citrus greening (a.k.a. Huanglongbing). CLas is acquired when the psyllid feeds, replicates in ACP tissues and persists throughout the life of the insect. The study of CLas has been hampered by the lack of a tractable in vitro culture system. We hypothesize that CLas could be cultured in ACP cell lines, which are lacking despite multiple previous attempts. For the current study, having optimized methods for dissection of ACP embryos, we evaluated a range of insect cell culture media and media combinations for their ability to support ACP embryonic cell growth in vitro. Of 10 media tested, all but

one supported initial cell attachment, but only two supported the long-term survival and division of ACP embryonic cells, resulting in six ACP primary cultures. These primary cultures are being maintained for the potential generation of continuous cell lines that would facilitate the study of both CLas and of viruses that infect ACP.

42. PARTIAL VALIDATION OF A TAQMAN REVERSE TRANSCRIPTION-QUANTITATIVE PCR ASSAY FOR THE DETECTION OF TURTLE FRASERVIRUS 1

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Florida is home to a wide variety of chelonian species, many of which are listed as threatened or critically endangered. Management of diseases affecting these species is vital for their conservation. A novel virus (i.e., Turtle fraservirus 1, TFV-1) isolated from diseased freshwater turtles from a Florida farm in 2007 and free-ranging populations of Florida softshell turtles (Apalone ferox), Florida red-bellied cooters (Pseudemys nelsoni), and peninsula cooters (Pseudemys peninsularis) in 2019. Ultrastructurally, viral particles were round to pleomorphic and acquired an envelope with prominent surface projections by budding from the cell membrane. Phylogenetic and genetic analyses showed that the TFV-1 is highly diverged from all known negative-sense RNA viruses and forms a deep branch within the phylum Negarnaviricota, which is not affiliated with any known group of viruses, even at the class level. This study sought to develop a Reverse Transcriptase quantitative Polymerase Chain Reaction (RT-qPCR) for the rapid and economical diagnosis of TFV-1 in farmed and free-ranging freshwater turtle populations. We developed a RT-qPCR assay for the detection of TFV-1 which targeted the virus's RdRp region. In silico primer and probe design returned forward and reverse primers and probe sequences with no mismatches compared to TFV-1 isolate 2019. The RT-qPCR assay was efficient (mean ± SD= 99.95 ± 1.82%) and the limit of detection (analytic sensitivity) was determined to be 102 viral copies. Analysis of 130 cloacal swabs from free-ranging turtles revealed 15 positive samples among three different species and 115 negative samples. The developed RT-qPCR assay allows for the rapid and

economical diagnosis of TFV-1. This partially validated assay can be integrated into surveillance programs aiming to mitigate TFV-1 and better understand the transmission, distribution, and prevalence of this virus among managed and free-ranging turtle populations.

43. ASSESSING THE IMPACTS OF HABITAT ON MOSQUITO DIVERSITY IN SELECTED AREAS IN NORTH-CENTRAL FLORIDA

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Introduction: Understanding the habitat use of mosquito species allows for more nuanced options of control, and can assess the risk of vector borne disease based on the knowledge of what species are supported by a certain habitat. Between three general habitat types represented in Gainesville and the surrounding area, we have performed surveillance to take inventory of the mosquito community compositions across this gradient. We expect to see a higher total abundance in forested sites, a higher diversity in residential sites, and more anthropophilic mosquito species in urban sites.

Methods: Monthly surveillance has been performed at each sites among our habitat types. Every site is visited within the same sampling event, where a CDC Light Trap, CDC Gravid Trap, and BG-Sentinel 2 have been used, baited with dry ice.

Results: Based on the current data, the diversity metrics have been calculated for each habitat type - Species richness, Shannon Diversity

Index, Simpson Diversity Index, as well as total abundance at each. The highest total abundance is found in forested sites, while the greatest species richness and diversity indices are found in residential sites. The relative abundances of species compositions are also reported, as well as total abundance ranked by species captured.

Conclusions: Our hypotheses are supported by our data. Future work planned with this system is to screen mosquito species known to be vectors of West Nile virus for an insect specific virus, Culex flavivirus, that is known to modulate West Nile virus within the mosquito, and investigating the relationship of habitat with the viral prevalence.

44. REVERSE TRANSCRIPTION LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (RT-LAMP) ASSAY FOR RAPID DETECTION OF MAYARO VIRUS

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Mayaro virus (MAYV) is a member of the genus Alphavirus that is transmitted by mosquitoes. Human infections with MAYV result in an acute febrile illness and with some symptoms similar to those due to infections by the alphavirus Chikungunya virus (CHKV), and flaviviruses Dengue virus (DENV) and Zika virus (ZIKV), making it difficult to differentiate MAYV infection from those caused by the other three viruses. Epidemiological data show that MAYV is an emerging virus in the Americas, suggesting an immediate need to develop effective diagnostic techniques for its detection. Up to now, serological tests such as ELISA and molecular techniques such as reverse-transcript polymerase chain reaction technique (RT-PCR) have been the common techniques used for detection of MAYV. These methods are time-consuming and must be performed by experienced staff using costly instruments. Additionally, serology methods are not useful during the early phase of infection, when

IgM and IgG antibodies have not yet reached detectable levels. Loop-mediated isothermal amplification (LAMP) has proven to be a rapid and sensitive technique for effective diagnostic of various virus infections. We developed an RT-LAMP assay for the detection of MAYV genomic RNA. The conserved region of the non-structural protein (NS1) gene of the MAYV genome was targeted for primer design. A set of six primers were designed using Primer Explorer V5. We then evaluated the real-time amplification of our MAYV genome target using our RT-LAMP assay. The specificity of our RT-LAMP assay has also been evaluated for three different flaviviruses: Dengue-1, Dengue-4, and Zika viruses. Our amplification curve reveals that the assay enables detection of MAYV genomic RNA withing 25 minutes, with 450 genome equivalents per reaction. Our studies indicate that there is no cross-reactivity of the MAYV RT-LAMP assay for the three flaviviruses we tested. We will next evaluate specificity of the MAYV assay for CHIKV. Finally, we employed our previously developed point-of-care (POC) diagnostic device integrated with the newly developed RT-LAMP assay for MAYV detection. The device integrated with RT-LAMP assay showed a potential to help address the challenges associated with infectious diseases outbreaks from MAYV.

45. MOLECULAR PROFILING OF THE INSECT-SPECIFIC VIRUSES IN URBAN AND RURAL AEDES AEGYPTI FROM PUERTO RICO

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Aedes aegypti mosquito is the main vector of dengue virus, which has a huge public health burden with over 40% of the world's population at risk of infection. With no human treatment or vaccines available, mosquito control remains the primary approach to prevent the spread of this mosquito-borne disease (MBD). A mosquito's biology and its microbiome can influence its vector competence (their ability to transmit pathogens). A novel approach is the study of insect-specific viruses (ISV), which are a group of viruses restricted to insects based on their inability to replicate in vertebrate cells. Isolated ISV's from the mosquito microbiome have shown the capacity to modify vector competence by enhancing or suppressing the transmission of viruses of medical importance. Natural acquisition transmission has been associated with transovarial (vertical) transmission from mother to offspring and venereal transmission (from male to female), however, it is unclear how mosquito ecology, spatial partitioning, and habitat can shape the mosquito virome and its impact on vector competence. This study will conduct an RNA-sequencing metagenomic analysis of field-caught Aedes aegypti from San Juan and Patillas, Puerto Rico, a dengue-endemic island, with the highest incidence of dengue in the entire U. S. We will compare the virome of individual Aedes aegypti mosquitoes from San Juan (urban, high dengue incidence) and Patillas (rural, low dengue incidence), evaluating the richness and diversity of ISV profiles between these geographically segregated mosquito populations. We hypothesize that ISV profiles will be characterized by their spatial partitioning, with individuals from rural habitats having a more diverse ISV population than urban individuals. This is the first study of an RNA-metagenomic screen of Aedes aegypti in

Puerto Rico. Our results are expected to provide better insights into the role ISVs play in diverse mosquito communities. This study provides foundational data on the characterization of the mosquito microbiome, which is necessary for the development of novel methods for the control and prevention of MBD transmission.

46. PLANT ESSENTIAL OILS ENHANCE THE TOXICITY AND KNOCKDOWN OF PERMETHRIN IN AEDES AEGYPTI

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As resistance to current insecticidal treatments continue to develop throughout the world, the development of new insecticides and insecticide synergists is critical to protect public health from mosquito-borne diseases. To identify new pest control technologies, we screened 21 different plant essential oils topically at 2 ug/insect and 10 ug/insect to determine their baseline toxicity and ability to synergize permethrin on a pyrethroid-susceptible strain of Aedes aegypti. We assessed knockdown at 1-hr and mortality at 24-hrs post-application. At 10 ug/insect, 6 of the 21 tested oils produced knockdown of > 50%. Patchouli oil at 10 ug/insect produced the highest 1-hr knockdown (100%). At 10 ug/insect, we found that 4 of the 21 oils tested produced mortality of > 50%. Calamus oil applied at 10 µg/insect produced the highest 24-hr mortality (96.6%). Plant essential oils were then applied at 2 ug/insect and 10 ug/insect in combination with an LD25 of permethrin (0.4 ng/insect). A co-toxicity factor as defined by Mansour et al. (J. Econ. Entomol. 1966, 59: 307–311) was used to as a benchmark to define whether plant essential oils had an additive effect, synergized, or antagonized permethrin activity. It is calculated from the following equation: (co-toxicity factor) = ((observed mortality – expected mortality)/(expected mortality)) X 100. For this analysis, values > 20 represent synergistic mixtures, values > -20 but ≤ 20 represent additive mixtures, and values < −20 represent mixtures that are

antagonistic. We found that 9 of 20 plant essential oils displayed synergistic effects when used in combination with permethrin. The oils with the highest co-toxicities were Juniper oil and Cascarilla oil. Juniper oil at 10 ug/insect produced a knock down co-toxicity of 338 and Cascarilla oil at 10 ug/insect produced a mortality co-toxicity of 227. Juniper oil however had the largest antagonistic effect on mortalities, producing a co-toxicity factor of -50 at 10 ug/insect. Overall, this study shows the potential for plant essential oils to augment the efficacy of current, readily available insecticidal products on the market.

47. MULTIPLE INTRODUCTIONS IN FLORIDA OF ZIKA VIRUS FROM THE CARIBBEAN AND CENTRAL AMERICA

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Introduction: In 2016, the Zika virus (ZIKV) gained traction in South America with infections being linked to microcephaly and other serious neurological disorders. In July 2016, the United States reported mosquito-borne transmission of ZIKV, and since its introduction, Florida has observed hundreds of locally acquired infections. In order to have better understanding of the local transmission reported in Florida, we analyzed the likely routes of the introduction of ZIKV into Florida and the source of their transmission. Recent studies examining local transmission in Florida have been very limited up to this point, due to the small number of samples collected (Grubaugh et al. 2017). By performing phylodynamic

analyses on a larger number of ZIKV isolates, we have a better insight on the origin, spread, and evolutionary dynamics of locally acquired infections reported during 2016 and 2017 in Florida.

Methods: We sequenced 25 locally acquired whole genome ZIKV isolates and 38 travel-related isolates collected in Florida by the FDOH. Then 455 whole genome ZIKV isolates collected from 2016-2017 were downloaded from NCBI's Genbank database. In order to analyze the introduction of ZIKV into Florida, we separated the isolates into different clades based on their clustering from a maximum likelihood (ML) phylogeny containing all collected ZIKV isolates. Bayesian phylogeography and ML analysis was performed on each of the five clades to determine the most recent common ancestor (tMRCA) and the approximate date when ZIKV was introduced into Florida.

Results: Our results from the phylodynamic analysis suggests multiple introductions of ZIKV into Florida from the Caribbean and Central America. We estimate the tMRCA of ZIKV's introduction into Florida could be as early as June 2015. The Dominican Republic may potentially be a major contributor for the introduction and subsequent local transmission observed in Florida. Honduras and Nicaragua also display a potential contribution to the local transmission in Florida, due to locally acquired isolates clustering closely with travel related isolates from Honduras and Nicaragua.

Conclusion: Since Florida is most likely unable to sustain long-term local transmission of ZIKV, outbreaks would be dependent on introductions from other locations. Although introductions into Florida from the Caribbean have been established, the role of Central America should also be considered as potentially driving local transmission in Florida. Introductions from Central America and the Caribbean were essential for local transmission occurring in Florida. Travel data should be collected and analyzed to further investigate the impact introductions have on local transmission of the ZIKV in Florida.

48. CHARACTERIZING THE SPATIOTEMPORAL DISTRIBUTION OF WEST NILE VIRUS TRANSMISSION IN FLORIDA USING ROUTINE MOSQUITO CONTROL AND PUBLIC HEALTH SURVEILLANCE DATA

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Introduction: West Nile virus (WNV) has been persistently transmitted in Florida since 2001, causing negative impacts on veterinary and public health. Culex nigripalpus and Culex quinquefasciatus mosquitoes are considered the main WNV vectors in the southeastern United States. WNV maintenance occurs across multiple avian species, and both vector species are opportunistic feeders and contribute to the potential for spillover events in equines and humans. The objective of this study is to characterize spatiotemporal distributions of WNV from 2015 to 2020, as a first step toward identifying possible environmental drivers of observed patterns, while providing new information for mosquito control and public health agencies.

Methods: In order to increase WNV and other arbovirus surveillance, in 1978 the Florida Department of Health (FDOH) and the Florida Mosquito Control Districts (FMCD) started a weekly arboviral testing program using sentinel chickens throughout Florida. This data collection has been reported in inconsistent format, which makes deliverability difficult. Various optical character recognition (OCR) technologies were used, as well as R and OpenRefine softwares to pull this data together into one

single format for further analysis . In this study, a total of six years are studied across all 46 Florida mosquito control districts currently participating in the arboviral surveillance program. We calculated the normalized weighted proportions of WNV sentinel chicken seroconversions to compare rates across all the participating counties in Florida and by region over the 6 year study period.

Results: Central and Northern Florida showed the highest sentinel chicken seroconversions throughout the study period. Seasonal distributions indicated high seroconversion across multiple geographic areas between the months of July and November. Interannual variation in transmission across the study period demonstrated higher seroconversion in 2015, 2018, and 2019, but varied across regions. County-level maps demonstrated variability in seroprevalence across the study period and across the state, with high WNV seroprevalence occurring in different counties each year.

Conclusions: Such evident results can help the FDOH and the FMCD to make informed decisions on further management strategies for integrated pest management (IPM) and potentially achieve better stakeholder engagement. This study demonstrates the need for additional sampling in districts without sentinel programs to determine WNV transmission risk.

49. BACTERIAL PATHOGENS ISOLATED FROM FLORIDA FARMED WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) DURING 2016 - 2021

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White-tailed deer (Odocoileus virginianus) farming is an emerging agricultural industry in Florida. Bacterial infections cause high mortality in fawn and yearling deer and are a source of significant production loss among Florida deer farms. Bacterial infection remained the most frequent cause of death among young farmed white-tailed deer aged 0-3 months from 2017 to 2021. The University of Florida Cervidae Health Research Initiative (CHeRI) provides a diagnostic service to Florida deer farmers to determine and monitor the proportion of animals that have died from bacterial infections, hemorrhagic disease-causing viruses, or other causes of death. From 2016 to 2021, 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 aerobic or anaerobic culture. Escherichia coli was the most frequently isolated bacteria in farmed deer from 2016 to 2021. Trueperella spp. remained the second most frequently isolated bacterial pathogen, with the percentage of cases increasing since 2018. Escherichia coli, Trueperella spp., Streptococci spp., and Pseudomonas spp. were identified as the most common bacterial pathogens isolated from necropsied farmed white-tailed deer. July to September is the peak of bacterial infection,

coinciding with the fawn season. Most bacterial pathogens have similar prevalence throughout Florida, while Trueperella spp. showed much higher prevalence in north Florida. Fawns (1-90 days) and yearlings (13+ months) have higher prevalence of Escherichia coli and Trueperella spp. infection than weaning deer (4-12 months). These data provide valuable information to improve preventive measurements and treatment in Florida farmed white-tailed deer, improving herd health, and reducing mortalities. Future works are aimed to identify the serotypes and possible antimicrobial susceptibility of the isolated bacterial pathogens.

50. AN ATYPICAL PRESENTATION OF CAT SCRATCH DISEASE IN A 3-YEAR-OLD CHILD

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Introduction: Bartonella henselae infection usually causes cat scratch disease (CSD), a mild disease presenting with a regional lymphadenopathy, fever, headache and malaise after a skin scratch or bite by a cat. We present a case of CSD complicated by skull base osteomyelitis and multiple central nervous system venous thrombosis.

Method: medical record review.

Results: A 3-year-old healthy female was admitted with left occipital pain, fever, headaches, photophobia and emesis 2 weeks after being scratched by a cat on her right cheek. Physical examination showed the presence of a right submandibular tender lymph node but otherwise it was within the normal limits, including neurological examination. Due to the history of a facial cat scratch, B. henselae serology was ordered and was positive: IgG > 1:1024 (NV: < 1:16) and IgM: 1:128 (NV: < 1:16). A computed tomography (CT) scans of head and neck revealed the presence of right submandibular lymphadenitis, left sigmoid and distal transverse sinus thrombosis extending to the left jugular vein and phlegmon on the retropharyngeal space. Patient was admitted to the PICU. A brain MRI/MRV confirmed the findings of the CT (Figure 1a) and showed the

presence of osteomyelitis of the left occipital bone and clivus without lytic lesions with regional suppurative lymphadenitis (Figure 1c). Patient was started on IV Rifampin, Ceftriaxone and Trimethoprim-sulfamethoxazole. Given elevated fibrinogen, PT, INR) and radiological evidence of thrombosis, continuous heparin effusion was started. After clinical improvement, patient was discharged home on day# 12 after admission on IV Rifampin and Trimethoprim-sulfamethoxazole, and subcutaneous Enoxaparin. Follow-up brain MRV on day # 38 of therapy revealed resolution of the left sigmoid and lateral sinuses thrombosis, partial resolution of the jugular thrombosis and unchanged left occipital bone and clivus osteomyelitis. Patient remains asymptomatic.

Conclusions: Infection caused by Bartonella spp. is an emergent infectious diseases. Neurological manifestations of Bartonella infection may include headaches, photophobia, venous sinus thrombosis and skull base osteomyelitis. Osteomyelitis may develop in a different site of the lymphadenitis, possible due to hematogenous dissemination of the bacteria.

51. IDENTIFYING RESIDUES OF THE FRANCISELLA TYPE VI SECRETION SYSTEM SHEATH THAT ARE REQUIRED FOR VIRULENCE

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Francisella are highly pathogenic intracellular bacteria that require the type VI secretion system (T6SS) for virulence. The T6SS is a contractile toxin secretion apparatus that is activated upon Francisella entry into host cells. Although the Francisella T6SS is absolutely required for virulence and has been an active area of investigation for over 15 years, the mechanisms that drive toxin secretion remain elusive. The contractile portion of the T6SS apparatus, known as the sheath, is essential for activity and comprised of two proteins, IglA and IglB. Blocking the interaction of IglA with IglB abrogates sheath formation and renders Francisella avirulent. The goal of this project is to identify residues in the sheath proteins that are required for heterodimer formation, T6SS activity, and virulence. To measure sheath assembly, we developed a split Renilla luciferase reporter by genetically linking two catalytically inactive fragments of the luciferase gene to iglA and iglB. Interaction of IglA with IglB leads to close physical proximity of the luciferase fragments, which reconstitutes a measurable, luminescent enzymatic activity. We inserted the N-terminal and C-terminal luciferase fragments into the Francisella novicida genome in-frame with iglA and iglB to generate all eight possible permutations of the split reporter. Upon measuring the luminescence of the reporter strains, we discovered four genotypes that resulted in robust luciferase activity. By utilizing these four reporter strains and comparing the Francisella sheath proteins to homologs in other bacteria, we identified IglA residues that are required for interaction with IglB. Specifically, the mutation of valines at positions 105 and 109 in IglA to tryptophan produced steric hinderance that blocked interaction with IglB.

The intramacrophage growth of this mutant was significantly attenuated, suggesting that these residues are responsible for sheath formation, T6SS activity, and virulence. These studies have enlightened our understanding of the mechanisms that drive T6SS activity. In the future, we will utilize these data and tools to identify genetic regulators of T6SS sheath assembly and small molecule inhibitors of virulence.

52. CHIRONOMID AS A NEW MODEL FOR VIBRIO CHOLERAE COLONIZATION IN AQUATIC INVERTEBRATES

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Cholera has been a human scourge since the early 1800s and remains a global public health challenge, which is caused by the toxigenic strains of the bacterium Vibrio cholerae. In its aquatic reservoirs, V. cholerae has been shown to live in association with various arthropod hosts, including the chironomids, a diverse insect family commonly found in wet and semi-wet habitats. The association between V. cholerae and chironomids is believed to be beneficial as the chironomid host could shield the bacterium from environmental stressors. But how the interactions between V. cholerae and chironomids determine the bacterium's persistence, evolution, and disease transmission is largely unknown. In this research, we use freshwater microcosms to test how different V. cholerae strains persist in the chironomid larvae and the effects of V. cholerae exposure on chironomid survival and the microbiome composition. Our results show that V. cholerae colonization in the chironomids varies by V. cholerae strain and concentration, with some strains more superior than others to persist in the larvae. V. cholerae infection appears to be non-pathogenic to the chironomid larvae, except at a very high concentration (10^8 cell per microliter). Interestingly, the presence of chironomid causes a more rapid decline in CFU in the water of some V. cholerae strains, suggestive of antagonistic interactions potentially between the pathogen and the chironomid microbiome. This is supported by our 16S rDNA amplicon sequencing data: during the

exposure to V. cholerae, the diversity of bacterial community in chironomid was reduced and a strain variation was involved in this process. Together, our results shed light on the importance of different bacterial and host factors in Vibrio Cholerae-chironomid-interaction.

53. PLASMID-MEDIATED DISSEMINATION OF NEW DELHI METALLO-B-LACTAMASE (NDM)-ENCODING KLEBSIELLA PNEUMONIAE BETWEEN HOSPITALIZED PATIENTS AND FERAL SWINE

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NDM-producing Enterobacteriaceae pose a great threat to public health globally. NDM-1 hydrolyzes beta-lactam antibiotics, including penicillins, cephalosporins, and carbapenems. NDM-producing Enterobacteriaceae are mainly associated with nosocomial infections but have also been isolated from the environments and animals. However, data on NDM-encoding microorganisms isolated from wildlife are limited. Recently, we reported one strain carrying the NDM-1 gene in Klebsiella pneumoniae, KCJ2K2161, isolated from feral swine. To understand the genomic features of KCJ2K2161, we conducted whole-genome sequencing and compared its genome to 16 human and three chicken K. pneumoniae isolates that encode the NDM-1 gene. Four strains (KCJK2161, KCJ3K292, KCJ3K293, and KCJ3K307) had similar chromosomal backbones (size 4.6 Mb) with a large fragment of indels (0.4 Mb). Genes related to antibiotic resistance (kpnE, kpnF, and ompK37) and virulence factors (pspA, and pspC), were not identified in KCJ2K2161, suggesting that these genes may be related host adaptation and selection in humans. However, plasmid pKC149K encoding the NDM-1 gene reported in three human isolates was

found in KCJ2K2161, indicating that plasmid transformation led to the dissemination of the NDM-1 into wildlife.

54. NEISSERIA GONORRHOEAE DRIVES CHLAMYDIA TRACHOMATIS INTO AN ABERRANT STATE DURING IN VITRO CO-INFECTION

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Chlamydia and gonorrhea are the top two most prevalent bacterial sexually transmitted infections in the world, caused by Chlamydia trachomatis (Ct) and Neisseria gonorrhoeae (Ng) respectively. Together they are a major public health concern and health system burden. In addition to single infections, these two bacterial pathogens are reported to frequently co-infect the same individual. Ct is an obligate intracellular pathogen with a unique biphasic developmental cycle, oscillating between infectious elementary bodies (EB) and replicative reticulate bodies (RB). However, during periods of environmental stress, Ct can transition into a temporary aberrant state and remain viable within the host cell until the stressor is removed. In contrast, Ng is a facultative intracellular pathogen, potentially interacting with Ct from inside or outside the host cell during infection. In this study we aimed to investigate how these organisms respond when grown in in vitro co-culture and better understand how this may translate to human disease. When cervical epithelial cells are pre-infected with Ct, then subsequently infected with non-invasive Ng, Ct transitions into an aberrant state characterized by the appearance of enlarged abnormal bacteria. We counted both Ct and Ng and comparing titers between single and co-infections. Using qPCR, we saw that Ct continued to replicate its genome whether grown with or without Ng, while the number of EBs decreased in co-infected cells. Ng titers remained equivalent between conditions. When gentamycin was used to kill Ng, the aberrant Ct returned to a normal phenotype. Our data suggest that during co-infection, Ng creates an environmental stress which drives Ct into a dormant state until the

competing pathogen is removed. We will examine if the stress factor is limited to live Ng and also examine bacterial gene expression data to identify the triggering factor in Ng causing this induced aberrant state.

55. SPATIAL ANALYSIS OF HUMAN AND LIVESTOCK ANTHRAX IN DIEN BIEN PROVINCE, VIETNAM (2010-2019) AND THE SIGNIFICANCE OF ANTHRAX VACCINATION IN LIVESTOCK

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Anthrax is a serious zoonosis caused by Bacillus anthracis, which primarily affects wild herbivorous animals with spillover into humans. The disease occurs nearly worldwide but is poorly reported in Southeast Asian countries. In Vietnam, anthrax is underreported, and little is known about its temporal and spatial distributions. This paper examines the spatio-temporal distribution and epidemiological characteristics of human and livestock anthrax from Dien Bien province, Vietnam from 2010 to 2019. We also define the role of livestock vaccination in reducing human cases. Historical anthrax data were collected by local human and animal health sectors in the province. Spatial rate smoothing and spatial clustering analysis, using Local Moran's I in GeoDa and Poisson model Space-time scan statistic in SaTScan, were employed to address these objectives. We found temporal and spatial overlap of anthrax incidence in humans and livestock with hotspots of human anthrax in the east. We identified three significant space-time clusters of human anthrax persisting from 2010 to 2014 in the east and southeast, each with high relative risk. Most of the

human cases were male (69%), aged 15-59 years (80%), involved in processing, slaughtering, or eating meat of sick or dead livestock (96.9%) but environmental and unknown exposure were also reported. Animal reports were limited compared to humans and at coarser spatial scale, but in areas with human case clusters. In years when livestock vaccination was high (>~25%), human incidence was reduced, with the opposite effect when vaccine rates dropped. This indicates livestock vaccination campaigns reduce anthrax burden in both humans and livestock in Vietnam, though livestock surveillance needs immediate improvement. These findings suggest further investigation and measures to strengthen the surveillance of human and animal anthrax for other provinces of Vietnam, as well as in other countries with similar disease context.

56. INABILITY TO SYNTHESIZE ANTHROSE LEADS TO PATHOPHYSIOLOGICAL CHANGES IN BACILLUS ANTHRACIS

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The Bacillus anthracis exosporium nap is the outermost portion of spore that interacts with the environment and host systems. Modification of this layer has the potential to impact wide-ranging physiological and immunological processes. The unique sugar anthrose normally coats the exosporium nap at its most distal points. We had previously identified several new mechanisms that render B. anthracis anthrose negative. In this work, several new B. anthracis strains that are ant - have been identified and the impact of anthrose negativity on spore physiology is

investigated. Lack of anthrose was previously limited to a sub-group of unique B. anthracis isolated in Chad, Mali, Cameroon and Nigeria that had been aptly dubbed the West Africa Group. These strains have a conserved SNP and nucleotide triplication event that renders them ant -. Our previous work found two strains of B. anthracis, one from Chile and another from Poland, in our own collection were genetically ant - via chromosomal deletions that encompassed the entirety of the anthrose biosynthetic operon [1,4]. A search of publicly available sequence records indicated B. anthracis strain Ba4599 Heroin, which was isolated from a European anthrax case linked to spore-contaminated heroin, had a novel SNP linked to the ant- genotype. These three observations expanded the mechanisms and geographic spread of anthrose negative strains beyond the original WAG observations placing more urgency on understanding their geographic origins and results of spore anthrose loss. We have continued to analyze anthrose negativity from an epidemiological perspective. By taking a closer look at recently deposited next generation sequencing files we are able to, again, greatly expand our knowledge of the geographic spread of anthrose negative strains and associate them with outbreaks of public health importance. We demonstrate that live-attenuated Sterne vaccines as well as culture filtrate anthrax vaccines generate antibodies that target non-protein components of the spore. The role of anthrose as a vegetative B. anthracis Sterne signaling molecule is implicated by luminescent expression strain assays, RNA-seq experiments, and toxin secretion analysis by western blot. Lastly, naturally ant – B. anthracis from Nigeria were genetically complemented to anthrose positivity using the Sterne anthrose biosynthetic operon to understand the consequence of adding anthrose back to an anthrose negative strain. PA and LF secretion levels were characterized and virulence in the G. mellonella model of infection were performed.

57. ACQUISITION OF TYPE III SECRETION SYSTEM BY V. CHOLERAE O1 STRAIN IN HAITI

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Toxigenic Vibrio cholerae strains are responsible for severe secretory diarrheal disease cholera and cholera remains a major public health threat particularly in poor and developing countries lacking safe drinking water, optimal sanitation, and hygiene. Although the virulence mechanisms of toxigenic V. cholerae serogroup O1 strains are well understood, the role of non-toxigenic V. cholerae O1 strains frequently circulating in cholera endemic countries is poorly defined and/or understood. Type III secretion system (T3SS) present in many Gram-negative bacterial pathogens, including many Vibrio spp encodes important virulence factor as demonstrated by many studies. However, V. cholerae O1 serogroup has never reported to have T3SS. During our ongoing environmental (surface water) monitoring of the presence of V. cholerae O1 serogroups in Haiti, we have recently isolated a nontoxigenic O1 strain encompassing a T3SS system. Whole genome sequencing (WGS) and sequence analysis of the non-toxigenic O1 strain revealed that this isolate has acquired ~42 kbp T3SS island incorporated between IS3 like transposons in the second chromosome of the genome. Comparative sequence analysis between our T3SS and Gen Bank sequences suggests that entire T3SS island of this newly isolated strain is highly similar to

T3SS2 of V. anguillarum PF4-E2-3 (CP031493.1). We anticipate that this Haitian non-toxigenic O1 strain acquired entire T3SS island from V. anguillarum through horizontal gene transfer event in Haiti. We are currently investigating if the T3SS of non-toxigenic O1 strain promotes virulence and/or environmental fitness and thereby justify the cost of acquiring this novel T3SS in non-toxigenic V. cholerae O1 strain.

58. SPATIO-TEMPORAL PATTERNS OF NATIONAL ANTHRAX IN VIETNAM, 1990 – 2015

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Anthrax is a priority zoonosis for control in Vietnam, but the geographic distribution of anthrax in the country remains to be defined, challenging our ability to identify target areas for control. Here we analyzed retrospective data (1990 – 2015) on human anthrax cases to obtain anthrax incidence at the national and provincial level. Nationally, results showed that the trendline for cases remained at approximately 61 cases per year throughout the 26 years of the study period. This indicates that control efforts are not effectively reducing disease burden over time. Most anthrax cases occurred in the Northern Midlands and Mountainous region, with the provinces of Lai Chau, Dien Bien, Lao Cai, Ha Giang, Cao Bang, and Son La experiencing some of the highest incidence rates. However, based on Spatial Bayes smoothed maps, every region of Vietnam experienced human anthrax cases over the study period. Understanding the geographic distribution of anthrax in Vietnam will allow us to better identify risk areas so that public health officers can

target those areas with educational campaigns and improved surveillance, apply better strategies for rapid clinical care, and run livestock vaccination campaigns.

59. PARASITIC BACTERIOPHAGES IN BURKHOLDRIA PSEUDOMALLEI GENOMES: A SUBSTANTIAL RESOURCE FOR PHAGE APPLICATIONS

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Introduction: Burkholderia pseudomallei is a Gram-negative bacterium residing in soil and fresh water and is the causative agent of melioidosis in both humans and animals in the tropics. Due to the development of antibiotic resistance and the cost of effective antibiotic treatments for up to 20 weeks, melioidosis is always considered untreatable in animals. In addition, the presence of B. pseudomallei in environments also complicates decontamination. Bacteriophage therapy is now being explored as an alternative treatment for this disease. Lysogenic or temperate phages are known as parasites of bacteria. They have mechanisms to incorporate their genomes into the host chromosome. This mechanism allows phages to take advantage of the bacterial host rather than demolish it.

Method: Here, we analyzed completed genomes of 131 Burkholderia pseudomallei species from GenBank database using bioinformatic tools, Linux local Blastn and PHASTER to identify the recombination sites of the phages. Then, the predicted phages were induced by UV exposure to exit the host.

Results: This method revealed 11 hot spots of phage recombination, most of which are associated with tRNA gene sequences. These recombination

sites, prophage islands, are conserved in terms of site-specific recombination sequences, integrase genes, and genes encoding structural proteins, while non-structural protein genes may vary. The use of the UV induction technique can release the predicted phages, which allows us to collect and analyze the phage genomes.

Conclusion: In this study we have found more than one hundred functional prophage islands which are inducible and provide a substantial resource for studying phage biology and for downstream applications, which may include phage engineering and therapy.

60. BEHAVIORAL PATTERNS LEAD METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS TRANSMISSION NETWORKS BETWEEN COMMUNITIES AND HOSPITALS: ATTESTATION TO THE BLURRING OF TRADITIONAL DEFINITIONS

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Introduction: Recent trends in methicillin-resistant Staphylococcus aureus (MRSA) infections have promoted uncertainty on transmission pathways, particularly among strains associated with nosocomial and community settings. To understand MRSA transmission networks, we performed longitudinal epidemiological and phylogenomic analyses on community and hospital-associated MRSA infections in North-Central Florida.

Method: From August 2015-January 2017, pediatric and adult patients presenting to UF Health Shands Emergency Department with acute skin and soft tissue infection (SSTI) were prospectively enrolled. Nasal and

SSTI specimens were collected, as well as patient-level geographic, social, and medical epidemiological data via self-reported survey. Hospital-based MRSA cultures collected as part of routine standard of care procedures were sampled before (2010), concurrently (2015-2017), and after (2019) prospective study period. Whole-genome sequencing (Illumina) was conducted on cultured S. aureus isolates. Multivariate analyses were used to test associations of geographic, medical, and social determinants of health with microbiological and molecular results. Following whole-genome assembly and alignment, a maximum likelihood tree was calculated using single nucleotide polymorphisms only. Transmission dynamics of MRSA within the community setting, and between the community and hospital facilities, were analyzed by Bayesian phylodynamic analysis.

Results: During prospective study period, 200 participants were enrolled where 182 (91%) subjects were included in the analysis. Of eligible subjects, 85 (46.7%) were female and 31(17.0%) reported living in rural communities. Fifty-three (29.1%) subjects had MRSA isolated from their SSTI. Kernel density estimation models identified high-density geographic clustering of community MRSA isolates. Subjects residing in rural census tracts and reported recent livestock exposure were 2.4 (95CI: 1.1-5.2, p=0.049) and 3.0 (95CI: 1.3-6.7, p=0.010) times more likely to have MRSA SSTI. Among community (n=42), pre-study hospital (n=6), concurrent hospital (n=13), and post-study hospital (n=18) MRSA isolates selected for whole-genome sequencing, 16 unique spa-types were identified where 57% (n=45) were spa-type t008. Phylogenetic analysis revealed two major clades distinguished by t008 and t002 spa-types, where 74% (31/42) community samples and 35% (13/37) hospital samples were t008. Phylogenetic analysis revealed multiple transmission patterns: lineages of nosocomial infections clustering with community isolates, as well as community infections clustering with hospital isolates.

Conclusions: In this longitudinal community assessment, MRSA transmission in hospital or community settings was not attributable to sustained strain-specific outbreaks but largely from distinct, previously unrecognized reintroductions in both settings. The non-uniform

geographic distribution of community MRSA isolates, as well as the identified epidemiological risk factors, suggest lifestyle and behavioral patterns may influence transmission in community settings more than traditional genotypic factors.

61. AN EMERGING ANTHRAX-LIKE PATHOGEN: TOXIGENIC BACILLUS CEREUS KILLS A KANGAROO IN SUMTER COUNTY, FL

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Bacillus anthracis, the classical anthrax bacterium, belongs to a species complex with Bacillus cereus; both are spore forming with environmental reservoirs. Most B. cereus strains are harmless, but a limited number are associated with food poisoning illness and other opportunistic diseases. Recently, B. cereus isolates harboring Bacillus anthracis toxin genes are known to cause anthrax-like disease in humans and animals. Here we present a case report and confirmation of an anthrax-like infection caused by B. cereus from a captive kangaroo living in a ranch setting from north central Florida. In August 2021, A healthy 1-year-old red kangaroo (Macropus rufus) with a history of progressive lethargy for one day was euthanized after ineffective treatment. Necropsy reports showed sepsis with interstitial pneumonia. Histopathological diagnosis revealed severe bacterial infection with massive diffusion of Gram-variable bacilli and occasional bacterial containing spores in several tissues. Bacterial culture

from a liver sample demonstrated colony morphology typical of B. anthracis. Genomic DNA extraction from pure culture was performed. PCR analysis specific to B. anthracis ruled out the species but confirmed the presence of lethal factor (lef), a B. anthracis pXO1 toxin gene. Whole genome sequencing revealed high similarity to B. cereus G9421 isolated from severe pneumonia in metal workers in Louisiana and Texas (in the 1990s) and BcFL2013 isolated from anthrax-like cutaneous lesion in a female Florida resident in 2013. BcFL2013 was the first report of the pathogen in Florida. The kangaroo isolate had 99.97% and 99.96% average nucleotide identity to G9241 and BcFL2013, respectively. In addition, the clinical isolate from the kangaroo harbors virulent plasmid pBCXO1 and pBC210 carrying several B. anthracis virulence genes. This is the first report confirming anthrax-like disease in an animal with a lef-positive B. cereus strain. The 2013 human case in 2013 was suspected as environmental exposure. Here we confirm lethal septicemia in a wildlife species from a similar part of Florida 9 years later from the same species of B. cereus and suspect an environmental transmission route. Since 2002, anthrax-causing B. cereus biovar anthracis, which carries both anthracis plasmids, has been associated with enzootic anthrax-like disease/death in primates in Africa, also associated with environmental transmission. Though we cannot determine the spatial distribution of this new pathogen in Florida or the US from available data, this strain of B. cereus should be considered an emerging anthrax-causing pathogen and a zoonosis present in Florida. Furthermore, veterinarians treating animals with unusual sepsis should include B. cereus in differential diagnosis when performing treatment and necropsy.

62. MEASURING BACILLUS ANTHRACIS REPLICATION AND PERSISTENCE ON ENVIRONMENTAL SUBSTRATES

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Anthrax is a zoonosis caused by the environmentally maintained, spore-forming bacterium, Bacillus anthracis, affecting humans, livestock, and wildlife nearly worldwide. Bacterial spores are ingested, inhaled, or may be mechanically transmitted by biting insects, or injected (human heroin-associated cases). Herbivorous hoofstock are most susceptible and serve to contaminate the environment after death. When these hosts die, a localized infectious zone (LIZ) is created at and surrounding the carcass where viable B. anthracis cells (likely mixed populations of vegetative cells and spores) are returned to the environment as the carcass is scavenged and decomposes. Necrophagous flies scavenge carcasses and contaminate the outer carcass, surrounding soils, and surfaces (leaves, sticks, rocks,) with viable pathogen. Field observations in Texas have confirmed this process and identified primary browse species (e.g. persimmon) are contaminated. However, those results suggest survival is less than 21 days. There are limited data available on B. anthracis survival on environmental substrates immediately following a host death at a LIZ. Toward this, we simulated fly contamination by inoculating untreated leaves and small rocks with 20 µl of bacteria in BHI broth measured viable spore and vegetative cell recovery at 0, 2, 5, 7 days for five strains. We found that live-attenuated, fully virulent laboratory-adapted and fully virulent wild strains (recovered from Texas LIZs) multiply for 2 or more

days post inoculation and persist on leaves and rocks for at least seven days with variation by strain. We found differences in sporulation rates between laboratory-adapted strains and wild isolates, with the live-attenuated strain sporulating fastest, followed by the wild isolates, then laboratory-adapted virulent strains. Replication outside of the carcass and rapid sporulation confirms the LIZ extends beyond the carcass for several days after formation and supports the necrophagous fly transmission pathway for amplifying cases during an outbreak (though individual fly emesis and fecal spots are smaller than our 20 µl inoculum). We note caution must be taken when extrapolating replication and sporulation rates from laboratory-adapted strains of B. anthracis.

63. DISEASE MITIGATION: NEW PARADIGM

Alain Michel

Mitigation fish diseases in aquaculture : proposal for a new paradigm. My objective with this post is to challenge pathologists, immunologists and farmers asking for a critical analysis on the application of a simple temperature manipulation, hyperthermia, to boost the fish innate immune system through the upregulation of the Heat shock proteins HSPs with chaperone and immunomodulatory roles functioning in networks . Many supporting data are available in recent scientific literature, and hyperthermia treatment is increasingly used also in human cancer therapy. Aquatic organisms are living in a world full of virus, bacteria and parasites some very harmful. In aquaculture the critical point is the production of fast growing and healthy juveniles in nursery systems. It means a domesticated broodstock under a selective breeding program and juveniles equipped to enter safely in the grow out systems. The present dominant paradigm is to consider the pathogens as devils and to do everything to eradicate them: pathogens are “persona non grata” in the ashore hatchery nursery systems. Strict biosecurity is the main strategy aiming at eradicating all the potential pathogens thanks to sophisticated water treatment, tight control of all the inputs and use of various functional feeds. Modern hatcheries are producing pathogen free juveniles equipped through vaccination against the main pathogens. Without vaccination they are naive and when they are encountering the local pathogens for the first time large outbreaks of mortalities are often occurring. The last 50 years intensive researches on vaccine development have been conducted and for a mature industry like salmon culture all the main pathogens have their vaccines, killed, attenuated, or recombinant. Oral vaccines are not common and juveniles have to be injected one by one mobilizing a lot of labour forces. Cost of developing and licensing new vaccines is high and for low market value species like catfish or tilapia they are often too expensive for commercial purposes. Live vaccines tend

to be more immunogenic in entering the host and stimulating cellular responses linked to both innate and adaptive immunity. What I am proposing is to use directly the pathogens as they are at the moment where they are invading the hosts to obtain the vaccination effect, not in reducing their virulence but in boosting the host innate immune system response which will stop immediately the mortality and will transfer to the adaptive immune system the memory of the first encounter. It can be done with each pathogen one by one but better with the mix of pathogens constitutive of the local pathobiome and giving a long term protection. Change in paradigm. Instead of a reductionist approach considering the pathogens as our enemies it should b bitter: - to live with them, recognizing they are an integral part of our environment and will always there. - to enroll them on our side to obtain the “live vaccination effect”. to recognize that their specific effect is mainly imbedded in their interrelations of all the components of the pathobiome with the host at a given moment and in a given environment. What I am proposing is to use an holistic approach considering the pathobiome as the main player attacking the fish and instead of weakening the pathogens let them with their full immunogenicity to get the best reaction of the host ending with a long term protection. This is immunotherapy in real-time and if evolution has conserved the heat shock protein response from bacterial to human cells as the first line of defense it is an evidence of their pivotal role. Practically it starts by a controlled infection of the juveniles using the pathobiome searched in cage environment, followed by non lethal heat shock treatments to end with immunization. This proposal of paradigm change is supported by all the results obtained the last 15 years mainly on fish pathogens (barramundi- tilapia-grouper-snapper) but some experiments have concerned invertebrates like shrimp and oysters. As each new innovative approach I know there will be three phases in the reaction: first « it is stupid » and I am eager to hear and discuss the arguments. Second « it is dangerous ». I am fully conscious that to infect voluntarily juveniles in nurseries is against all the politically correct of the biosecurity dogma. And third, I hope « it is evident » for the benefit of the farmers. For me biosecurity which is necessary means to have a well controlled husbandry no more. Keywords of underlying science are :

innate immune system-toll like receptors- chaperon heat shock proteins-interferon….. Results obtained so far by thermal manipulation: - Mitigation of VNN, Iridovirus ISKNV, SDD virus and herpesvirus in barramundi, groupers and other tropical species in Indonesia and Srilanka Mitigation of the emerging Iridovirus on tilapia in Ghana farms. Positive results at experimental scale for invertebrates: oysters and shrimps acquiring immunity ?

64. PREVALENCE OF NEOVAGINAL AND PENILE HUMAN PAPILLOMAVIRUS (HPV) AMONG TRANSGENDER WOMEN IN CENTRAL BRAZIL

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Introduction: Human papillomavirus (HPV) is the most common sexually transmitted infection (STI) worldwide. It causes genital warts and can also lead to cancer. Transgender women are at high risk for acquiring STIs such as HPV infection. In Brazil, gender-affirming surgery is available via the Brazilian Public Health System. However, data on HPV in this population is limited and there are no guidelines for HPV screening in neovagina among transgender women. The objective of this study was to estimate the prevalence of neovaginal and penile HPV DNA among transgender women in Central Brazil.

Method: A cross-sectional study was conducted between April 2018 and August 2019 among 207 transgender women in Goiânia - Brazil recruited using Respondent-Driven Sampling. Neovaginal and penile samples were self-collected with a sterile brush (HC2 DNA collection device - QIAGEN). Valid neovaginal (n=22) and penile (n=185) samples were tested for HPV by PCR using the SPF-10 primer. Also, HPV typing was performed for ten penile samples by reverse hybridization (INNO-LiPA HPV Genotyping Extra® - Fugirebio®)

Results: For the transgender women who reported gender-affirming surgery with valid samples the mean age was 40.0 years (SD=9.4), 4.5% were sex workers and 22.7% had HIV infection. For the transgender women who had male genitalia and valid samples, the mean age was 24.5 years (SD=5.1), 77.3% were sex workers and 33.0% had HIV infection. The prevalence for neovaginal HPV was 54.5% while the prevalence for penile HPV was 38.4% (p=0.144). Of the ten penile samples tested for specific HPV type, nine had high risk HPV (linked with the development of cancer) and multiple infections with high risk HPV were detected in three samples. The most common HPV subtypes were 52 (high risk;n=4) followed by HPV-16 (high risk;n=3), 58 (high risk;n=3), and 11 (low risk;n=3).

Conclusions: The HPV infection was more common among transgender women that reported gender-affirming surgery than among those who had male genitalia, however there is no statistically significant difference between the prevalence of neovaginal and penile HPV infection. Since the consequences of HPV infection in the neovagina are unclear, further studies should be conducted to characterize the types of HPV to help guide the development of public health policies for monitoring the incidence of HPV-associated neoplasms.

65. MENTAL HEALTH SYMPTOMS AND RISKY SEXUAL BEHAVIORS AMONG A COHORT OF WOMEN LIVING WITH HIV IN FLORIDA

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Introduction: Mental health symptoms impact health outcomes in women living with HIV (WLHIV). Risky sexual behaviors are also known to impact WLHIV's health outcomes. The relationship between mental health symptoms and risky sexual behaviors in WLHIV is poorly understood and merits further investigation. The purpose of this study was to investigate the associations between mental health symptoms with risky sexual behaviors in a cohort of WLHIV in Florida

Methods: A cross-sectional analysis was done on survey data collected from 2014-2017 in 317 adult WLHIV. Risky sexual behaviors were defined as self-report of one or more of the following: 1) at least one sexually transmitted infection (STI) diagnosis in the past 12 months, 2) two or more sexual partners in the past 12 months or 3) any inconsistent condom use in the setting of transactional sex, sex with a partner living with HIV, or sex with an unknown partner in the past 12 months. Mental health symptoms were assessed using the PHQ-8 to determine depressive symptoms and the GAD-7 for anxiety symptoms. A score of 10 or more for either scale was indicative of mental health symptoms. Descriptive statistics and bivariate analysis using chi-square analysis ($p< 0.05$) were done using SAS 9.4.

Results: Mean age was 48 years, 32% reported at least one or more risky sexual behavior,13% reported at least one STI, 15% reported two or more sexual partners, and 13% reported inconsistent condom use. Neither anxiety nor depression was significantly associated with self-reporting

one or more risky sexual behavior (p=0.16, p= 0.19 respectively). Neither anxiety nor depression was significantly associated with at least one STI (p= 0.13, p=0.40 respectively) or two or more sexual partners (p=0.54, p=0.40 respectively). However, inconsistent condom use was more common in women with anxiety symptoms vs. no anxiety symptoms (20% vs. 10%, p=0.01), and those with symptoms of depression vs. no symptoms of depression (18% vs. 10%, p=0.04).

Discussion: Anxiety and depression symptoms were each associated with inconsistent condom use. Future intervention studies should evaluate the impact of mental health interventions on condom use and condom use interventions for WLHIV with mental health symptoms.

66. ADHERENCE TO ANTIRETROVIRAL TREATMENT IS ASSOCIATED WITH MEDICATION-HIDING BEHAVIORS AMONG PEOPLE LIVING WITH HIV: EARLY RESULTS FROM THE FLORIDA COHORT STUDY

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Background: Antiretroviral therapy (ART) access is critical to ending the HIV epidemic. It is unknown whether medication-hiding behaviors to prevent others from discovering one's HIV status influence ART adherence. The purpose of this study is to describe medication-hiding behaviors and factors associated with these behaviors among a cohort of people living with HIV (PLWH) and to assess whether medication hiding is associated with ART adherence or running out of medication.

Methods: The ongoing Florida Cohort Study has been enrolling local adult PLWH since October 2020. In this analysis, participants (n=309, mean age 50, 56% male, 44% Non-Hispanic Black, 16% Hispanic) were asked about having at least 90% ART adherence in the preceding 30 days and running out of ART medications for more than 24 hours in the past year. Participants were also asked if they engaged in at least one of five medication-hiding behaviors to prevent others from finding out their HIV status in the past year.

Results: Thirty percent of the sample had less than 90% ART adherence and 14% ran out of their medication. Many (44%) reported engaging in at least one behavior to hide their ART medications. The most common medication-hiding behaviors were hiding medication bottles (35%), removing prescription labels (25%), moving medications to another bottle (16%), changing where they got their medications (6%), and traveling at least 30 miles to obtain medications (4%). PLWH who were under 50 years old had greater odds of engaging in medication-hiding behaviors (OR=2.47, 95% CI=1.54-3.97, p=0.002).There was no association between medication-hiding behavior and running out of medication. However, PLWH who engaged in medication-hiding behaviors were more likely to have less than 90% ART adherence (OR=3.10, 95% CI=1.41-6.79, p=0.006)

Conclusions: Taking action to conceal HIV medications to hide one's HIV status was common. PLWH under 50 were more likely to engage in medication-hiding behaviors. Medication-hiding behaviors were associated with less than 90% ART adherence. These findings suggest that PLWH may want to receive ART medications in ways that ensure privacy. Further research is necessary to determine the impact of other behaviors to hide one's HIV status on ART adherence and health outcomes.

67. CHARACTERIZATION OF A NOVEL SUNSHINEVIRUS FROM A COLLECTION OF SNAKES IN FLORIDA

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The family Sunviridae includes the genus Sunshinevirus and a single species, Reptile sunshinevirus 1. The founding member of the family is an enveloped, negative-sense, single-stranded RNA virus with a linear genome of 17,187 base pairs (bp). The virus was isolated during an outbreak of neurorespiratory disease in a collection of Australian pythons along the Sunshine Coast of Australia. More recently, a second sunshinevirus species was characterized from moribund sidewinder rattlesnakes (Crotalus cerastes) that were part of a snake collection in the United States. Herein, we report a mortality investigation of venomous snakes at a commercial facility in Florida. The event resulted in the loss of approximately 70 captive snakes including at least four species in the family Viperidae. After PCR assays targeting typical snake viruses were negative, the RNA extracts from the tissues of affected snakes were used to generate cDNA libraries for sequencing on an Illumina MiSeq. The complete genome sequence of the Florida sunshinevirus is 17,164 bp and a total of 7 putative open reading frames were predicted. The phylogenetic analysis based on RNA dependent RNA polymerase (RdRP) protein revealed that the Florida sunshinevirus formed a well-supported clade with the sunshine coast virus. This data suggests that Florida sunshinevirus is likely to represent a separate species within the genus Sunshinevirus. A conventional RT-PCR targeting the RdRP gene of the Florida sunshinevirus was developed and confirmed all tested snakes (n =5) were positive. Future research including the development of in situ

hybridization and quantitative RT-PCR assays are needed to determine the tissue tropism, host range, and pathogenicity.

68. GENOME SEQUENCING OF A ROTAVIRUS A STRAIN FROM A DISEASED FLORIDA RACING PIGEON (COLUMBA LIVIA DOMESTICA)

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A 2-year-old Florida racing pigeon was submitted for necropsy to the state diagnostic laboratory. The gross pathology examination showed that the intestine was distended, flaccid, and congested. Frozen intestinal tissue was sent to the Wildlife and Aquatic Veterinary Disease Laboratory in Gainesville, FL, for viral discovery using a next-generation sequencing approach. RNA from the intestinal tissue was extracted using a RNeasy Mini Kit and served as the template for the generation of a cDNA sequencing library using a NEBNext Ultra Directional RNA Library Prep Kit. The library was sequenced using a v3 chemistry 600-cycle Kit on a MiSeq sequencer. A total of 2,955,154 paired-reads with an average read length of 240 bp were assembled de novo in SPAdes v3.13.0. The 5' end of the coding sequences of segments 2 and 4 were determined using a Rapid Amplification for cDNA End PCR Kit and Sanger sequencing. Gaps within segments 2 and 3 were closed by RT-PCR followed by Sanger sequencing. The total length of the complete coding sequences of the 11 RTA segments was 17,794 bp, with a G+C content of 34.98%. BLASTN analysis of the 11 complete coding sequences showed the highest nt identity (99.55-99.89%) to RVA/Pigeon-wt/USA/K1802315/2018/G18P[17]

isolated from a dead racing pigeon in California, genotype G18P[17]-I4-R4-C4-M4-A4-T4-N4-E19-H4. Using the Rotavirus Classification Tool, the complete genotype of the Florida racing pigeon RVA was determined to be G18P[17]-I4-R4-C4-M4-A4-T4-N4-E19-H4. Further study is needed to determine the distribution of pigeon RVA G18P[17] in Florida and its potential impact on the global industry.

69. PATHOBIOMIC SURVEY OF THE BAY SCALLOP (ARGOPECTEN IRRADIANS) FROM THE GULF COAST OF FLORIDA, USA

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The bay scallop Argopecten irradians once supported a commercial fishery in Florida but their populations declined and the fishery was closed in 1994. Despite evidence of further population decline, a recreational fishery remains along the Florida west coast from Gulf to Pasco County. Disease is increasingly recognized as a major threat to bivalve fisheries, so to advance our understanding of its impact on the bay scallop, we examined the relationship between their pathobiome, density, and geographic location across their fished range. The 'pathobiome' refers to an organism's pathogenic microbes, which cause disease. Three study sites were chosen within Florida's recreational scallop fishery: St. Joseph's Bay in the northern Gulf of Mexico, offshore of the Steinhatchee River in the Florida Big Bend, and offshore of Hernando County, Florida at the southern end of the fishery. Each site was visited prior to the opening of the fishery to survey the abundance and distribution of scallops, and to collect samples (n = 50) for the creation of pathogen profiles. To further investigate the effect of density on their pathobiomes, caged scallops (n = 150) were collected from cages stocked at varying densities in St. Joseph's Bay. Using a combination of traditional histological methods and molecular diagnostics, a suite of 15 pathogens were identified as part of the bay scallop pathobiome, across their geospatial range. Select samples collected in St. Joseph's Bay were examined using DNA and RNA metagenomics. From the RNA-seq data, four novel +ssRNA viral genomes were isolated: three picorna-like viruses

and one hepe-like virus. From the DNA-seq library, a novel Mycoplasma sp. was discovered. Additionally, histological evaluation revealed protozoan, helminth and crustacean infections were also common in A. irradians. Understanding the role these marine pathogens play is necessary to prevent ecological and economical losses pertaining to the bay scallop.

70. LACTATIONAL STATUS IS ASSOCIATED WITH CHANGES IN MATERNAL GUT MICROBIOME COMMUNITY AND COMPOSITION

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Background: Breastfeeding (BF) is associated with reduction in maternal risk of cardiometabolic diseases (CMD). We propose that lactation's physiological effects lead to gut microbiome modifications and, may in part, mediate the association between lactation and CMD.

Objective: To determine the influence of BF on gut microbiome diversity and composition in postpartum women during the first 6 months postpartum.

Study Design: Demographics, clinical data and maternal stool were analyzed longitudinally from 94 women in the first 6 months postpartum. We classified stool samples into 2 periods based on median sample collection timepoint (T1: <=130 days and T2: >130 days). Samples were divided into two BF groups. Exclusive breastfeeding (EBF) implies 100% BF (N=148) and non-EBF implies less than 100% BF (N=50). Gut microbes

were estimated using 16S rRNA gene amplicon sequencing. Data was analyzed using nf-core/ampliseq V1.1.3 pipeline. Beta diversity (between groups) was calculated. Alpha diversity (within samples) indices were analyzed for gut microbiota richness and diversity.

Results: We observed differences in the maternal diet ($p<0.001$) and BMI ($p=0.003$) according to lactational status and adjusted subsequent modeling for these covariates. We found significant differences in gut microbiota community (beta diversity) between the BF groups at T1 and T2. Linear mixed models showed that none of the covariates (lactational status, BMI, or diet) had a significant influence on gut microbiota alpha diversity or richness. Regarding microbiome composition, we found significant changes ($p<0.05$) in Enterobacteriaceae according to lactational status at T1. At T2, we found significant differences ($p<0.05$) in Prevotellaceae, Preveotella 9, and Ruminococcus 2 according to lactational status.

Conclusion: BF has a large influence on gut microbial community structure in lactating persons. The differentially abundant bacteria between BF groups, Prevotellaceae, Preveotella 9, and Ruminococcus 2, are associated with various cardiometabolic measures such as glucose tolerance, blood pressure, and BMI. Next steps are to assess the influence of microbial composition on postpartum cardiometabolic measures. BF offers a key preventative strategy and gut microbes may be a mediator between maternal BF status and CMD.

71. BIOACCUMULATION OF BREVETOXIN IN TWO FAMILIES OF FILTH FLY LARVAE

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Harmful algal blooms (HABs) are common events in the Gulf of Mexico that result in significant environmental and economic damages to the adjacent coastal states. The most common HAB in the Gulf of Mexico is the marine dinoflagellate, Karenia brevis. These are ubiquitously referred to as red tide events. Karenia brevis blooms are often responsible for mass mortality in marine and terrestrial fauna. There are two known mechanisms by which Karenia brevis blooms can cause mass mortality. Blooms of K. brevis often produce lethal anoxic conditions to marine life. More importantly, K. brevis produces a potent class of phycotoxins known as brevetoxins. Brevetoxins (PbTx) are highly lipophilic, polyether molecules. They have a high affinity for acceptor sites on the voltage-sensitive sodium channels (VSSC), and their binding causes hyperexcitation and depolarization of the membrane potential in vertebrate nerve and muscle. This biotoxin causes neurotoxic shellfish poisoning in humans from consuming brevetoxin-contaminated shellfish, as well as causing major respiratory illnesses as it is aerosolized at the shoreline. Manatees and dolphins can succumb to brevetoxin poisoning from ingesting contaminated seagrass or fish, respectively. The mass mortality events produced from these HABs attract many shoreline scavengers, and to data research on the effects of brevetoxins on terrestrial organisms has focused only on vertebrates. This study investigated brevetoxin exposure on one of the first major colonizers of carcasses, filth flies. Using a commercially available competitive Enzyme-Linked Immuno-Sorbant Assay (ELISA), larvae collected from two different

red tide events in St. Petersburg, Florida in August 2021 and October 2021 tested positive for brevetoxins. Larval concentrations of brevetoxin ranged from just below the oral LD50 of mice (i.e., 0.5 ng/kg) to four times the LD50 in mice. All larvae tested were positive for brevetoxins. This is the first study to confirm bioaccumulation of brevetoxins in invertebrates along the Gulf Coast during red tide events and it foreshadows potential trophic effects at the interface of the marine and terrestrial food webs.

72. GOING WITH THE FLOW: APPLICATION OF BAYESIAN BELIEF NETWORKS TO PRODUCE VALUE CHAINS IN SUB-SAHARAN AFRICA

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Bayesian Belief Networks (BBN) are effective tools in quantifying probabilistic relationships in food systems. They have the potential to enhance decision-making in areas where uncertainty and lack of data are key characteristics. BBNs accomplish this by defining the complex interdependences between variables and outcomes through probabilities. The work presented here describes a novel application of BBNs for the evaluation of food value chains (VC). Specifically, we developed models to (1) understand the flow of tomatoes through VCs in two African countries, and (2) evaluate tomato quality (intact vs. damaged) and food loss as tomatoes move through these VCs. In sub-Saharan Africa, lack of food security and safety are major contributors to poor health outcomes which are concentrated in marginalized communities, and children disproportionally carry this burden. We applied these models to data collected in Burkina Faso and Ethiopia to identify the most probable scenarios of tomato flow through VC actors and diagnose VC relationships that give rise to poor tomato quality and food loss. BBNs were developed with a user-defined structure and parameter estimates were based on VC assessments and quantitative surveys. Our preliminary results indicate that in Ethiopia, the probability of consumption of tomatoes was highest

at home (0.74), followed by informal restaurants (0.19), and formal restaurants (0.08). The probability that home consumed tomatoes originated from distant and local producers was, 0.73 and 0.27, respectively. Because the majority of tomatoes consumed in the region were produced in the Rift Valley, collectors and wholesalers played an important role in the flow of tomatoes. The probability that tomatoes flow through collectors and wholesalers was 0.40 and 0.68, respectively. We estimated that at the time tomatoes reached retailers, the probability of tomatoes being intact was only 0.66. Probabilities of tomatoes being damaged but still edible or damaged to such an extent that they were categorized as food loss was 0.22 and 0.12, respectively. In Burkina Faso, the majority of tomatoes were also consumed at home, but with a probability of 0.58. This was related to an increased share of informal restaurants (0.24) and formal restaurants (0.18). The probability of tomatoes consumed at home having originated from distant producers was 0.57, compared to 0.43 from local producers. Long-distance transport of tomatoes in Burkina Faso was observed less than in Ethiopia, hence the probability of tomatoes flowing through collectors was lower at 0.28. However, wholesalers did play a significant role in the VC with a probability of 0.62 of tomatoes flowing through this VC actor. Despite a shorter VC, the probability of a tomato entering retail and being intact was low at 0.54, while the fraction damaged was 0.25 and the fraction food loss was 0.21. Differences in loss of tomatoes in the countries' VCs can be attributed to harvesting practices, where tomatoes in Burkina Faso are harvested at a riper stage than in Ethiopia, and hence are more likely to be damaged. We conclude that BBNs can be utilized as tools for decision-making to target interventions in food VCs.

73. HISTOLOGICAL ASSESSMENT OF SYMBIONTS AND DISEASE AMONG FISHERY-CAPTURED FLORIDA STONE CRABS (MENIPPE MERCENARIA)

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The stone crab Menippe mercenaria fishery is regularly ranked in the top three fisheries in Florida, USA. It employs the practice of claw removal wherein claws are the only product harvested and live crabs are returned to the water where they can survive and regenerate claws. While studies suggest that only 20-40% of harvested animals survive the trauma of two claw harvest, and only 4-13% survive to regenerate their claws and re-enter the fishery, the effects of returning sublegal and harvested crabs on the spread of parasites and diseases must be explored. Very little is known about parasitism in this species. We therefore conducted parasite screening via histology and molecular diagnostics on n = 276 crabs collected at two locations in the Gulf of Mexico coast of Florida. We documented new parasite associations between M. mercenaria and 1) bacterial hepatopancreatic necrosis, 2) a putative microcell-like parasite in connective tissues, and 3) Mesomycetozoa on the gills. We are working to identify the microcell-like parasite via metagenomics and transmission electron microscopy. We also documented the apicomplexan gregarine Nematopsis sp. in the gut and Hematodinium perezi via histopathology and conventional PCR. Bacterial hepatopancreatic necrosis and H. perezi infection are potentially highly consequential for this species and should continue to be monitored.

74. VIRUS DISCOVERY IN THE SMALL HIVE BEETLE

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Introduction: The western honey bee (Apis mellifera) is a critical pollinator in both natural and managed habitats. Globally, the success of ~75% of crop species relies on insect pollinators.1 There has been a steady decline in managed honey bee populations in the US and elsewhere. The small hive beetle (SHB, Aethina tumida) is an invasive parasite of the honey bee. Though native to sub-Saharan Africa, this pest is now pervasive in nearly every continent. Due to limitations with current SHB management tools, new approaches for management are needed. Because of their close association with honey bees, SHB may vector honey bee viruses, such as Deformed wing virus, Israeli acute paralysis virus, and Sacbrood virus. Characterizing viruses in SHB is critical for understanding which viruses infect SHB and the potential role of SHB in honey bee virus transmission. Because viruses have been successfully employed previously to achieve long term control of pest beetle populations, it is possible they can be used for the SHB.3 Additionally, viruses would be ideal for this application because they can be highly specific to certain hosts, are stable in the hive setting, environmentally friendly, and are favorable for large-scale production.

Methods: SHB adults and larvae were collected from Illinois, Ohio, and Florida. RNA extracted from SHB was sequenced using Illumina sequencing. BLASTx was performed on contigs from the assembled transcriptome to search for viral sequences. Contigs were visualized in SnapGene.

Results: Sequences from several honey bee viruses were found in the Florida SHB samples, including Black queen cell virus, Acute bee paralysis virus, Deformed wing virus, Sacbrood virus, and Israeli acute paralysis

virus. However, no honey bee virus sequences were found in the Illinois and Ohio SHB samples. This is likely because the SHB from Illinois and Ohio were lab-reared and not exposed to virus-containing hive products, suggesting that honey bee viruses may not replicate in SHB. Sequences from a putative novel phasmavirus were also discovered (order: Bunyavirales). Their genome consists of a segmented, negative-sense single-stranded RNA. The entire S and M segments (encoding the nucleocapsid and glycoprotein, respectively) were represented in the transcriptome, while only fragments of the L segment (encoding RdRp) were assembled. Segment S RNAs were more abundant in the transcriptome than those of segments M and L.

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

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An estimated 35,000 individuals in the U.S. alone are killed by antibiotic-resistant bacteria every year. This number is expected to increase ten-fold by 2050 if no effective treatments are found. Bacteriophages (phages), viruses that infect and kill bacteria, provide a promising tool for the treatment of antibiotic-resistant bacterial infections. Despite recent advancements, selecting phages that exhibit specificity for bacteria has been challenging mainly because of the knowledge gaps in understanding virus-host interactions and the high mutations rates of both organisms. Our study investigates phages that target Pseudomonas aeruginosa, a

ubiquitous multidrug-resistant opportunistic pathogen that commonly infects ill, hospitalized patients. We aim to develop a universal phage cocktail that is specific to a broad spectrum of clinical, environmental, and animal isolates of P. aeruginosa. Furthermore, we aim to identify bacterial genes that affect phage specificity. To date, we isolated ten unique bacteriophages from UF wastewater. The cocktail of isolated phages targets 49 of 55 clinical isolates of P. aeruginosa, each with a unique antibiotic resistance profile and a sequenced genome. We tested the efficacy of our cocktail in combination with 24 antibiotics and found that chloramphenicol and colistin enhanced bacterial killing when combined with phages. We are currently identifying the specificity of these phages against additional 117 unique strains of P. aeruginosa. Once we assess the specificity of the isolated phages against a broad spectrum of bacterial isolates, we will begin to mine the bacterial genomes to identify regions linked to phage sensitivity. Through this project, we aim to develop a unique phage cocktail that will target a broad range of P. aeruginosa and reveal genes that influence phage-bacteria interaction, which will help to move phage therapy a step closer to clinical application.

76. BETA-LACTAM ANTIBIOTIC RESISTANCE DEVELOPMENT IN INTENSIVE CARE PATIENTS

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Beta-lactams are the most commonly used class of antibiotics due to their broad-spectrum antibacterial activity. Due to their frequent usage, bacteria may develop resistance to these antibiotics through different mechanisms. In this study, we evaluated data gathered from patients treated with beta-lactams between 2016 and 2019 at UF Health Shands ICU to determine how often bacteria developed resistance to the beta-lactams administered. We included adult patients who received beta-lactam therapy, had its plasma concentration measured, and had multiple samples collected for culture and susceptibility testing for up to 30 days after beta-lactam therapy. We compared the bacterial minimum inhibitory concentration (MIC) from cultures taken before therapy to that of cultures taken during or after therapy completion. Resistance was defined as any increase in the MIC. The minimum beta-lactam concentration (Cmin) was calculated and correlated with the development of resistance. Out of 1,456 patients, 173 had sufficient data to determine the incidence of resistance and calculate free Cmin. The mean (SD) age was 54 years (±18), weight 82.7 kg (±33.0), and serum creatinine 1.2 mg/dL (±1.3). The beta-lactams used were cefepime (63.1%), meropenem (19.6%), piperacillin/tazobactam (7.8%), oxacillin (6.7%), ampicillin (2.2%), and cefazolin (0.6%). The mean (±SD) calculated free Cmin/MIC was 26.55 (±48.66). A total of 65 patients (37.6%) had bacteria that developed resistance within 30 days to the beta-lactams they received. The median free Cmin/MIC was similar in patients who developed resistance and those who did not (8.6 vs. 8.4, p=0.7). This

research highlights the need for more investigation in this area to improve therapy outcomes and minimize bacterial resistance.

77. PREVALENCE AND CORRELATES OF HIV INFECTION AMONG TRAVESTIS IN GOIÁS-BRAZIL

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Introduction: Travestis are people in Brazil who are born male and have a female gender identity. They do not undergo sex reassignment surgery, and many are exposed to unfavorable situations such as low education, low income, sex work, stigma, violence, multiple sexual partners, and unprotected sex. Thus, the travestis are disproportionately affected by sexually transmitted infections (STI) and are 12 times more likely to be infected with HIV (19.1%) than the general population aged 15 to 49 years (0.8%). There are limited data on the sociodemographic and behavioral characteristics of travestis in this region. Thus, this study aimed to estimate the prevalence and correlates of HIV infection among travestis in Goiás- Brazil.

Methods: A Cross-sectional study was conducted with travestis (N=166) in three cities in Goiás (Goiânia, Jataí, and Itumbiara), Central Brazil, between April 2018 to August 2019. Respondent Driven Sampling (RDS) methods were implemented for recruitment. All participants were interviewed face-to-face in private locations. A standardized

questionnaire was used to collect data on the sociodemographic characteristics and risk factors for HIV. All participants were tested for anti-HIV-1 and 2 using the Abon-HIV kit rapid test. All variables with $p<0.20$ in the bivariate analysis were included in the multivariate analysis model, and values of $p<0.05$ were reported.

Results: The majority of the participants lived in Goiânia (71.0%), of mixed race (73,8%), over 25 years of age (37.9%), single (87.9%), with education between 10-12 years (63.8%). Of the total, 28.9% (n=48) were HIV-positive. In multivariate analysis, a history of STI ($p < 0.002$; OR: 7.2; 95% CI: 2.1-24.4) predicted HIV infection and had only one sexual partner in the last seven days ($p < 0,026$; OR: 0.2; CI 95%: 0.03-0.8), was independently associated with HIV infection.

Conclusions: The study results show a high prevalence of HIV in travestis in Central Brazil. Travestis with any STI diagnosis also need HIV testing and may benefit from strategies such as PrEP. Therefore, it is essential to emphasize prevention and health promotion among this population.

78. ISOLATION OF DNA AND RNA FROM ARCHIVAL BAT GUT TISSUES OF MUSEUM SPECIMENS PRESERVED IN ETHANOL AND FORMALIN

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Background: Museum collections of archival tissue samples represent a vast collection of untapped genetic information. The Florida Natural History Museum maintains a collection of frozen preserved, ethanol preserved, and formalin preserved whole bat specimens which could serve as a valuable source of such genetic information for insight into the history of Florida's bat populations and ecologically connected species. Specifically, the preservation of the microbiome in these samples will provide direct insight into the diet and disease state of these populations of bats.

Methods: Stomach, gut, and rectum tissue were resected from frozen preserved, ethanol preserved, and formalin preserved museum bat samples and stored in RNAlater at -20°C. Rectum samples from each bat underwent DNA and RNA extractions which were tailored to optimally collect genetic material in accordance with their respective preservation method. All procedures were conducted in accordance with manufacturer protocol with minor adjustments. DNA and RNA were extracted from frozen preserved samples via the QIAGEN AllPrep DNA/RNA Mini Kit. DNeasy Blood & Tissue Kit was utilized to extract DNA from Ethanol preserved samples following the protocol. DNA was extracted from Formalin preserved tissues via the QIAamp DNA FFPE Tissue Kit. To

identify presence of bacterial DNA corresponding to sample tissue microbiome, amplification of the V3/V4 region of the r16S gene was preformed utilizing total DNA extracted from each sample and visualized on agarose gel.

Results: DNA and RNA were successfully extracted from rectum samples of frozen preserved, ethanol preserved, and formalin preserved bat samples. DNA extracted from frozen preserved rectum samples ranged in concentration from 90.8 ng/uL to 98.6 ng/uL. DNA extracted from ethanol preserved bats ranged in concentration from 0.102 ng/uL to 0.434 ng/uL. DNA extracted from formalin preserved rectum samples ranged from 0.322 ng/uL to 0.329 ng/uL. PCR amplification utilizing primers targeting the V3/V4 region of the r16S gene successfully amplified a 464bp amplicon in each of the extracted DNA samples.

Conclusion: RNA and DNA were successfully obtained from archival bat samples preserved in ethanol, formalin , or frozen. Furthermore, successful amplification of the V3/V4 region from total DNA isolated from archival bat samples indicated the presence of a preserved bacterial microbiome, and the successful extraction of their DNA. Future metagenomic sequencing of extracted DNA will permit analysis of the bacterial microbiome composition.

79. PROFITABILITY, PERCEPTIONS AND DETERMINANTS OF THE PESTE DES PETITS RUMINANTS (PPR) VACCINE ADOPTION AMONG FEMALE GOAT KEEPERS OF DIFFERENT ETHNICITY IN DHADING, NEPAL

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Goats, known as “poor man’s cow,” are important for poverty alleviation, especially among women who are primary goat caretakers in Africa and Asia. In Nepal, 73.2% of women are considered as primary goat caretakers (Regmi, 2019), who contribute to the family wellbeing. Goats are susceptible to different diseases, including the deadly peste des petits ruminants (PPR) disease, which is endemic in Nepal. Among approximately 11 million goats only 15% were vaccinated against PPR in 2017 (MoALD, 2017). Vaccinators target commercial goat farmers and households with large goat herd rather attending to goats of subsistence farmers (Gangga, 2019), while majority of women are subsistence goat farmers who neither have access to vaccines nor have adopted to the PPR vaccination campaigns organized by the Government of Nepal as part of the PPR eradication strategy by 2030. Moreover, female goat keepers of lower castes and marginalized ethnic groups lack access to and awareness about PPR vaccine. Therefore, it is urgently needed to identify and rank the determinants of PPR vaccine adoption among female subsistence goat keepers of different caste/ethnicity in Nepal. The study will be conducted in two municipalities in Dhading district. The cross-sectional mixed methods (quantitative and qualitative) research design will be applied. Eight focus group discussions (FGDs) will be conducted with female goat keepers from indigenous group to understand female goat keepers’ roles in PPR vaccination, and their perceptions of adopting the PPR vaccine. 14 key informant interviews (KIIs) will be done with government officials, agro-veterinary practitioners, the staff of veterinary hospital and livestock service expert centers, and vaccine suppliers in Dhading and Kathmandu. Finally, 120 household surveys with female goat keepers will be done to gather data around determinants about PPR vaccination and vaccine adoption, goat production and profitability. FGD and KII data will be analyzed using thematic analysis, while individual surveys will be analyzed using an SPSS program. Statistical inferences will

run around identifying differences among female goat keepers of different caste/ethnicity in terms of goat production, profitability, and determinants about adopting PPR vaccination. The findings of this research will glean light into the perceptions and determinants about the PPR vaccination among females of different ethnicity/caste groups, and rank the determinants of PPR vaccine adoption. The findings will help identify strategies to maximize profit and increase vaccine adoption among female goat keepers that empower women and eradicate PPR disease in Nepal.

80. THE INTERPLAY OF HOST STAGE STRUCTURE AND INFECTIOUS DISEASE CAN STABILIZE DYNAMICS AND ELEVATE HOST POPULATION SIZE

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Introduction: All individuals transition through various life stages over the course of their development and nearly all organisms must contend with infectious disease at some point in their lives. Yet the intersection of these two universal features of life – stage structure and infectious disease – and their joint effects on population dynamics, are poorly understood.

Methods: Here we develop a two-stage population model in which density dependence acts on juvenile maturation, and infectious disease affects either juveniles or adults via reduction in maturation, reproduction or survival. Using the Routh-Hurwitz criterion for stability, we examine whether infectious disease stabilizes (dampens population oscillations), or further destabilizes (augments amplitude of oscillations), the oscillatory population dynamics generated via stage structure in the absence of disease.

Results and Conclusions: We find that, for moderate transmission rates (a proxy for disease incidence), infectious disease can stabilize dynamics. In contrast, fast disease transmission is not generally stabilizing, which is, at least in part, due to disease overexploitation of the infectious class.

Hydra effects, the phenomenon by which altering vital rates (such as increasing mortality) counter-intuitively increases long-term population size, are possible in the model due to density overcompensation. They occur when disease increases juvenile mortality or decreases adult fecundity (but do not occur when disease augments adult mortality). This suggests that infectious disease could at times surprisingly increase host population size, which could have consequences for management and conservation.

81. EFFECTS OF PESTICIDE EXPOSURE TO AEDES AEGYPTI AND IMPLICATIONS ON VECTOR COMPETENCE

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Introduction: A variety of larvicides were bioassayed to produce dose-response curves against Aedes aegypti.

Method: Adults were generated by exposing larvae to concentrations that would yield sub-optimal (i.e., ≥LC25 , ≤LC50) mortality. Mosquito wings were measured and compared to one another to identify changes in size based on the correlation to wing length and dry weight. Surviving exposed adults were fed dengue virus-1 via bloodmeal, and their vector competence was compared to unexposed adults.

Results: Our results describe the difference in survivorship between larvicides and potential effects that larvicide exposure has on the vector and vector competence in the adult stage, as well as the effect sublethal concentrations of larvicide has on mosquito size and M:F ratios.

Conclusions: Sublethal (LC50) larvicide exposure can influence the size and surviving sex ratio of Aedes aegypti adults. This may impact the vector competence of surviving adults.

82. EPITOPE MAPPING OF PATHOGENIC AUTOANTIGENS AND SMALL MOLECULE SCREENING ON SJÖGREN'S SYNDROME-SUSCEPTIBLE HLA USING IN-SILICO TECHNIQUES

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Sjögren's syndrome (SjS) is characterized by lymphocytic infiltration and the dysfunction of the salivary and lacrimal glands. The autoimmune response is driven by the effector T cells and their cytokines. The activation of the effector helper T cells is mediated by autoantigen presentation by human leukocyte antigen (HLA) class II molecules of antigen-presenting cells. Studies using familial aggregation, animal models, and genome-wide association demonstrate a significant genetic correlation between specific risk HLA and SjS. One of the key HLAs is the HLA-DRB1*0301 allele, one of the most influential associations in patients with primary SjS and having the highest odds ratio and occurrence in different ethnic groups. The specific autoantigens attributed to SjS remain elusive, especially the specific antigenic epitopes presented by HLA-DRB1*0301. In this study, we applied a high-throughput in-silico mapping technique to identify antigenic epitopes. Furthermore, we identified specific binding HLA-DRB1*0301 epitopes using structural modeling tools such as IEDB, AutoDock Vina, and COOT. By deciphering the critical epitopes of autoantigens presented by HLA-DRB1*0301, we will better understand the origin of the antigens, determine the T cell receptor function, learn the mechanistic insights of disease progression, and develop therapeutic applications.

83. ANTIBODY RESPONSE TO MOSQUITO SALIVARY PROTEINS AS A MARKER FOR EXPOSURE: A LITERATURE REVIEW AND META-ANALYSIS

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Introduction: Arboviruses (arthropod-borne viruses) are responsible for a massive global burden of disease in humans. Aedes aegypti and Aedes albopictus mosquitoes spread the majority of human mosquito-borne viruses. Mosquito population surveillance is key to assessing mosquito/vector population but is cumbersome. The need for more efficient mosquito surveillance that quantitatively measures the risk of exposure to mosquito bites (and possible arbovirus exposure) is needed; the use of antibody (Ab), specifically Ig, response to mosquito salivary proteins (MSP) is being examined as a potential alternative.

Methodology: Using PRISMA guidelines, a meta-analysis was performed to assess the efficacy of detection of human Ab response to MSP as presented in the literature. The aims of this review/meta-analysis are to characterize the methods used to classify an individual's Aedes spp. exposure and identify and extract individual level data on immune response to quantify sources of variation in ELISA based measure of IgG to MSP.

Results: A total of 1083 studies were screened by two reviewers; 100 articles were included in the narrative and data extraction portion, as they met the eligibility requirements. Variation in exposure (24 papers), allergy (49 papers), and infection/disease (15 papers) were common themes throughout the literature. Research included 15 species of Aedes that were identified, with Ae. aegypti being the most common; Ae. albopictus, Ae. communis, and Ae. vexans were also commonly cited.

Some studies utilized the most common mosquito species known to be endemic where subjects live, some cited specific entomological data, and others collected entomological data during the course of their studies. Extracted data will be pooled and analyzed further.

84. ANCESTRAL ORIGIN IS ASSOCIATED WITH SUSCEPTIBILITY TO SARS-COV-2 IN A FLORIDA PATIENT POPULATION

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COVID-19 is caused by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2). The severity of COVID-19 is highly variable and related to known (e.g., age, obesity, immune deficiency) and unknown risk factors. The widespread clinical symptoms encompass a large group of asymptomatic COVID-19 patients, raising a crucial question regarding genetic susceptibility, e.g., whether individual differences in immunity play a role in patient symptomatology and how much human leukocyte antigen (HLA) contributes to this. To reveal genetic determinants of susceptibility to COVID-19 severity in the population and further explore potential immune-related factors, we performed a genome-wide association study on 284 confirmed COVID-19 patients (cases) and 95 healthy individuals (controls). We compared cases and controls of European ancestry and African American ancestry separately and identified two loci on chromosome 5q32 and 11p12, which reach the significance threshold of suggestive association ($p<1x105$ threshold adjusted for multiple trait testing) and associated with the COVID-19 susceptibility in the European ancestry (index rs17448496: odds ratio

[OR] = 0.173; 95% confidence interval [CI], 0.08–0.36 for G allele; p=5.15× 106; and index rs768632395: OR = 0.166; 95% CI, 0.07–0.35 for A allele; p= 4.25×106, respectively), which were associated with two genes, PPP2R2B at 5q32, and LRRC4C at 11p12, respectively. To explore the role of HLA in COVID-19 severity and susceptibility, we applied fine-mapping analysis on chromosome 6 to dissect the association in the HLA. Due to the differences in the binding of different HLA molecules to individual structural proteins of the virus, a higher frequency of less-protective allele presence in certain ethnicity may explain the imbalance in the affected population. Although this study is limited to comparing SARS-CoV-2 positive and negative subjects, these data suggested possible differences in susceptibility to COVID-19 in different ancestral origins in the genetic background, which may provide new insights into the pathogenesis and clinical treatment of the disease.

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