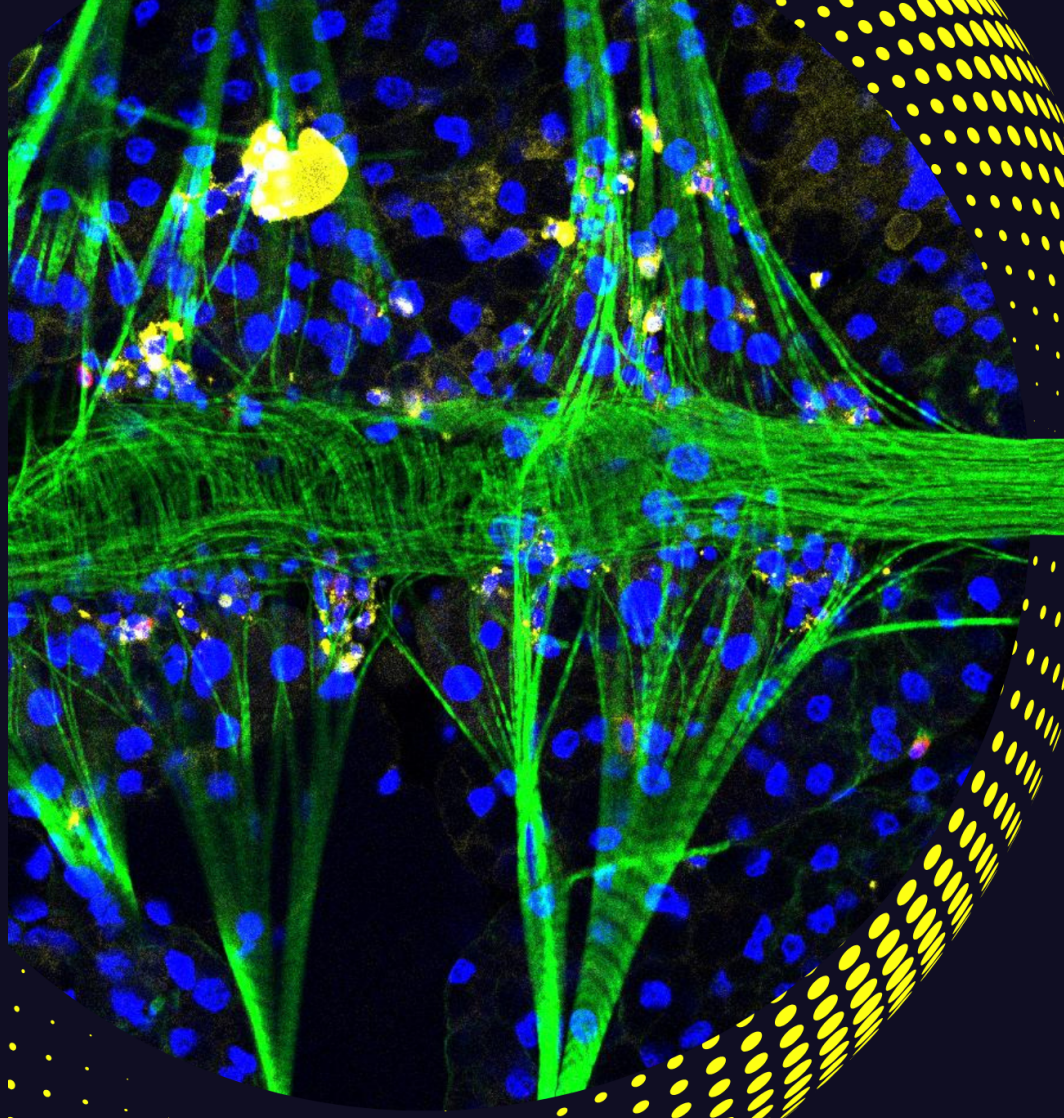
The image shows one segment of the Aedes aegypti dorsal vessel, or heart, that is infected with dengue virus 2 (DENV2). In the image, the vessel musculature (green), mosquito hemocytes (yellow), and DENV2 (red) are present and indicate mosquito hemocytes are involved in suppressing DENV replication in the vector. Ongoing work in the Swale Lab aims to test the relevance of circulatory homeostasis to DENV replication and infection of the mosquito vector in an effort to identify novel approaches to prevent arbovirus transmission.

EMERGING PATHOGENS INSTITUTE

UNIVERSITY OF FLORIDA

EPI RESEARCH DAY BOOK OF ABSTRACTS 2023



*Emerging Pathogens Institute*

# RESEARCH DAY

Book of Abstracts | February 2023

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## Letter from the director

Welcome to the 16th annual EPI Research Day! This year, we have returned to an "in-person" setting; the virtual format has worked well for the past few years, but it will be nice to see and meet people without an intervening video screen. We are pleased to have a total of 123 abstracts being presented today, with a wide range of topics reflecting the interdisciplinary nature of the research done by EPI members and collaborators. Abstract submissions came from 27 different departments in eight UF colleges, as well as from collaborators from other U.S. and international universities and state and federal agencies. Additionally, we have some exciting new aspects for this year, including poster competitions for trainees and early investigators, information booths from different UF units, and live social media engagement during the event.

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

Amadou A. Sall, Ph.D., will be speaking on "Emerging infectious pathogens in Africa: from vulnerability to resilience." Sall is the CEO of Institut Pasteur de Dakar in Senegal and the director of the WHO collaborating center for arboviruses and viral hemorrhagic fever. He has been chairman of the Global Outbreak Alert and Response Network and a member of the Coalition for Epidemic Preparedness and Innovation (CEPI) Scientific Advisory Board.

Tulio de Oliveira, Ph.D., will be speaking on "Challenging the status quo and making the global south to lead in genomics surveillance." Oliveira was the director of the genomics program at the Wellcome Trust Africa Center for Health and Population Studies and was an affiliate senior lecturer at the Division of Infection and Immunity at the University College London (UCL). He currently serves as a professor at UKZN, and he founded the KwaZulu-Natal Research Innovation and Sequencing Platform at the Nelson R. Mandela School of Medicine.

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

***J. Glenn Morris, Jr., MD, MPH & TM***
EPI Director and Professor of Medicine

## Schedule of events

**9:00 AM – 10:00 AM**

Registration, breakfast and poster setup

**10:00 AM – 1:00 PM**

Poster session
*Presenters, please stand by your posters*

**12:00 PM – 12:45 PM**

Lunch

**12:45 PM – 1:00 PM**

Keynote assembly

**1:00 PM – 1:10 PM**

Welcome and introductions
*Dr. J. Glenn Morris, Jr., Director, EPI*

**1:10 PM – 3:15 PM**

Keynote speeches

**3:15 PM – 4:00 PM**

Poster removal

*1:10 p.m. – 2:10 p.m.*

## Amadou Alpha Sall, PhD

*CEO, Institut Pasteur de Dakar*
Dakar, Senegal
*Director, Center for Arboviruses and Viral Hemorrhagic Fever*
WHO Collaborating Center

## *"Emerging infectious pathogens in Africa: from vulnerability to resilience"*

*2:10 p.m. – 3:10 p.m.*

## Tulio de Oliveira, PhD

*Founder, KwaZulu-Natal Research Innovation and Sequencing Platform (KRISP)*
School of Laboratory Medicine and Medical Sciences
University of KwaZulu-Natal
Durban, South Africa

## *"Challenging the status quo and making the global south to lead in genomics surveillance"*

## 01. ACQUISITION AND CLEARANCE DYNAMICS OF CAMPYLOBACTER IN CHILDREN IN LOW- AND MIDDLE-INCOME COUNTRIES

**Dehao Chen** - University of Florida; **Arie H. Havelaar** - University of Florida; **James A. Platts-Mills** - University of Virginia; **Yang Yang** - University of Georgia

The burden of Campylobacter infection is high in children under five years of age in low- and middle-income countries (LMIC), but its acquisition and clearance process is understudied due to scarcity of longitudinal data. We aim to quantify this process using a statistical modeling approach, leveraging data from a multi-nation study.

Motivated by the MAL-ED study in which children from eight low- and middle-income countries were followed up for enteric infections during their first two years of life, we developed a two-stage Markov model to compare the dynamics of acquisition and clearance of Campylobacter in children across countries and to explore antibiotic effectiveness on Campylobacter clearance. This model was validated using simulations and applied to the longitudinal data from MAL-ED.

The clearance rate was higher than the acquisition rate at most sites and times, but the temporal trend of these rates varied across countries. For Campylobacter jejuni/coli, clearance was faster than acquisition under two years of age at all sites. For Campylobacter spp., the acquisition rate surpassed the clearance rate in the second half of the first year in Bangladesh, Pakistan and Tanzania, leading to high prevalence in these countries. Bangladesh had the shortest (28 and 57 days) while Brazil had the longest (328 and 306 days) mean times to acquisition for Campylobacter spp. and C. jejuni/coli, respectively. South Africa had the shortest (10 and 8 days) while Tanzania had the longest (53 and 41 days) mean times to clearance for Campylobacter spp. and C. jejuni/col respectively. The use of macrolides was associated with accelerated

clearance of C. jejuni/coli in Bangladesh and Peru and of Campylobacter spp. in Bangladesh and Pakistan. The use of fluoroquinolones showed statistically meaningful effectiveness only in Bangladesh but for both C. jejuni/coli and Campylobacter spp.

Higher burden of Campylobacter infection was mainly driven by high acquisition rate that was close to or surpassing the clearance rate. Acquisition usually peaked in 11-17 months in the LMIC setting, indicating the importance of targeting the first year of life for effective intervention.

**02. ANALYZING TRANSLOCATION OF SALMONELLA SOPB INTO HOST CELLULAR COMPARTMENTS**

**Valeria Molinary** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Liberal Arts and Sciences, University of Florida; **Saloni H. Bhimani** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Lisa E. Emerson** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Samantha Enslow** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Mariola Edelmann** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Non-typhoidal Salmonella enterica is responsible for a significant burden of illness in the world population. Salmonella enterica is a gram-negative bacterium that causes severe gastrointestinal disease. Salmonella has a role in infecting multiple cell types such as epithelial cells and immune cells. SopB is a Salmonella type III effector that enhances infection, and our group has identified via the proteomics approach that macrophages release some virulence factors, including SopB, via extracellular vesicles during infection. However, the mechanism of SopB entry into the extracellular vesicles and accessing the function of SopB released via these vesicles remains unknown. This study aims to elucidate the

presence of SopB proteins in Salmonella infected macrophages and their secreted extracellular vesicles. Towards this goal, we infect RAW264.7 macrophages and HeLa cells with wildtype Salmonella and a strain in which the SopB contains an HA tag expressed from a plasmid. We conduct Western blots to view protein contents of the Salmonella-infected macrophages and HeLa cells. Specifically, we viewed the SopB-HA band in SopB infected cells. Overall, our results suggest that Salmonella SopB is included in the trafficking of the bacterial protein into host compartments. These results have the potential to lead to the development of improved preventative strategies against Salmonella infection. A vaccine against Salmonella infection is also a possible outcome since SopB is a candidate protective antigen, and our group has previously shown that extracellular vesicles can be protective against this infection. Future work will include examining the mechanism of SopB trafficking within extracellular vesicles from Salmonella infected cells.

**03. COMPARATIVE WHOLE-GENOME CHARACTERIZATION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS FROM HUMANS AND FOOD ANIMALS**

**Grace Oldham** - 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; **Kartikeya Cherabuddi** - Department of Infectious Disease, University of Florida; **Nicole Iovine** - Department of Infectious Disease, 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) is a typical human and animal pathogen, causing various diseases that pose great concerns to public health. We aim to understand genetic features that enable MRSA for successful colonization in humans and animals. We conducted whole-genome sequencing of 50 MRSA strains isolated from hospitalized patients (HMRSA), as well as the genome sequences of 50 food animal MRSA (FAMRSA) from the NCBI database and identified their sequence types. Then, we constructed a core-genome based maximum-likelihood

phylogenetic tree for FAMRSA and HMRSA. Strains isolated from the same host species have closer phylogenetic relatedness. Only two strains isolated from humans were clustered with four other strains from food animals. We further conducted comparative genome analysis to identify the antibiotic resistance and virulence profiles of these strains, with an emphasis on the differences between human and animal hosts. Multiple antibiotic resistant mechanisms were identified in the MRSA strains. Regardless of the host types, methicillin resistance and efflux pump encoding genes were shown in all the strains. There was no specific host-related resistance gene identified. Various virulence factors were also identified in the MRSA strains. Interestingly, strains isolated from the same host species have more similarity in their virulence profiles. In the future study, we are going to investigate the mobile genetic elements of these MRSA strains to further understand the dissemination and specificity of MRSA in different hosts.

**04. COMPARISON OF MICROBIOME COMPOSITION BETWEEN AQUATIC LARVAE AND THE INFLUENCE OF LARVAL MICROBIOME ON DEVELOPMENT RATE**

**Aria Deluna** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Adam Chun Nin Wong** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida

**Introduction:** Research concerning the microbiome composition and function in aquatic insects remains limited. Chironomids and mosquitoes are two distinct insect families that are both widespread and occupy similar aquatic habitats during the larvae stage. The proximity of their larval habitats and the potential overlapped route of microbial acquisition raise many questions as to how their microbiomes may overlap or differ in composition and function. In mosquitoes, the gut microbiomes have been shown to be highly variable depending on host species, geography, seasons, etc., but interestingly, different bacterial species may be equally capable of stimulating larval development. Less is known about the environmental variability and developmental function of the chironomid microbiome, despite that the chironomid larvae have been implicated as

a natural environmental host for Vibrio cholerae, a significant human pathogen that causes cholera.

**Methods:** In this study, we inoculated the microcosms of chironomids with the microbiome of chironomids and mosquitoes, then their development time to pupation will be compared. Axenic chironomids were also reared alongside these for comparison of development in the absence of a microbiome. Then, we use 16S rRNA gene sequencing to examine how the microbiome composition and temporal variability differ between the mosquito and chironomid larvae collected from the same microenvironments (using experimental potholes) at different times.

**Results:** 16S sequencing results suggest that the microbiomes differ substantially, with a greater seasonal variation in the mosquito microbiome compared to that of chironomids. Development data showed that development time increased with deviation from the native chironomid microbiome.

**Conclusions:** Microbiome composition of both chironomids and mosquitoes differ between species and between collection events, implying that many factors ranging from diet to temperature are likely to influence composition. Additionally, the native microbiome contributes to healthy development in these insects, but they are still capable of developing to pupation when the microbiome is disrupted.

**05. DISCOVERING THE MICROBIAL LANDSCAPE: AN INVESTIGATION OF CAMPYLOBACTER IN INFANTS AND HOUSEHOLD ENVIRONMENTS IN RURAL EASTERN ETHIOPIA**

**Amanda Ojeda** - Department of Microbiology and Cell Science, University of Florida; **Loic Deblais** - Ohio State University; **Bahar Mummed Hassen** - Haramaya University; **Mussie Brhane** - Haramaya University; **Kedir Hassen** - Haramaya University; **Belisa Usmael** - Haramaya University; **Yenenesh Demisie** - Haramaya University; **Arie H. Havelaar** - Institute of Food and Agricultural Sciences, University of Florida; **Luiz Roesch** - Department of Microbiology and Cell Science, University of Florida; **Gireesh Rajashekara** - Ohio State University

**Introduction:** High prevalence of Campylobacter infections in low-resource settings is a major contributor to environmental enteric dysfunction (EED) and stunting in children. In 2018, our cross-sectional study detected Campylobacter in 88% of child stools (8-367 days of age) collected in rural eastern Ethiopia using Meta-total RNA sequencing. An average of 11 Campylobacter species (thermophilic and non-thermophilic) were detected per positive stools. Therefore, a longitudinal study of the Campylobacter Genomics and EED (CAGED) project was conducted to identify Campylobacter species associated with EED/stunting and to define reservoirs associated with early infections of Campylobacter spp. in infants.

**Methods:** Infant stool samples (n=1,073) were collected monthly from birth to 12 months. Environmental samples (soil and water), livestock feces (cattle, chicken, goat, and sheep), and human stools (mother and sibling) were collected biannually (n=1,744). Campylobacter genus detection was done using TaqMan real-time PCR targeting 16SRNA in field samples. Species-specific quantitative PCR (cpn60 and hipO) was done on one thermophilic and three non-thermophilic Campylobacter species to assess prevalence and diversity. In-house validation of primer specificity and sensitivity was done.

**Results:** Campylobacter was detected in all households selected in this study (n=106), with 71% of field samples being positive for

Campylobacter at the genus level. To date, (1,439/2,817) have been tested using species-specific qPCR. Non-thermophilic Candidatus C.infans and thermophilic C.jejuni were predominant in human samples (23-44%), followed by C.upsaliensis (11%). Of these, C.infans was prevalent in 42% of infant stools and in 51.7% of mother/sibling stools. C.jejuni was common in environmental samples (24%). Other Campylobacter species were rarely detected (<2%). Detection of C. infans increased as the child aged, with 20% positivity (51/251) at <6 months of age which surged to 58% (219/380) at >6 months of age. Similarly, C.jejuni prevalence increased as the child aged, with 6% positivity (16/251) in infants <6 months of age and 38% (148/389) at >6 months of age.

**Conclusion:** Our study found a high prevalence of Campylobacter in infants in Eastern Ethiopia, with non-thermophilic species being the most predominant. Further testing is underway to determine the prevalence, distribution, and sources of infection and assess dietary and WASH risk factors. Our findings provide new insight into the relationship between Campylobacter and infants in Eastern Ethiopian households and hold the potential to inform interventions that reduce the transmission of Campylobacter to infants, thereby improving their health by mitigating the risks of EED and stunting.

**06. EVALUATION OF SURFACE WATER TREATMENT EFFICACY PROTOCOL USING SALMONELLA SPP. IN FLORIDA WATER**

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

**Introduction:** Microbial pollution can be a significant threat to surface water and has been implicated as a source of contamination on produce resulting in outbreaks. Growers are under market-driven and regulatory pressure to treat surface water prior to use in contact with produce. The study aimed to evaluate the applicability of the FDA's revised water treatment efficacy chemical labeling protocol using agricultural water.

**Methods:** Surface water from a West Central Florida Farm Pond (98 ml) was inoculated with 1 ml of a ca. 10 log CFU/ml Rifampicin resistant seven strain Salmonella cocktail. Water (99 ml) was equilibrated at 12 or 32°C for 30 minutes. Calcium hypochlorite was ground into powder and mixed with PBDW to create a stock solution (150-200 ppm). Stock chlorine solution (1 ml) was added to 99 ml of water to achieve 2-4 ppm of free chlorine. Following the addition of chlorine, at 1 and 5 min, Salmonella populations were determined by serial dilutions in sodium metabisulfite, plating onto non-selective media with rifampicin, and incubating 35±2°C for 24±2 h. Colonies were counted by hand and expressed as log CFU/ml; student t-tests were performed (n=3).

**Results:** At 32°C log reductions of <6.2 and <6.5 log CFU/ml, at chlorine concentrations 3 and 3.5 ppm, for 1 and 5 minutes, respectively. At 12°C log reductions of < 6.2 and<6.5 log CFU/ml, at chlorine concentrations 3 and 3.5 ppm, for 1 and 5 minutes, respectively. The greater than 6 log

reductions under all treatment conditions were statistically significant. No significant differences were found between 12 and 32°C (P value = 0.967).

**Conclusions:** Calcium hypochlorite, at concentrations of 2-4 ppm free chlorine, is an effective surface water treatment to reduce Salmonella for growers using ponds in West Central Florida.

**07. GROUP 3 INNATE LYMPHOID CELL PYROPTOSIS REPRESENTS A HOST DEFENSE MECHANISM AGAINST SALMONELLA INFECTION**

**Lifeng Xiong** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Shifeng Wang** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Joseph W Dean** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Kristen N Oliff** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Christian Jobin** - Department of Medicine, College of Medicine, University of Florida; **Roy Curtiss III** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Liang Zhou** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida

Group 3 innate lymphoid cells (ILC3s) produce interleukin (IL)-22 and coordinate with other cells in the gut to mount productive host immunity against bacterial infection. However, the role of ILC3s in Salmonella enterica serovar Typhimurium (S. Typhimurium) infection, which causes foodborne enteritis in humans, remains elusive. Here we show that S. Typhimurium exploits ILC3-produced IL-22 to promote its infection in mice. Specifically, S. Typhimurium secretes flagellin through activation of the TLR5-MyD88-IL-23 signaling pathway in antigen presenting cells (APCs) to selectively enhance IL-22 production by ILC3s, but not T cells. Deletion of ILC3s but not T cells in mice leads to better control of S. Typhimurium infection. We also show that S. Typhimurium can directly invade ILC3s and cause caspase-1-mediated ILC3 pyroptosis independently of flagellin. Genetic ablation of Casp1 in mice leads to increased ILC3 survival and IL-22 production, and enhanced S.

Typhimurium infection. Collectively, our data suggest a key host defense mechanism against S. Typhimurium infection via induction of ILC3 death to limit intracellular bacteria and reduce IL-22 production.

**08. INFANT CAMPYLOBACTER INFECTION, GROWTH FALTERING, AND THEIR DETERMINANTS IN EASTERN ETHIOPIA**

**Dehao Chen** - University of Florida; **Yang Yang** - University of Georgia; **Amanda Ojeda** - University of Florida; **Bahar Mummed Hassen** - Haramaya University; **Loic Deblais** - The Ohio State University; **Ibsa A. Ahmed** - Haramaya University; **Ibsa Aliyi Usmane** - Haramaya University; **Gireesh Rajashekara** - The Ohio State University; **Arie H. Havelaar** - University of Florida; **Sarah L. McKune** - University of Florida

**Introduction:** Campylobacter is a zoonotic enteric pathogen associated with both environmental enteric dysfunction and malnutrition in children. Campylobacter infection could be a lynchpin between household risk factors for fecal exposure and child health outcomes in low-resource settings where livestock are ubiquitous.

**Method:** In a birth cohort of rural smallholder households in eastern Ethiopia, infants were followed to 13 months of age. We measured anthropometry quarterly and surveyed socio-demographic determinants at baseline and each monthly visit, when infants' stool samples were also collected to assay for Campylobacter using qPCR methods. The primer set employed is not restricted to the well-studied species C. jejuni and C. coli but is able to detect all species in the Campylobacter genus. We employed linear (mixed) models to assess the associations between household determinants, Campylobacter colonization, and growth faltering in 106 infants who completed the follow-up. Campylobacter load was calculated as a linear function (based on the standard curve of qPCR) of cycle threshold.

**Results:** The mean Campylobacter load had increased around two-fold at the last visit when the infants aged around 12-13 months (day range: [353-376], concurrent prevalence = 89% [95% CI: 81%-94%]) compared to the first visit after birth (day range: [7-39], concurrent prevalence = 32% [95% CI: 24%-42%]). The mean length-for-age z-score (LAZ) decreased

from -0.45 around months 3 and 4 (day range: [79-121]) to -2.06 after the thirteenth month (day range: [389-513]), and the concurrent prevalence of stunting (LAZ < -2) increased from 3% (95% CI: 1%-11%) to 51% (95% CI: 40%-61%). Pre-lacteal feeding was associated with increasing Campylobacter load at the first visit after birth. Longitudinally, complementary feeding and raw milk consumption were associated with increasing Campylobacter load, while food insecurity was associated with higher risks of both Campylobacter load and growth faltering. A higher Campylobacter load was associated with more frequent diarrhea. Despite a sex- and location-adjusted model suggesting a significant effect of cumulative Campylobacter load on growth faltering, this effect was no longer significant after adjusting for age, possibly due to small sample size.

**Discussion:** Despite the existence of many risk factors related to exposure to livestock feces, most significant risk factors increasing Campylobacter infection in infants in our setting are food related, signifying the need to consider strengthening infant food safety. Analyses are ongoing to associate the outcomes of Campylobacter load and growth faltering with other putative determinants as specified by a modified UNICEF framework for child undernutrition, accounting for impacts of enteric pathogens on gut health as additional determinants of child nutrition.

**09. ISOLATION AND CHARACTERIZATION OF TOXIGENIC VIBRIO CHOLERAE O1 IN GOMA IN DEMOCRATIC REPUBLIC OF CONGO**

**Meer Taifur Alam** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **S. Nazmus Sakib** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Soung Iballa F. B.** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Felicien Masanga Maisha** - Emerging Pathogens Institute, University of Florida; **R. Kabangwa Kakongo Senga** - Appui Medical Integre aux Activites de Laboratoire (AMI-LABO), Goma, Democratic Republic of Congo; **J. Glenn Morris, Jr.** - Department of Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Afsar Ali** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

**Introduction:** African subcontinent, including Democratic Republic of Congo (DRC) reported first cases of cholera in early 1970s. Since then, cholera is regularly reported from African countries, including DRC. Indeed, WHO documented over 90% of cholera cases from Africa with majority of the recent cases are from DRC. High incidences of cholera in Africa are attributed to lack of safe drinking water, optimal sanitation and hygiene and mass migration of population due to wars and natural disasters. Cholera is caused by toxigenic strains of V. cholerae O1 that are natural inhabitant of aquatic reservoirs. Despite the highest cholera burden in Africa, the aquatic reservoirs of V. cholerae O1 therein, even during ongoing epidemics, remain elusive. To determine if V. cholerae O1 has aquatic reservoirs in Goma, we have attempted to isolate the pathogen from aquatic reservoirs in Goma using environmental survey. Here we report the isolation and characterization of toxigenic V. cholerae O1 from aquatic reservoirs in Goma.

**Methods:** To detect toxigenic V. cholerae O1 from aquatic reservoirs in Goma, water samples (250 ml) were collected monthly from 15 fixed sites

and immediately transported to the microbiology laboratory in Goma. Using conventional microbiological culture, serological and PCR techniques each water sample was processed for the isolation and characterization of V. cholerae O1 as described previously. On receiving strains at the Emerging Pathogens Institute (EPI) in the University of Florida, we further confirmed V. cholerae strains.

**Results:** We processed 321 water samples from 15 sentinel sites for the detection of V. cholerae O1 strains; of 321, 5 (1.56%) water samples were positive for V. cholerae O1 Ogawa. While 2 V. cholerae O1 were isolated from two distinct fixed sites, one site yielded 3 strains at three different months. To our knowledge, we are the first to report the isolation of toxigenic V. cholerae O1 from aquatic reservoirs in Goma.

**Conclusions and future directions:** Detection of toxigenic V. cholerae O1 from multiple sites suggests that V. cholerae has established aquatic reservoirs in Goma. We are performing whole genome sequencing of these 4 isolates; the genome of these environmental strains will be compared to that of contemporary clinical V. cholerae O1 strains collected in Goma. Comparative whole genome sequence analysis will allow us to determine the relationship between environmental and clinical strain and help us design preventative/predictive approaches to mitigate cholera in Goma and potentially globally.

**10. MECHANISM OF PERSISTENCE OF VIBRIO CHOLERAE O1 IN HAITI DURING CHOLERA LULL PERIOD**

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

**Introduction:** The ancient pandemic disease cholera is a major public health threat for socially and economically disadvantaged people lacking safe drinking water, sanitation, and hygiene. Despite one of the poorest countries in the world, Haiti, however, never witnessed cholera in at least 100 years. That changed in 2010 when Peace keeping troop of Nepal introduced cholera in Haiti. Initial wave (2010-2019) caused an estimated 820,000 cholera cases with 10,000 deaths. Cases were not reported between 2019-2022 contributing over 3 years of lull period; the disease has re-emerged in September 2022. V. cholerae is a natural component of aquatic reservoirs and our group has monitored the presence of V. cholerae in Haitian aquatic reservoirs effective 2012. Based on our environmental survey, we here provide evidence of dynamic mode of persistence of the pathogen while contributing to the recent wave of the disease.

**Methods:** Using environmental survey we have collected water samples from ~80 environmental sites across Haiti effective 2012-2021. The water sample from each site was processed for V. cholerae O1 strains using conventional culture, serology and PCR techniques as described previously. Once the strains were confirmed, each strain was subjected to its ability to form biofilm formation and lytic phage susceptibility testing as described previously.

**Results:** We isolated 70 V. cholerae O1 strains from Haitian aquatic reservoirs with 33 toxigenic and 37 non-toxigenic O1 strains. Of toxigenic strains 29 and 4 were found as Ogawa and Inaba serotype, respectively. Of non-toxigenic strains 9, 11 and 17 were serotyped as Ogawa, Inaba and polyvalent-positive, respectively. Intriguingly, decline of cholera cases was correlated with increasing number of non-toxigenic strains. Biofilm assay exhibited that Inaba serotype, regardless of its toxigenic status, produced poor biofilm compared to Ogawa serotype suggesting its weak adaptability to aquatic reservoirs in Haiti. Polyvalent positive Ogawa serotype produced the highest biofilm. Strikingly, all non-toxigenic O1 strains (Ogawa, Inaba, and polyvalent-positive) were resistant to lytic phage infection. In summary, V. cholerae maintains a subset of toxigenic V. cholerae O1 in aquatic reservoirs while promoting increased numbers of non-toxigenic O1 strains during cholera lull period, the latter might provide shelter (using robust biofilm and resistant to phage infection) of toxigenic strains to initiate next wave of cholera.

**Conclusion:** V. cholerae Inaba serotype appears to be not adaptable to Haitian aquatic reservoirs. However, the presence of both toxigenic and non-toxigenic V. cholerae O1 might be of benefit for recurrent cholera in Haiti.

## 11. PROTECTIVE ANTIBODY RESPONSES PRODUCED BY EXTRACELLULAR VESICLES ISOLATED FROM SALMONELLA INFECTED MACROPHAGES

**Lisa E. Emerson** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Hailey Barker** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Terri Tran** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Samantha Barker** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Samantha Enslow** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Mark Ou** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Carol Hoffman** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **Andrew L. Rainey** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **David W. Pascual** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **Mariola Edelmann** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

**Introduction:** Non-Typhoidal Salmonella (NTS) causes over 95 million infections each year, and despite the severity and disease burden there is no approved vaccine to combat infection. A potential vaccine strategy that has not been deeply explored, are extracellular vesicles (EVs) produced during NTS infection.

**Methods:** To study the role of EVs isolated after NTS infection, we infected macrophages with Salmonella and isolated EVs using ultracentrifugation. Next, we treated 35 mice with either PBS control, live Salmonella vaccine (ΔaroA) control, EVs isolated from NTS infection of

macrophages, or disrupted EVs; and collected blood and stool samples to assess the effect of EVs on mucosal immunity. We performed ELISAs to measure IgG in serum and IgA in stool. Finally, we challenged mice orally with NTS.

**Results:** Our analysis of serum IgG and fecal IgA showed a significant increase in Salmonella-specific antibody responses in the EV treated mouse group. IgG and IgA increased over time for 12 weeks in both live vaccine and EV treatment groups. Challenge results showed that mice with protective IgA and IgG responses had a lower bacterial burden and increased survival compared to PBS control mice and mice given disrupted vesicles.

**Conclusions:** Our results display the ability of EVs isolated during Salmonella infection to generate Salmonella-specific protective antibody responses. Our findings demonstrate a previously unknown role of EVs in bacterial infection and their potential as a vaccine strategy. Understanding the full immune potential of EVs will aid in EV vaccine approaches to fight the bacterial infection.

**12. PROTECTIVE IMMUNE REPONSES GENERATED FROM ANTIGEN-CONTAINING EXTRACELLULAR VESICLES**

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Salmonella Typhimurium is a gram-negative intracellular bacteria that causes foodborne illness. Salmonella infects a variety of cells, including antigen-presenting cells such as macrophages. Our laboratory has found that Salmonella-infected macrophages secrete extracellular vesicles (EVs) that can have immunomodulatory functions. EVs can package various cargo including proteins, nucleic acids, metabolites, lipids, and Salmonella antigens. We hypothesized that EVs that package Salmonella antigens are sufficient on their own to stimulate immune responses in study animals. Our previous work has characterized antigen contents from Salmonella-infected macrophages; however, the extent to which EVs generate protective antibody response has not been fully explored. IgA is an

immunoglobulin produced in response to a specific antigen. The presence of IgA responses may indicate immune protection against an organism, such as Salmonella. The purpose of this project was to determine if EVs made by macrophages during Salmonella infection generate pathogen-specific IgA responses. To study this, we dosed mice with EVs, a live vaccine strain of Salmonella as a positive control, or PBS as a negative control. Mouse stool was collected each week and analyzed for IgA responses. Fecal IgA titers showed similar responses between EV and live vaccine treatment groups, while PBS mice had no measurable IgA tigers. Overall, our results suggest EVs from Salmonella-infected macrophages are able to induce Salmonella-specific fecal IgA responses, which could be protective. Future work will investigate the level of protection provided by the IgA antibodies.

**13. RE-EMERGENCE OF CHOLERA IN HAITI LINKED TO ENVIRONMENTAL V. CHOLERAE O1 OGAWA STRAINS**

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**Introduction:** The single-source introduction of toxigenic V. cholerae O1 Ogawa strain on October 2010 in Haiti started a cycle of seasonal outbreaks resulting in nearly 10,000 deaths by January of 2019. However, between February 2019 and August 2022, no further cholera cases were registered. On September 25th, 2022, a new outbreak occurred in the Ouest Department of Haiti, where our group previously demonstrated the establishment of an environmental reservoir of toxigenic V. cholerae O1 in the aquatic ecosystem and its active role in fuelling the epidemic during lull periods.

**Methods:** We investigated the origin of the new outbreak by analyzing the full genome sequence of V. cholerae O1 toxigenic Ogawa strains isolated on October 3rd and 4th, 2022, from two infected individuals with profuse watery diarrhea, vomiting and hypovolemic shock. We performed maximum likelihood phylogenetic analysis of 2,129 toxigenic V. cholerae O1 sampled worldwide, from 1937 to 2022, and in-depth Bayesian phylogenetic and molecular clock analysis of 31 new strains obtained from Haiti in 2018, at the nadir of the previous outbreak, as well as 294 Haitian strains sampled between 2010 and 2017.

**Results:** Our phylogenetic analysis firmly shows the new strains clustering within the Haitian monophyletic clade that emerged at the time of the 2010 outbreak. Our Bayesian phylogenetic also demonstrated that the new strains of V. cholerae cluster shared a most recent common ancestor with a 2018 Haitian Ogawa strain isolated from the aquatic ecosystem in Jacmel, in the South-East of the country, and cluster with the previous Ogawa clade that was circulating in 2015-2016.

**Conclusions:** Our results show that the new outbreak strains originated from strains that have been circulating undetected at sub-epidemic levels in the aquatic environment. Our data strongly indicates that re-emergence of cholera in Haiti is the likely result of a spill-over event at

the aquatic-human interface related to persistence of V. cholerae O1 in the environment.

## 14. ROLE OF CHOLERA INDEX CASE PATIENT IN SPREADING INFECTION AMONG ASSOCIATED HOUSEHOLD MEMBER(S) AS INVESTIGATED IN HAITI

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**Introduction:** Cholera, a secretory diarrheal disease, is caused by toxigenic strains of V. cholerae O1 strains. Once in humans, cholera rapidly spread in household members and in community. While symptomatic cholera is easier to monitor, asymptomatic cases (~75%) is difficult to track for the lack of techniques; tracking asymptomatic cholera

is very important to control cholera. Using a cholera index-household studies in Haiti, we provide evidence that cholera index case transmits the pathogen among family member(s). We performed the study in rural (Gressier) and urban (Gheskio) settings.

**Methods:** Index case and associated household members were enrolled for this study using approved IRB. Stool samples were collected from week 1-4 from cholera Index cases (CICs) who were positive for V. cholerae O1, diarrheal index cases (DICs) who were negative for V. cholerae O1 and associated household members (HHMs). Samples were investigated for V. cholerae O1 using culture, Nested PCR (N-PCR) and 16S rRNA sequencing (16SrRNA-S) assay.

**Results:** Gressier: We enrolled 20 index cases in Gressier with 15 CICs and 5 DICs. All CICs were 100% positive for both N-PCR and 16SrRNA-S suggesting the similarity in three methods. In DICs, 60% and 37.5% were positive for N-PCR and 16SrRNA-S, respectively. We included 41 HHMs from CICs; of 41, 31 (75.6%) members yielded positive result for N-PCR. Of 32 samples subjected to 16SrRNA-S, 12 (37.5%) were positive. We included 20 HHMs from DICs; of 20, 4 (20%) were positive for N-PCR. Of 4 samples subjected to 16SrRNA-S, none yielded positive results. From CICs household members (n=41), we detected 5 (12.2%) V. cholerae O1 strains by culture method; all 5 were positive with N-PCR while 2 of 4 (50%) were positive by 16SrRNA-S. Gheskio: We enrolled 20 index cases in Gheskio with 13 CICs and 7 DICs. All CICs were 100% positive for both N-PCR and 16SrRNA-S. In DICs, 5 of 7 (71.4%) and 4 of 7 (57.1%) were positive for N-PCR and 16SrRNA-S, respectively. We included 44 HHMs from CICs; of 44 HHMs, 29 (65.9%) members showed positive result for N-PCR. Of 18 samples subjected to 16SrRNA-S, 5 (27.8%) were positive. We included 8 HHMs from DICs; 7 of 8 HHMs (87.5%) were positive for N-PCR. Of 4 samples subjected to 16SrRNA-S, 2 (50%) were positive.

**Conclusion:** Our data indicate that cholera has transmitted among household members and there is a clear need for asymptomatic monitoring of cholera in household and community settings.

## 15. SALMONELLA CROSS-CONTAMINATION RISKS BETWEEN TOMATOES AND HARVEST BINS DURING HARVESTING

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**Introduction:** Fresh tomatoes have been frequently associated with salmonellosis in the United States. Contaminated food contact surfaces are a concern as they can be a source for cross-contamination. The purpose of this study was to evaluate the Salmonella's transfer potential between three common harvest bin materials, high-density polyethylene (HDPE), wood and cardboard, and tomatoes.

**Method:** Tomatoes or a HDPE, wood, or cardboard coupon (5x5 cm) were spot inoculated with a Rifampicin-resistant Salmonella cocktail (10$^5$) and dried for 1h. Uninoculated tomatoes or coupons were placed into contact with inoculated items and a 1lb weight was placed on top of each tomato to mimic the pressure of a 25 lb bin. The tomatoes and coupons with the weight were left for 10 min., 3, 6, and 24h at ambient temperature (70°C). At each sample time, the coupons and tomatoes were placed into separate WhirlPak bags with 0.1% peptone. A shake, rub, shake, (30 sec. each) was followed by dilutions, plating onto non-selective media with Rifampicin, and incubation (35°C, 24h). The experiment was replicated 3 times with 3 samples (n=9). Transfer coefficients (TCs) were calculated by dividing the CFU/mL Salmonella on the uninoculated surface by the CFU/mL Salmonella on the inoculated surface and reported as log %TCs.

**Results:** Salmonella transfer between tomatoes and HDPE were significantly higher ($P \leq 0.05$) than between tomatoes and wood or cardboard; tomato to HDPE: 1.4 to 4.2, and HDPE to tomato: 0.2 to 2. Transfer between tomatoes and cardboard ranged from -1.3 to 2 (tomato to cardboard) and -0.34 to 1.5 (cardboard to tomato); no transfer was seen after 6h in either case. Transfer from tomatoes to wood ranged from-1.9 to 1.5; transfer did not increase after 3h. Transfers from wood to tomato ranged from -0.6 to 1.3; transfer did not increase after 10 min.

**Conclusion:** The TCs between the tomatoes and HDPE were higher than transfers from cardboard and wood, indicating that the use of HDPE may result in higher cross-contamination. HDPE remains a viable choice for a harvest bin material as it is considered a more cleanable and sanitizable surface than cardboard or wood.

## 16. SIMULATION OF THE RISK OF MICROBIAL CONTAMINATION FOR DROPPED AND DROOPING GRAPEFRUITS AND STRAWBERRIES WITH INK

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**Introduction:** The Produce Safety Rule of the Food Safety Modernization Act (FSMA), states that growers must not distribute dropped covered produce. The rule does not mention drooping produce (produce in contact with ground but still attached to the plant), but FDA guidance directs growers to consider drooped produce the same as dropped produce. The objective of this study was to investigate the risk of contamination associated with drooped and dropped strawberries and grapefruits, due to the increased possibility of produce damage and contact with the ground.

**Methods:** Two trials (4 fruits per treatment, n=8) for both strawberries (field-packed) and grapefruits (washed but not waxed), were conducted where fruits were drooped or dropped on black ink pads. Strawberries were drooped for 0 (touch), 0.16, 1, and 24 h and dropped through PVC pipe (7.62 cm diameter) from 15.24, 30.48, 60.96, 121.96 cm. Grapefruits were drooped for 0 (touch), 24, 72, 168, 336 h and dropped through PVC pipe (15.24 cm diameter) from 15.24, 30.48, 60.96, 121.92, 182.88 cm. Pictures of each fruit were taken and the percentage of the inked area (PIA; area with risk of microbial contamination) were measured using Image J program.

**Results:** Grapefruit PIA by dropping (16.9±9.8 to 34.3±12.0%) and drooping (1.8±0.6 to 17.5 ± 2.4%), were significantly higher than

strawberry PIA for dropping (8.5±2.7 to 18.9±10.11%) and drooping (2.8±2.2 to 4.4±1.2%). When drooping, time did not significantly ($p<0.05$) impact the PIA for strawberries, but did for grapefruit at 72 h and all times afterwards. When dropped, PIA significantly increased ($p<0.05$) for both fruits as the heights increased. No correlation was found between the weight and the PIA, during drooping or dropping for either fruit.

**Conclusions:** Evaluating the area of fruits impacted by drooping or dropping is an important first step in understanding the difference in food safety risks between drooped and dropped produce.

## 17. SPATIAL AND TEMPORAL PATTERNS OF CAMPYLOBACTER INFECTION AND PROJECTED HABITAT SUITABILITY OF THE GENUS CAMPYLOBACTER IN EASTERN ETHIOPIA

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Infections with Campylobacter species have been associated with environmental enteric dysfunction (EED) and stunting among children in

low-resource settings. However, previous studies on prevalence of Campylobacter infection primarily focused on diarrheal children, which could underestimate the actual prevalence given asymptomatic infections are also very common in such settings.

Here, we leverage the data collected from the Campylobacter Genomics and Environmental Enteric Dysfunction (CAGED) project to characterize the spatial and temporal patterns of Campylobacter infections among infants with/without diarrhea in rural Eastern Ethiopia. A total of 106 infants were randomly enrolled from 10 kebeles of Easter Ethiopia at birth and followed up until approximately 13 months of age. Fecal samples were collected from enrolled infants monthly and from mothers, siblings, livestock (i.e., chicken, cattle, goat, and sheep), and the environment (soil and drinking water) biannually during December 2020 to June 2022.Genus-specific Taqman real-time PCR was used for detection and quantification of Campylobacter, with a cycle threshold (Ct) value of 35 as the cut-off for positive cases. The dataset was divided into 4 groups based on the child age using the k-means clustering approach. Then the Disease Mapping and Analysis Program (DMAP) was employed to generate smoothed prevalence surfaces for each month in each age group with the monthly genus-specific presence/absence data. Temporally, an upward trend of prevalence was observed as the children grew older. Spatially, high-prevalence areas were distributed across the whole study area. Four kebeles in the north, southwest, and south covered regions with persistently higher prevalence over time.

To predict the potential distribution of Campylobacter at the genus level in the study area, we used MaxEnt with multiple environmental covariates to model habitat suitability. Of 106 sites where Campylobacter was recovered from environmental samples, 93 were spatially unique at 1km x 1km, the resolution of covariates. These input data were randomly split into 80% training and 20% testing, and we ran 10 replicates with bootstrapping. The average training AUC of these models was 0.841, with a standard deviation of 0.011. Elevation, slope, and vegetation index were the most important environmental contributors in the models. MaxEnt-

based distribution models identify kebeles in the north were more likely to support the genus, though the distribution was ubiquitous.

These results inform Campylobacter infection patterns and identify target areas with higher risk of Campylobacter in low-resource settings. This further contributes to developing effective interventions to combat this enteric pathogen in the future.

**18. TRANSCRIPTOME ANALYSIS REVEALS THAT AVOIDING THE ENDOCYTIC PATHWAYS IS CRITICAL FOR THE COLONIZATION OF EXTENDED-SPECTRUM BETA-LACTAMASE PRODUCING ESCHERICHIA COLI IN HOSTS**

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Extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae causes numerous infections in humans and animals worldwide. In the previous study, we found that two genes, fliD and pglA, were necessary to break the host barrier, resulting in the colonization of E. coli in multiple hosts. In this study, we investigated the functions of these two genes using an animal model Caenorhabditis elegans. The C. elegans infected with fliD and pglA mutants showed better survival compared to wildtype (WT), indicating that these two genes are necessary for bacterial colonization. Then, transcriptomic analysis was conducted to understand the roles of the fliD and pglA genes that contribute to the survival and adaptation of ESBL-producing E. coli in C. elegans. Genes involved in lysosome activity in C. elegans were the most significantly altered by the infection of both mutants. Furthermore, the fliD and pglA mutant bacteria showed significantly reduced replication within human macrophages. The fliD and pglA mutants showed defective function to avoid the endocytic

pathway, resulting in co-colonization with lysosomes. The results indicate that these two genes are critical for colonization of ESBL-producing E. coli by avoiding the endocytic pathways in the host cells.

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

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Methicillin-resistant Staphylococcus aureus (MRSA) has been identified in hospitalized patients, livestock, wildlife, companion animals, food products, and the environment. Due to its high transmission and evolution rates, MRSA infection poses a substantial threat to public health and veterinary medicine. However, the host specificity and genetic features enabling MRSA to adapt to a broad host range are still poorly understood. Here, we sought to understand the host-associated genetic

features by applying a machine learning (ML) approach. We included 2,485 MRSA genomes from human (n=2,186) and non-human hosts (n=299) to develop a source-attribution considered ML model. The Support Vector Machine (SVM) model accurately predicted the origin of MRSA based on their genomic contents indicating that the genetic features are important for MRSA host specificity. These important genetic features encode recombinases and virulence and immune evasions factors, such as the tyrosine type recombinase XerC, transposases, staphylokinase (SAK), staphylocoagulase, chemotaxis inhibitory protein (ChIPS), staphylokinase (SCN), and staphylococcal complement inhibitor (SCIN), which were associated with prophages. Notably, seven prophages were suggested to play important roles in MRSA host adaptation. The ϕN315- and ϕNM3-like prophages (belonging to the ϕSa3 family) showed a high prevalence in human-sourced MRSA, whereas StauST398-2-like prophages were greatly prevalent in non-human hosts. Moreover, our in vitro experiments suggested that the excision of ϕN315- and ϕNM3-like prophages may facilitate the infection of MRSA in human hosts by expressing virulence factors. Overall, the ML analysis, revealed that MRSA is a host specialist, mediated by specific genetic features that are associated with prophages and immune modulators, shedding light on the understanding of host-adaptive evolution and complex epidemiology of MRSA infection.

## 20. VIBRIO CHOLERAE INVASION DYNAMICS IN THE CHIRONOMID HOST ARE STRONGLY INFLUENCED BY AQUATIC CELL DENSITY AND CAN VARY BY STRAIN

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**Introduction:** Cholera has been a human scourge since the early 1800s and remains a global public health challenge, 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 may shield the bacterium from environmental stressors and amplify its dissemination. However, the infection dynamics between V. cholerae and chironomids remain largely unknown.

**Method:** In this study, we have developed freshwater microcosms housed with the Chironomus columbiensis to test the effects of cell density and strain variation on V. cholerae-chironomid interactions.

**Result:** Our results show that 1) Chironomid larvae can be exposed to V. cholerae up to a high inoculation dose without observable detrimental effects, regardless of the strain. 2) Successful invasion of V. cholerae into larvae was positively related to inoculation dose and remained stable in a 72-hour test window. 3) Inter-strain variability in host bacterial load after the invasion was highly cell-density-dependent. 4) Chironomid larvae could accelerate the decrease of free-living V. cholerae in microcosms. While the acceleration rate is cell-density-dependent.

**Conclusion:** Taken together, our study has provided novel insights into V. cholerae invasion dynamics in the chironomid larvae with respect to varying doses and strains. The findings suggest that aquatic cell density is a crucial driver of V. cholerae invasion success in the chironomid larvae. Our results also show that V. cholerae-chironomid interactions happen both inside and outside the larvae body. This study provides a novel

system to investigate V. cholerae-arthropod interaction. It paves for future work examining the effects of a broader dose range and environmental variables (e.g., temperature) on such interaction.

## 21. A MULTISTATE ASSESSMENT OF POPULATION NORMALIZATION FACTORS FOR WASTEWATER-BASED EPIDEMIOLOGY OF COVID-19

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**Background:** Wastewater-based epidemiology (WBE) has become a valuable tool for monitoring SARS-CoV-2 infection trends throughout the COVID-19 pandemic. Population biomarkers that measure the relative human fecal contribution to normalize SARS-CoV-2 wastewater concentrations are needed for improved analysis and interpretation of community infection trends. The Centers for Disease Control and Prevention National Wastewater Surveillance System (CDC NWSS) recommends using the wastewater flow rate or human fecal indicators as population normalization factors. However, there is no consensus on which normalization factor performs best. In this study, we provided the first multistate assessment of the effects of flow rate and human fecal indicators (crAssphage, F+ Coliphage, and PMMoV) on the correlation of SARS-CoV-2 wastewater concentrations and COVID-19 cases using the CDC NWSS dataset of 182 communities across six U.S. states.

**Methods:** Obtained CDC NWSS data includes lab processing methods, SARS-CoV-2 wastewater concentrations, estimated population served, flow rate, fecal indicator wastewater concentrations, and corresponding daily COVID-19 clinical case counts. We first analyzed the correlation between the normalization categories and estimated sewershed population to determine which normalization parameter is an accurate population marker. We then assessed the SARS-CoV-2 wastewater concentration with COVID-19 clinical cases from each of our normalization categories. We stratified the correlation coefficients with the COVID-19 clinical cases by molecular analysis method used, i.e., real-time reverse transcription quantitative polymerase chain reaction (rRT-qPCR) and reverse transcription droplet digital polymerase chain reaction (RT-ddPCR), to determine if the analysis technique used can improve the correlation between SARS-CoV-2 wastewater concentrations and COVID-19 cases.

**Results:** We observed a very strong correlation between the sewershed-level flow rate (L/Day) and estimated population served across all study sites (R=0.76-0.96). The flow normalized SARS-CoV-2 wastewater concentrations also produced the strongest correlation with COVID-19 cases (R=0.13-0.90). Correlations from the three human fecal indicators were significantly lower than the flow normalized correlation with COVID-19 cases. Additionally, using RT-ddPCR significantly improved correlation values over samples that were analyzed with rRT-qPCR (Mean difference=0.21, P-value=0.008).

**Conclusions:** Our study has provided the first multistate assessment of population normalization factors for WBE of SARS-CoV-2 using the CDC NWSS dataset. The dataset of 12,445 wastewater surveillance observations across 182 communities located throughout six U.S. states provided depth, breadth, and robustness to our study. Our assessment shows that utilizing flow normalization with RT-ddPCR generates the strongest correlation between SARS-CoV-2 wastewater concentrations and COVID-19 cases. Overall, our assessment has demonstrated an effective population normalization approach for WBE of SARS-CoV-2.

## 22. A SYSTEMATIC REVIEW OF ANTIBODY RESPONSES TO THE LIVE-ATTENUATED INFLUENZA VACCINE

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**Introduction:** The immune response to influenza in humans, and the protection gained by infection and vaccination, varies across time and between individuals. In the 1940s, the first inactivated influenza vaccine was developed to combat this deadly virus. Since then, many iterations of the influenza vaccine have been developed without attaining the “holy grail” – a vaccine that provides long-lasting, broad protection against infection and disease. In our study, we will be focusing on the antibody response to Live-Attenuated Influenza Vaccines (LAIV). LAIVs contain live but weakened influenza viruses and are typically administered through intranasal routes. As is the case with other influenza vaccines, the antibodies generated from LAIV neutralize viruses via hemagglutinin and neuraminidase binding. However, when compared to other flu vaccine technologies, LAIV offers the possibility for a more long-lived immune response against a greater variety of influenza strains.

**Methods:** This systematic review aims to describe the antibody response to LAIVs in humans. Particular questions of interest include the response of different antibodies (e.g. neutralizing, immunoglobulin), antibodies in

different compartments (e.g. serum, mucosal), the induction of cross-reactive antibodies, and predictors of antibody response. We plan to conduct a pooled analysis using meta-regression to estimate seroconversion rates to strains contained and not contained in the vaccine, and correlation between antibody responses in different compartments.

**Results:** Based on the presence of key search terms, 1,625 studies were selected for inclusion in the initial title and abstract screening. This resulted in 391 studies that were included in the full text review. From this pool, 257 (including 209 primary sources) will undergo data extraction. In this poster, we describe the search terms, excluded papers, a brief overview of included studies, and an initial description of extracted data.

**Conclusions:** Analysis of the extracted data will aid in understanding how LAIV initiates antibody responses across various subpopulations. This insight into the short and long-term protection offered by LAIV can be a step forward in developing the most protective immunization regimens and recommendations.

## 23. ANALYSIS OF SARS-COV-2 INTRA-HOST EVOLUTION IN PERSISTENTLY INFECTED PATIENT WITH LONG READ SEQUENCING AND PHYLOGENETIC ANALYSIS

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**Introduction:** The current COVID-19 pandemic has resulted in over six million deaths globally. Despite the development of effective vaccines, new cases continue to precipitate due in part to the emergence of Variants of Concern (VoC) which have necessitated the development of new vaccine boosters. It is likely that VoC emerge within the context of persistently infected COVID-19 patients, whereby virus is not efficiently cleared by the immune system and subsequently has ample time to accumulate immune escape mutations and other VoC defining mutations. Intra-host evolution in these patients leads to a heterogeneous viral quasi-species which necessitates investigation at the single molecule level to elucidate patterns of evolution contributing to the emergence of these mutations.

**Methods:** Here we investigate the spike (S) gene coding region at the level of single viral copies by employing a method first developed in Ko et al. 2021 which utilizes Pacific Biosciences (PacBio) Single Molecule Real Time (SMRT) long read sequencing combined with Unique Molecular Identifiers (UMI) to sequence longitudinal samples collected from a persistently infected (over 300 days) COVID-19 patient. A series of saliva samples were collected from the patient at 47, 54, 70, 78, 280, and 297 days since initial positive test from which RNA was extracted, and a 4.2kb

region containing the spike coding region was reverse transcribed to generate cDNA which included the 8bp UMI. SMRT bell adapters were ligated to amplicon inserts generated from the 8bp UMI containing cDNA which were subsequently sequenced via PacBio SMRT sequencing with a 30-hour movie time on a Sequel IIe machine in CCS mode. Resulting Hi-Fi sequences were quality filtered and binned by the unique UMI sequence corresponding to reads originating from individual viral particles. Representative sequences for each UMI bin from each time point were aligned with MAFFT and maximum likelihood phylogenetic trees were generated using IQ-TREE.

**Results:** The resulting phylogenetic tree indicates that there is not a clear intra-host evolutionary structure until day 297, at which time a monophyletic clade is seen to emerge and replaces earlier variants. Additionally, it appears that the viral population experienced multiple bottleneck and emergence events over the course of the infection, as seen in shifting diversity between subsequent time points in conjunction with continual divergence along longitudinal sampling.

**Conclusions:** Future studies will seek to further characterize the intra-host evolution of SARS-CoV-2 in additional patients as well as in persistently infected immunocompromised patients, in which it is likely that Intra-host evolution unchecked by immune pressures leads to enhanced accumulation of immune escape mutations which may contribute to the emergence of VoCs.

**24. ANTIBODY RESPONSES TO SARS-COV-2 VACCINES IN CANCER PATIENTS OVER THE FIRST TWELVE WEEKS OF TREATMENT**

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**Introduction:** Cancer patients are at risk for grave outcomes if infected with SARS-CoV-2. We have been studying the vaccine efficacy in cancer patients to SAR-CoV-2 vaccines in a cohort of solid tumor (ST) and lymphoma (LYM) patients.

**Methods:** We enrolled 19 ST patients, 10 LYM patients, and 37 healthy controls (CTRLS). All had to be vaccinated against SARS-CoV-2 (prime dose series for mRNA or one dose for Johnson and Johnson (J&J) vaccines. Cancer patients had to be within one month of starting treatment. Blood samples were collected at enrollment, 4-6 weeks, and 10-12 weeks after initial draw. For antibody responses, samples were tested by in-house ELISAs to the receptor binding domain (RBD) of the alpha (□) and omicron (□) variants as well as the nucleoprotein (N) of SARS-CoV-2.

**Results and Conclusions:** Sixty people were enrolled; 53 returned for the second collection, and 47 for with 8 cancer patients remaining for the third collection. One subject had one vaccine dose, 14/66 had two, 41/66 had three, 6/66 had four and 3/66 had 5 vaccine doses. All three recipients that received 5 doses were cancer patients. Sixty-two subjects received mRNA vaccines from either or both manufacturers. The remaining had either the J&J vaccine only (3) or a combination J&J and mRNA vaccine (1). Seventeen of 66 people reported having COVID-19

infection and 10 were cancer patients. Over the time course, there was little change in the mean OD in each respective group for each protein and there was no difference in responses between groups. However, the mean OD values to the □-variant decreased over time in the LYM group from 2.9 to 1.5. Two enrollees had low responses to both the alpha and omicron variants throughout the study. The N protein is a marker for probable infection. Ten cancer patients (1 LYM, 9 ST) reported as having COVID-19 and all had high ODs to the N protein, however six (3 LYM, 3 ST) additional patients had high N ODs. The eight CTRL reported as having COVID-19 had high N protein ODs and another 10 also had high N OD values. Based on these findings, cancer patients, if vaccinated before receiving treatment continue to have consistently high OD values during the first three months of treatment. However, there may be a decrease in the repertoire of responses in some patients. Additional work is ongoing to determine neutralization responses to the vaccines.

**25. CROSS-REACTIVITY BETWEEN RECEPTOR BINDING DOMAINS OF FELINE CORONAVIRUSES AND SARS-COV-2: THE FOUNDATION TO DEVELOPING MINIMALISTIC PAN-COV VACCINES FOR ANIMALS AND HUMANS**

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Feline coronavirus (FCoV), when mutated into fatal feline infectious peritonitis virus (FIPV), displays pathogenic features similar to SARS-CoV-2 (SCoV2), and all these viruses infect cats. The current study was initiated when our specific-pathogen-free, laboratory toms developed unexpectedly high levels of cross-reactive antibodies to the human SCoV2 receptor binding domain (RBD), upon mating with FCoV-positive queens. Multi-sequence alignment analysis of SCoV2 Wuhan RBD and FCoV serotypes 1 and 2 (FCoV1, FCoV2) demonstrated 11.5% amino acid sequence identity and 31.8% similarity with FCoV1 (12.2% identity and 36.5% similarity with FCoV2). The sera from toms and queens cross-reacted with SCoV2 and FCoV1 RBDs, FCoV2 spike-2, nucleocapsid, and membrane proteins, but not with FCoV2 RBD. However, the plasma from FCoV2-inoculated cats reacted with FCoV2 and SCoV2 RBDs, but not with FCoV1 RBD. Additionally, group-housed laboratory cats possessed persistent serum cross-reactivity with SCoV2 RBD over 15 months. Such cross-reactivity was also observed in FCoV1-positive pet cats from two separate households. The SCoV2 RBD at a high non-toxic dose and FCoV2 RBD at 60-400-fold lower doses blocked the in vitro FCoV2 infection, demonstrating their close structural conformations. Remarkably, such cross-reactivity was detected by the T cells from FCoV1-infected cats. Conversely, the cross-reactivity with the FCoV1 RBD was detected by the sera from COVID-19-vaccinated humans. Such cross-reactivities in humans are most likely attributed to the human common cold CoVs (HCCoV-NL-63, HCCoV-229E). Hence, a pan-CoV vaccine for humans should be formulated with HCCoV-NL-63 and HCCoV-229E, instead of FCoV1 and FCoV2. The broad cross-reactivity between human and feline RBDs and the induction of pan-CoV-specific T-cell responses are essential to our vaccine approach for animals. We will combine FCoV1, FCoV2, and SCoV2 RBDs, each in a configuration of trimer RBD-HR (heptad repeats) with highly-conserved, SCoV2 stem helix core (SHC). More importantly, the full-length stem helix (SH) also has known highly-conserved β-CoV-

specific neutralizing antibody epitopes. The addition of conserved pan-CoV cytotoxic-T-lymphocyte epitopes from highly-conserved SCoV2/FCoV major-protease and RNA-dependent RNA polymerase enzymes to the triple RBD-SH-HR should promote pan-CoV-targeted sterilizing immunity in animals, preventing the development of future zoonotic variants. A minimalistic pan-CoV vaccine, with multimodal antiviral immunity, is essential to decreasing the vaccine-induced adverse effects which are prevalent in the FDA-approved SCoV2 spike mRNA vaccines, while generating sterilizing immunity against SCoV2 in humans.

## 26. DETECTION AND ISOLATION OF INFECTIOUS SARS-COV-2 OMICRON VARIANTS COLLECTED FROM RESIDENTIAL SETTINGS

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**Introduction:** Airborne transmission of infectious (viable) SARS-CoV-2 is increasingly accepted as the primary manner by which the virus is spread person to person. The risk of inhalation exposure to the virus is high in enclosed and poorly ventilated spaces. We present a study focused on air sampling within residential environments occupied by individuals with COVID-19.

**Method:** Air samplers (BioSpot-VIVAS, VIVAS, and BC-251) were positioned in primary- and secondary-occupancy regions in the homes of seven volunteers. Additionally, surface swab samples were collected from high-touch surfaces. Air and surface samples were processed by RT-qPCR. Isolation of SARS-CoV-2 in Vero E6 cells and LLC-MK2 cells was attempted for samples with detectable virus. Viable virus was quantified by plaque assay, and sequencing of SARS-CoV-2 was conducted for select samples according to the sampling day.

**Results:** SARS-CoV-2 was detected in 25 of 129 samples (19.4%) by RT-qPCR and isolated from 15 (11.6%) in cell cultures. It was detected in 81.8% (18/22) and cultured from 63.6% (14/22) of samples that were collected using water condensation air samplers. No statistically significant differences existed in the likelihood of virus detection by RT-qPCR or amount of infectious virus in the air between areas of primary and secondary occupancy within residences. The SARS-CoV-2 isolated from all residences was determined by Sanger sequencing to belong to the omicron-lineage variant of concern.

**Conclusions:** Our work provides information about the presence of SARS-CoV-2 in the air within homes of individuals with COVID-19. Information herein builds knowledge about the existence of infectious SARS-CoV-2 in the air. The demonstrated presence of virus beyond primary-occupancy spaces provides health agencies with information to apply to recommendations regarding airborne exposure risks in homes of sick individuals, such as enhancing air exchange rates, operating air purifiers, and using personal respiratory protection devices.

## 27. DETECTION OF SARS-COV-2 IN FLORIDA'S FARMED WHITE-TAILED DEER

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COVID-19, the cause of the recent pandemic, continues to be a substantial threat to global health. SARS-CoV-2 is the novel coronavirus that causes COVID-19 and is capable of infecting multiple domestic and wild animal species. Due to being the most abundant and widely distributed large mammal in North America, the shared homology of their ACE2/S protein binding motif, and their close association with humans, white-tailed deer (WTD) create large concern for anthropozoonosis. Evidence of either current viral infection or presence of SARS-CoV-2 neutralizing antibodies has been found in both wild and farmed WTD in the United States and Canada. Much of the conclusions made from these studies is that zoonotic transmission is occurring in some form between humans and WTD. If this is true, it is likely that transmission of SARS-CoV-2 is occurring between Florida farmhands and their deer. We therefore hypothesize that farmed Florida WTD are, or previously have been, infected with the SARS-CoV-2 virus. Respiratory secretion swabs (n=231), lung tissue (N=203), and serum (N=350) collected from farmed Florida

WTD between November 2019—June 2022 have been analyzed for either current SARS-CoV-2 infection by RT-qPCR, or for the presence of SARS-CoV-2 neutralizing antibodies using a surrogate viral neutralization test (sVNT). Our results indicated low prevalence of SARS-CoV-2 infection within Florida's farmed WTD. From the samples analyzed using RT-qPCR, 1.29% (N=3/231) of respiratory secretion swabs tested positive for SARS-CoV-2. It was determined that the viral RNA from the positive samples were of the Delta lineage B.1.617.2, the dominant strain circulating within Florida's human population at that time. Further, the sVNT results showed 0.003% (N=1/350) of serum contained specific neutralizing antibodies, indicating exposure of farmed WTD to SARS-CoV-2 is low. Additional surveillance will be conducted using samples collected from wild Florida WTD to compare viral prevalence of the two populations and to better understand the factors influencing SARS-CoV-2 infection in WTD.

**28. EVALUATING TARGETED COVID-19 VACCINATION STRATEGIES WITH AGENT-BASED MODELING**

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**Introduction:** During outbreak or pandemic situations, public health agencies respond with various interventions to contain and mitigate spread of the pathogen. When available, vaccination can be a useful strategy to reduce both transmission and infection severity. However,

different deployment strategies for vaccination vary in effectiveness, and in ways that may depend on vaccine performance and the natural history of the infection.

**Method:** We evaluate approaches to vaccine distribution using an agent-based model of human activity and COVID-19 transmission calibrated to detailed trends in cases, hospitalizations, deaths, seroprevalence, and vaccine breakthrough infections in Florida, USA. We compare the incremental effectiveness for four different distribution strategies (Infection-risk prioritization, age prioritization, risk prioritization and a standard mass vaccination) at four different levels of vaccine availability, reflecting different income settings' historical COVID-19 vaccine distribution. Three were chosen to represent low-, middle- or high-income countries world-wide, and we also evaluated strategies based on data specifically for the USA, which had particularly fast early uptake of the vaccine, followed by slower-than-HIC uptake during the second half of 2021.

**Results:** Our analysis indicates that the best strategy to reduce severe outcomes is to actively target high disease-risk individuals. This was true in every scenario, although the advantage was greatest for the middle-income-country availability assumptions, and relatively modest compared to a simple mass vaccination approach for rapid, high levels of vaccine availability. Ring vaccination, while generally the most effective strategy for reducing infections, ultimately proved least effective at preventing deaths. We also consider using age group as a practical, surrogate measure for actual disease-risk targeting; this approach still outperforms both simple mass distribution and ring vaccination. We also find that the magnitude of strategy effectiveness depends on when the assessment occurs (\eg, after delta vs. after omicron variants). However, these differences in absolute benefit for the strategies do not change the ranking of their performance at preventing severe outcomes across vaccine availability assumptions.

**29. PRELIMINARY CHARACTERIZATION OF A NOVEL RODENT PARAMYXOVIRUS**

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The family Paramyxoviridae is comprised of enveloped RNA viruses that infect mammals, birds, reptiles and fish. There are several paramyxoviruses that affect humans, including measles -, mumps -, and parainfluenza viruses. Paramyxoviruses pose particular risks to both humans and animals as they are frequently associated with respiratory infections and inter-species transmissions. It is important to identify and characterize these viruses because of their potential to jump species and cause serious infections in humans, as exemplified by Hendra and Nipah viruses. A novel paramyxovirus was opportunistically isolated from the kidneys and spleen of a dead rodent found in Gainesville, and its genome determined through next-generation sequencing. Preliminary phylogenetic analyses established the paramyxovirus group to which the novel virus likely belongs to. Fifteen different cell lines including bat, canine, deer, human, non-human primate and rodent cells, were inoculated to examine cell and host tropism. Avicel cellulose plaque assays were performed on supernatant samples obtained six days post-inoculation to determine the viral titer produced in each cell line, and we developed an RT-PCR test to detect the viral RNA. Preliminary results indicate that this novel paramyxovirus can complete its life cycle in human, non-human primate, and rodent cells, suggesting that the virus may be able to infect humans. We next wish to perform serology tests to explore whether Gainesville, Florida, residents have been exposed to this virus.

**30. VENTILATION AND SARS-COV-2 EXPOSURE IN HOSPITALS AND RESIDENCES: A META-ANALYSIS**

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As SARS-CoV-2 continues to sweep throughout the globe, ventilation has been emphasized by CDC and WHO as an important means to reduce exposure risk. Thousands of publications were screened, and 93 field sampling studies that took place in residences and hospitals were selected and compiled to assess whether higher ventilation rate leads to lower exposure risk. Concentrations of virus in air samples as determined by RT-PCR and the percentage of air samples in which SARS-CoV-2 was detected were the metrics used to infer exposure risk. Locations were categorized by type (hospital and residence) as well as the frequency with which a space was occupied (primary and secondary). Air changes per hour (ACH) representing ventilation rates were averaged within each of the two resultant primary locations. The mean ACH was higher in hospitals (10.6) compared to isolation homes (1.4), and the mean virus concentration in hospital settings (102 copies/L) was lower than that in residences (1.9 x 10^7 copies/L). The mean positivity rate in air samples collected in hospital settings (24.1%) was lower compared to that collected in residential settings (38.0%). Our analysis indicates that SARS-CoV-2 on average may be present in greater concentrations in residential spaces than in hospital rooms, supporting that a higher ventilation rate can reduce the potential exposure risks from the virus in ambient air.

### 31. A FRAMEWORK TO ANALYZE DECISION-MAKING FOR EMERGING EPIDEMICS, APPLIED TO AVOCADO LAUREL WILT

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**Introduction:** The mitigation of emerging diseases, like COVID-19 and citrus greening, often requires the collective action of a community. Here we evaluated how the informal exchange of information about epidemic and economic outcomes can influence the management decisions of individuals and the resulting epidemics in the context of the avocado laurel wilt epidemic in south Florida. We addressed how socioeconomic networks, epidemic networks, policy incentive structures, and social behaviors, combine to influence a) information exchange, b) growers' management decisions, and c) regional avocado health.

**Methods:** We built an agent-based model to simulate laurel wilt epidemic expansion and establishment across south Florida over a 10-year period. The model used parameters specific to observed patterns and the locations and sizes of avocado orchards in south Florida. We simulated disease expansion and information dissemination through multilayer

socioeconomic and epidemic networks and evaluated the effects of "carrot" and "stick" policy incentive structures and behaviors like "stubbornness" in decision making.

**Results:** We found that an increase in social connections resulted in decreased crop health due to the increased sharing of information which reinforced selection of less expensive but less effective management choices. This information exchange was particularly impactful during the lag phase of epidemic expansion, when the cost of disease management outweighed the cost of disease. Managers who were resistant or "stubborn" against adopting these cheaper and less effective management strategies, particularly during the lag phase of epidemic expansion, contributed to greater regional health. In these scenarios, growers responded to policies that imposed penalties on individuals more than to policies which offered financial incentives to individuals.

**Conclusions:** By quantifying varying degrees of stubbornness, we represented key aspects of decision making and its many influences on regional collective action in this novel agent-based model. The model demonstrates the caveats of information exchange across social networks during epidemics, and the valuable role that policy makers and informed educators can have, particularly during the lag phase of epidemic expansion.

## 32. ASSESSING THE IMPACT OF SUCCESSIVE SOIL CULTIVATION ON MELOIDOGYNE ENTEROLOBII INFECTION AND ON SOIL BACTERIAL ASSEMBLAGES

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**Introduction:** Soil cultivation may change the soil microbiome and alter interactions between plants and parasites. The objective of this work was to evaluate temporal changes in plant health, microbiome abundance, bacterial diversity and the plant-parasitic nematode, Meloidogyne enterolobii incidence in two soil fields with different agricultural uses.

**Method:** Soil samples were collected from a commercial tomato production field (agricultural soil) and a single-cultivation strawberry field (native soil). Samples for the second experiment were collected from the same fields the following year. Tomato plants cv. Yearly Girl were grown in a greenhouse and inoculated with M. enterolobii. After 45 days, plants were evaluated for the plant growth parameters, nematode

reproduction, and soil bacterial assemblages were assessed using cultivation-independent sequencing methods (V3/V4 region of the rRNA 16S).

**Results:** Overall the average of fruit fresh weight in the second experiment was 2.4-fold to 14-fold higher than the first experiment. Moreover, there was a 80.5% decrease in eggs present per root system from the first experiment to the second. The relative abundance of bacterial assemblages from Experiment 1 to Experiment 2 changed for most of the top phyla (eg. Actinobacteria, Bacteroidetes, and Chloroflexi) and genera (eg. Bacillus, Streptomyces, and Flavisolibacter) and there was no change in microbial diversity between the two experiments.

**Conclusions:** This study suggests that soil management can lead to an overall decrease in nematode reproduction and better crop yield, as well as a shift in the overall bacterial assemblages.

**33. CURVULARIA AND THE BRAIN: CASE DEMONSTRATION OF OPTIMAL MANAGEMENT**

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**Background:** Curvularia is a ubiquitous fungus found in tropical climates and has been reported to grow on marijuana leaves. Rarely, it can infect humans and propagate from the nasal sinuses into the brain.

**Case:** What appeared to be an invasive mass growing through the ethmoid and sphenoid sinuses into the anterior cranial fossa.

**Results:** Neurosurgery and otolaryngology was planned. Surgeons used a bifrontal craniotomy and endonasal approach for gross total resection. Following resection, the patient was placed on 4 weeks of amphotericin treatment followed by 12 months of voriconazole based on recommendations by infectious disease. The patient has been stable since surgery.

**Conclusion:** Curvularia is a rare but potentially life threatening central nervous system infection that can be acquired from inhalational

marijuana use. This illustrative case shows the importance of aggressive debridement followed by broad spectrum antifungal treatment to optimize outcome. With marijuana's increasing popularity, Curvalaria should be included on the differential diagnosis.

**34. DESCRIPTION OF AMOEBIC DISEASE IN A COLONY OF WESTERN HONEY BEES (APIS MELLIFERA)**

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The amoeba Malpighamoeba mellificae has been associated with clinical disease (amoebic or amoeba disease) in the western honey bee (Apis mellifera) since its discovery in 1916. This amoeba has been shown to damage the Malpighian tubules, which ultimately weakens and may kill the host bee. Here, we describe the detection and characterization of this protozoan in a colony of honey bees in the Yukon Territory, Canada. There was gross melanization of the Malpighian tubules in approximately 14% (7/50) of the adult worker bees dissected for tissue collection. All 15 bees tested positive for M. mellificae via Polymerase chain reaction (PCR). Histologically, nosemosis, a disease caused by microsporidians in the genus Nosema, was diagnosed in all bees examined, and amoebic cysts could be observed in 45% (9/20) of the bees. The Malpighian tubules of heavily infected bees were packed with amoebic cysts, causing dilation of the tubules and attenuation and loss of the tubular epithelium. Our analysis included the phylogenetic description of M. mellificae as a new clade, sister group to the Entamoebidae family, which includes several enteric parasites of vertebrates. Ultimately, this work provides a

foundation for further investigation into the distribution, prevalence, and pathology associated with M. mellificae infection in honey bees.

### 35. GXM-INDUCED INHIBITION OF MICROGLIAL CELL MIGRATION AND CONTROL OF CRYPTOCOCCUS NEOFORMANS BRAIN INVASION IN VIVO

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Cryptococcal meningitis is an opportunistic disease particularly affecting immunocompromised patients. After inhaling yeasts/basidiospores, the encapsulated fungus Cryptococcus neoformans migrates from the respiratory system into the circulatory system. Ultimately this systemic infection has a predilection for the central nervous system, where the fungus invades and colonizes the parenchyma of the brain tissue. The mechanisms of crossing the blood-brain barrier have been characterized, however it is not well defined how the fungus is able to persist in the brain tissue to develop cryptococcal lesions or cryptococcomas. It is this mechanism of brain colonization that is the central question in this study. As the resident immune cells of the brain, microglia are the critical in this process. To study how the fungus interacts with microglia during colonization, intracerebral infections were employed as the model to evaluate the immune response against fungal brain invasion. We hypothesized that the release of the fungal polysaccharide capsule, which is mainly made of glucorunoxylomannan (GXM), prevent microglial migration and activation. CXCR3-GFP-labeled mice were used to determine the microglial responses to the infection focusing on morphological and cell distribution changes. The cross-section analysis was conducted using confocal microscopy and fiji analysis software along with H&E and periodic-acid schiff stain. In comparison to the uninfected and acapsular cap59 strain infection, brains infected with the wild-type H99 strain displayed an increased density of microglia around the site of infection. In addition, there were microglia morphological alterations.

Future studies will describe the overall immune profile at the site of infection to determine how cerebral cryptococcosis dynamics over time.

**36. IDENTIFICATION AND MANAGEMENT OF BIPOLARIS SPECIES ASSOCIATED WITH FOLIAR DISEASE ON INVASIVE AND WEEDY GRASSES IN FLORIDA**

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Non-native grasses that have been introduced for food, feed or forage have later become invasive weeds. Following introduction, invasive weeds can serve as reservoirs of pathogens capable of causing disease on nearby cultivated crops. A study was conducted to determine if Bipolaris species causing foliar lesions on common weeds and invasive grasses in Florida have the potential to cause disease on crops. Grasses occurring in disturbed areas and with foliar disease symptoms were sampled from four different counties of Florida. We made isolations of conidia characteristic of Bipolaris and identified the resulting isolates using multi-locus sequencing. We identified four Bipolaris species associated with foliar lesions on sampled grasses. Inoculation of wheat seedings with a representative isolate from each resulted in moderate to severe disease. During isolation, we frequently observed co-occurrence of Cladosporium with Bipolaris conidia on same lesion. Further studies were conducted to understand the interaction between these two fungi using competition bioassays and co-inoculation onto wheat seedlings. The results suggest Cladosporium can restrict colony growth of Bipolaris and reduce disease severity. This suggests invasive and weedy grasses serve as reservoirs of Bipolaris that have potential to cause disease epidemic on agronomic crops, while being limited by Cladosporium.

**37. IMPACTS OF ASYNCHRONOUS EMERGENCE OF BATRACHOCHYTRIUM DENDROBATIDIS AND RANAVIRUS IN FLORIDA AMPHIBIAN ASSEMBLAGES**

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As emerging pathogens expand and overlap in geographic ranges, novel interactions can potentially exacerbate declines in already fragile host populations. Alternatively, heightened immune responses of infected hosts may suppress future infections, providing a mechanism for population persistence during new outbreaks. Here we track the interactions between two emerging pathogens impacting North American amphibians: the chytrid fungus Batrachochytrium dendrobatidis (Bd) and Frog-Virus 3-related Ranavirus. We investigate the co-occurrence of these pathogens in amphibian assemblages in Florida over two and a half years, identified factors associated with their emergence using zero-inflated negative binomial models, and quantified the impacts of subsequent disease outbreaks. The emergence of Bd and Ranavirus was synchronous, but patterns of infection and disease varied among sites and species. Ranavirus infections were more prevalent and caused lethal episodes of ranavirosis, resulting in severe population declines of striped newts (Notophthalmus perstriatus). However, when Bd emerged first, subsequent Ranavirus, infections were milder and disease was limited. Co-infections of Bd and Ranavirus were common within striped newts and cricket frogs (Acris gryllus), but the species showed opposite relationships between infection intensities. Our findings provide strong evidence that Ranavirus has driven declines of threatened species in Florida, permanently changing host community composition of sites post-outbreaks. Overall, our results highlight that immune-mediated competition between Bd and Ranavirus may be associated with dampened ranavirosis and population stability. Characterizing

interactions between pathogens can help us design management strategies to change the course in natural outbreaks.

**38. INFECTION WITH A MICROSPORIDIAN PARASITE ALTERS INVASIVE CRAYFISH IMPACTS ON ECOSYSTEM METABOLISM**

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Parasites can have density- and trait-mediated effects on their hosts, which can have indirect effects on ecosystem function [BC1] and structure. We explored how a pathogen may alter the ecosystem impacts of the invasive rusty crayfish (F. rusticus) through these effects. Ecosystem impacts of F. rusticus are well documented with dense populations causing greater impacts (density-mediated) and more active crayfish causing greater impacts (trait-mediated). A microsporidian outbreak in F. rusticus was found to cause a decrease in crayfish activity and corresponded with a significant decline in the population of F. rusticus in Trout Lake, WI. We conducted a 3-week mesocosm experiment to test density- vs trait-mediated indirect effects of the microsporidium on ecosystem metabolism by varying crayfish density and parasite presence. We hypothesized microsporidian infection would reduce F. rusticus ecosystem impacts through a decrease in crayfish activity leading to increased ecosystem metabolism. Gross primary productivity was measured using standard light- and dark-incubation methods. A water sample and cobble from each mesocosm was used to measure water column and benthic metabolism, respectively. We found ecosystem metabolism increased with crayfish density and in mesocosms without

microsporidian infected individuals. Therefore, invasive crayfish impacts on ecosystem function can be reduced by the presence of a pathogen through both trait- and density-mediated indirect effects.

**39. INVESTIGATING THE INFLUENCE OF RUSTY CRAYFISH INVASION HISTORY ON CRAYFISH SYMBIONT 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 symbiont communities and how these impacts may affect invasion success. Our study focused on crayfish symbiont communities in northern Wisconsin, a region where the invasive rusty crayfish (Faxonius rusticus) replaces native virile crayfish (Faxonius virilis). Our goal was to determine whether symbiont richness, prevalence, and composition differ among lakes that vary in rusty crayfish abundance and impacts. We dissected and histologically prepared over 450 crayfish to evaluate symbiont richness, prevalence, and composition, with ad hoc use of molecular tools. We identified pathogens from nine taxonomic groups and found most crayfish harbored at least one symbiont. We found native crayfish harbored a greater number of symbionts groups when they occurred in lakes with rusty crayfish. They were also more likely to be a host to trematodes and psorosperms and had a higher infection burden of these symbionts. Rusty crayfish harbored more symbiont groups in lakes which they occur with virile crayfish and lakes in which their population has recently declined compared to lakes in which they occur alone at high densities. We also found rusty and virile crayfish harbored distinct symbiont communities with specific symbionts more commonly found in each crayfish species. Overall, these data suggest that symbionts play a

role in the success of rusty crayfish invasions and the impacts of rusty crayfish on native crayfish.

**40. JUVENILE COQUI FROGS MOUNT AN IMMUNE RESPONSE TO CHYTRID FUNGUS AT THE COST OF FUTURE FITNESS**

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**Introduction:** Host immunity in amphibians is a complex trait influenced by seasonality and ontogeny. Although theoretical changes in energy allocation are expected, the long-term consequences in fitness have not been addressed in the context of growth and defense. Here, we investigate these trade-offs focusing on the direct-developing coqui frog Eleutherodactylus coqui, a species that recovered from population declines caused by the pathogenic chytrid fungus Batrachochytrium dendrobatidis (Bd). We combined empirical with theoretical approaches to evaluate mechanisms allowing juvenile frogs to survive infections, as well as the carry-over effects of the Bd-seasonality interaction. First, we hypothesize that if individuals secreted defenses that inhibit Bd growth (e.g., anti-microbial peptides–AMPs), individuals with higher concentrations will carry lower infections. Second, we predicted that this immune response will vary across life-stages and seasons.

**Methods:** We monitored a coqui population in Puerto Rico for more than one year to measure Bd-burden, -prevalence, and AMPs on the host skin. Then, we explored the effect of the interaction between the pathogen

and seasonality in future fitness using dynamic models. We modeled grow-immunity energy allocation during seasons varying in foraging success and Bd-exposure, where state variables were time from hatching, size (proxy of fitness), and pathogen burden. We used the field Bd-prevalence to parametrize the probability of getting Bd-infected between cool and warm seasons. Using the set of strategies that maximized fitness, we ran four simulations corresponding to frogs hatching at different times of the year to compare growth rates and time to maturity between individuals' mean infections and the time of the year when they hatched.

**Results:** Consistent with our previous studies, Bd-prevalence was higher in the warm season, but Bd-burden increased during the cool season. Surprisingly, earlier life stages often carried higher pathogen loads and also secreted more AMPs per body mass than adults. The dynamic models showed that investing energy in mounting an immune response delayed maturity, which became exacerbated during the cool season.

**Conclusions:** Our findings from terrestrial direct-developing frogs support the idea that juveniles mount immune responses, allowing them to survive. However, investments in immunity had consequences for future fitness, and the additional environmental stress resulted in less growth and a higher Bd-burden. Understanding potential trade-offs between key processes is important because models predict an increase in emerging diseases driven by climate change. Moreover, our theoretical model approach can be extended to simulate disease-mediated trade-offs under future climate change scenarios and across taxa.

**41. NECROPTOSIS IN PLACENTAL MALARIA IS ONLY FOUND IN CHRONIC INFECTION**

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Placental damage and dysfunction are prominent in malaria infection, and are associated with poor birth outcomes. Necrotic death and shedding of the syncytiotrophoblast from the villus core may be a critical factor that influences placental function in this context. The objective of this study was to investigate whether the syncytiotrophoblast undergoes necroptosis during placental malaria. Primary human trophoblast and placental tissue explant as well as placental tissues samples from women and mice exposed to malaria were used to assess markers of regulated necrosis by RT-PCR, western blot and immunostaining.

Our results indicate that necroptosis occurs in placental malaria. Two key markers for this regulated cell death pathway, receptor interacting protein kinase 3 (RIP3) and phosphorylated mixed lineage kinase domain-like protein (pMLKL), were significantly higher in human placenta with active malaria infection, and RIP3 levels were inversely correlated with infant birth weight. Furthermore, in vitro exposure of syncytialized primary human trophoblast to the malaria toxin, hemozoin, induced elevated transcripts for RIP1 and RIP3, which are required for propagation of necroptosis. However, in vitro activation of human primary cytotrophoblast and syncytiotrophoblast as well as primary placental villous explants through the death receptor pathway (treatment with antibodies to tumor necrosis factor, plus cycloheximide and carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]- fluoromethylketone (Z-VAD)), which in other epithelial cell types drives necroptosis, did not induce this cell death pathway; while expression and redistribution of RIP1 within the villus was observed, no RIP3 or pMLKL was detected in cells or explants. Instead, trophoblast apoptosis was observed with this

treatment. Only treatment conditions that included the caspase inhibitor Z-VAD were able to restore syncytiotrophoblast viability to control levels.

While the trophoblast is resistant to necroptosis in vitro under the conditions used in these studies, in vivo this cell death pathway could play an important role in the pathogenesis of placental malaria. Regulation of cell death pathways in the syncytiotrophoblast requires further investigation to establish critical triggers that can precipitate placental loss of function and pregnancy compromise.

**42. NEUTROPHIL ACTIVATION ASSOCIATES WITH MALARIA IN THE HUMAN PLACENTA**

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**Introduction:** Placental malaria (PM) is characterized by accumulation of inflammatory leukocytes in the placenta, leading to poor pregnancy outcomes. Understanding of the underlying mechanisms remains incomplete. Neutrophils respond to malaria parasites by phagocytosis, generation of oxidants, and externalization of Neutrophil Extracellular Traps (NETs). NETs drive inflammation in malaria but evidence of NETosis in PM has not been reported. Neutrophil activity in the placenta has not been directly investigated in the context of PM and PM/HIV-co-infection.

**Methods:** Using peripheral and placental plasma samples and placental tissue collected from Kenyan women at risk for malaria and HIV infections, we assessed granulocyte levels across all gravidities and markers of neutrophil activation, including NET formation, in primi- and secundigravid women, by ELISA, western blot, immunohistochemistry and immunofluorescence.

**Results:** Reduced peripheral blood granulocyte numbers are observed with PM and PM/HIV co-infection in association with increasing parasite density and placental leukocyte hemozoin accumulation. In contrast,

placental granulocyte levels are unchanged across infection groups, resulting in enhanced placental: peripheral count ratios with PM. Within individuals, PM- women have reduced granulocyte counts in placental relative to peripheral blood; in contrast, PM stabilizes these relative counts, with HIV coinfection tending to elevate placental counts relative to the periphery. In placental blood, indicators of neutrophil activation, myeloperoxidase (MPO) and proteinase 3 (PRTN3), are significantly elevated with PM and, more profoundly, with PM/HIV co-infection, in association with placental parasite density and hemozoin-bearing leukocyte accumulation. Another neutrophil marker, matrix metalloproteinase (MMP9), together with MPO and PRTN3, is elevated with self-reported fever. None of these factors, including the neutrophil chemoattractant, CXCL8, differs in relation to infant birth weight or gestational age. CXCL8 and MPO levels in the peripheral blood do not differ with infection status nor associate with birth outcomes. Indicators of NETosis in the placental plasma do not vary with infection, and while structures consistent with NETs are observed in placental tissue, the results do not support an association with PM.

**Conclusions:** Granulocyte levels are differentially regulated in the peripheral and placental blood in the presence and absence of PM. PM, both with and without pre-existing HIV infection, enhances neutrophil activation in the placenta. The impact of local neutrophil activation on placental function and maternal and fetal health remains unclear. Additional investigations exploring how neutrophil activation and NETosis participate in the pathogenesis of malaria in pregnant women are needed.

### 43. DIAGNOSTIC DIFFICULTIES IN NON-TUBERCULOUS MYCOBACTERIAL INFECTION IN LUNG TRANSPLANT RECIPIENTS

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Despite antimicrobial prophylaxis, 34 to 59% of lung transplant recipients experience severe life–threatening opportunistic infections, sometimes caused by Non-tuberculous Mycobacteria (NTM) and Nocardia. Although differentiating these infections is of utmost importance for effective treatment, it can be challenging as they share morphological and growth characteristics. Therefore, culture remains the gold standard for laboratory confirmation. With the aid of novel molecular methods performed on the cultured organisms, diagnosis may be accomplished rapidly and precisely. We present a case of a lung transplant recipient with a pulmonary infection where long, thin, beaded, branching filamentous organisms were seen with Acid-Fast Bacilli (AFB) and Modified Gomori's Methenamine Silver (GMS) stains in bronchoalveolar lavage sample. Cytological characteristics led to the suspicion of a Nocardia species infection. However, culture and the PCR-restriction fragment length polymorphism analysis (PRA) identified M. fortuitum. Additionally, antibiotic resistance was detected, which aided in choosing the appropriate treatment. Therefore, to overcome such diagnostic difficulties to differentiate NTM and Nocardia, a multidisciplinary approach including culture, molecular methods, and cytology is needed to enhance clinical outcomes.

**44. EFFECT OF POST-SUBCULTURE STORAGE CONDITIONS ON THE GENETIC STABILITY OF MYCOBACTERIUM TUBERCULOSIS.**

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Whole genome sequencing (WGS) of Mycobacterium tuberculosis (Mtb) is instrumental to TB control efforts. Retrospective analysis of archived clinical Mtb isolates by WGS requires subculture to extract high molecular weight genomic DNA for sequencing. Commonly, all samples for a project are pulled from the freezers and subcultured, but isolates may be stored at room temperature while DNA extraction is completed on a subset at the time. There is currently limited data on the effect of storage at room temperature on the stability of Mtb bacterial population structure. In this analysis, we used 29 paired isolates to investigate the effect of room temperature storage on changes in the within-sample genetic diversity. All isolates were subcultured in Mycobacteria Growth Indicator Tubes (MGIT) for six weeks. There was a two-week difference between when DNA extraction on the first and second sets was completed. The samples were sequenced together using Illumina 2x150 chemistry and the Novaseq system. We compared the bacterial genetic diversity between sample pairs using the pairwise genetic distance and a minimum spanning tree (MST) to test for an accumulation of single nucleotide polymorphisms (SNPs) between paired isolates. We observed zero SNP difference between the paired samples. In addition, the MST topologies were similar. Overall, the data suggest there was not a significant accumulation of SNPs between sample pairs, confirming that temporary storage at room temperature did not compromise the Mtb bacterial population genetic stability.

## 45. A HAND-HELD DEVICE INTEGRATING SAMPLE PREPARATION WITH ISOTHERMAL AMPLIFICATION FOR REAL-TIME 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 designed an RT-LAMP assay targeting MAYV's non-structural protein (NS1) and demonstrated a limit of detection at 10 genomic copies in 30 min with no cross-reactivity with DENV, CHIKV, and ZIKV. Additionally, we developed two point-of-care (POC) diagnostic devices to carry out the RT-LAMP assay either in real time or at the endpoint. The devices enable

sample preparation at POC. Further, the sensitivity and specificity of the RT-LAMP assay integrated with the devices were studied, and its utility was demonstrated using clinical samples (plasma) and whole blood. We showed that our real-time detection system has the potential to provide quantitative information associated with MAYV viral load and can be used in those regions with limited resources where MAYV infection often takes place.

**46. AN INTERACTOME OF THE FRANCISELLA TYPE VI SECRETION SYSTEM REVEALS NOVEL PROTEIN-PROTEIN INTERACTIONS**

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Francisella tularensis is a causative agent of tularemia, a lethal disease transmitted through aerosolization and arthropod vectors. Owing to it remarkable pathogenicity and ease of transmission, this infectious pathogen is highly concerning to the counter bioterrorism community. Francisella encode a type VI secretion system (T6SS) on a genomic locus termed the Francisella pathogenicity island (FPI). This T6SS injects toxic proteins directly into host cells to facilitate intracellular bacterial growth and virulence. The Francisella T6SS is distinct from other bacterial secretion systems because it is composed of unique proteins that are absent in other bacterial genera. To characterize this system, we set out to define the protein-protein interactions (PPIs) of the Francisella T6SS apparatus. We engineered strains of Francisella novicida to encode epitope fusions with each of the FPI-encoded genes and validated that these strains retain apparatus activity by maintaining their ability to infect cultured macrophages. For the strains that retained T6SS activity, we immunoprecipitated the FPI-encoded proteins, performed quantitative mass spectrometry, and constructed an interactome to map the PPIs of

the Francisella T6SS. To determine if the interaction of two of these proteins are required for T6SS activity, we constructed point mutations in a protein that comprises the T6SS sheath, IglA. These mutations abrogated interaction with the second component of the sheath, IglB, and this blocked T6SS activity and Francisella pathogenesis. These results have provided us with amino acid-level details of T6SS sheath assembly and the interactome we have generated will drive future studies on T6SS PPIs that can serve as future drug targets to block Francisella pathogenesis.

**47. ANALYZING SPATIAL AND TEMPORAL PATTERNS OF DESIGNATED MALARIA RISK AREAS IN NEPAL FROM 2018 TO 2021**

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**Background:** Nepal is preparing to eliminate malaria by 2026. To evaluate the progress of vector control and prioritize areas for targeted intervention, understanding the recent changing distribution of high and moderate malaria risk areas is vital.

**Methods:** Patterns of designated high and moderate malaria risk wards in Nepal between 2018 and 2021 were analyzed to identify stable and newly generated High- and Moderate-risk wards, using the Spatial Temporal Analysis of Moving Polygons (STAMP) method.

**Results and Conclusions:** High-Risk and Moderate-Risk wards decreased by about 55% and the number of districts containing these wards also decreased from 20 to 14. However, several stable and new High- and Moderate-Risk wards, mostly in the northwest and the southwest of the country, are apparent, despite intervention efforts. Public health officials should prioritize those wards for malaria surveillance and vector control, and future studies should explore the underlying reasons for persistent risk wards.

## 48. ANALYZING THE IMMUNITY AND ANTIGENIC CHANGES IN DENGUE VIRUSES OVER DECADES

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Dengue is a Flaviviridae virus that is known for causing disease in humans. Recent work by our group has found that dengue viruses vary antigenically, with infection with one virus providing heterogenous protection to other dengue viruses (Science, 2021). Using blood samples from African Green Monkeys, we found that viruses collected in Bangkok, Thailand collected over a 20-year period were antigenically growing more distant from each other over time. In our present work, we aim to characterize the antigenic relationship of viruses using the neutralization response of human sera in order to validate the information from the African Green Monkey map and confirm that DENV are growing more distant from each other as characterized by human immune responses. Blood samples have been collected from individuals living in Thailand for which DENV infection history is observed and well characterized. Using dengue viruses systematically sampled from a viral archive ranging from 1975 to 2015, we will characterize immune responses to viruses collected during individual's life spans and viruses from future and past time periods. Viruses will be selected from ~4000 sequenced viruses from our viral archive. We will use the Plaque Reduction Neutralization Test (PRNT) to characterize the immune responses of forty-four sera samples from individuals who have had detected DENV detected during longitudinal follow-up from 1998-2002. We will use PRNT responses to build antigenic maps of viruses to see if viruses show similar antigenic relationships in human sera compared to responses from African Green Monkey. We will also assess whether immune responses from this cohort (exposed to viruses from the 1990s) show reduced immune responses to viruses from the 2000s and 2010s compared to those from the 1990s and 1980s. We

will also assess whether genetic sequence information can be used to predict antigenic relationships between viruses. Understanding how dengue viruses evolve will provide insight into controlling and predicting outbreaks as well as how immunity plays a role in the severity of a dengue infection.

## 49. ANTIBODY RESPONSE TO MOSQUITO SALIVARY PROTEINS AS A MARKER FOR EXPOSURE

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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 diseases (MBDs). Mosquito population surveillance is key to assessing mosquito/vector population and determining appropriate interventions when MBD outbreaks occur. However, traditional methods are cumbersome and costly; therefore, there is a need for more efficient mosquito surveillance. The quantitative measuring exposure to mosquito bites (and possible arbovirus exposure), via technologies such as ELISA, has been demonstrated as a promising alternative to trap-based surveillance. Using PRISMA guidelines, a systematic review and pooled analysis were performed to assess the efficacy of detection of human antibody (Ab) response to mosquito salivary proteins (MSP) as presented in the literature. A total of 1353 studies were screened by two reviewers; 103 articles were included in the qualitative synthesis. The pooled analysis included 23 papers met our inclusion criteria, provided individual level human IgG response to MSP via ELISA. We assessed how subject age, Aedes spp. mosquito, antigen type, collection season, population level of mosquito exposure, and Koppen-Geiger climate impact OD values

in separate univariate analyses as well as a multivariate analysis. We found that OD values correlated positively with antigen complexity as well as population level of mosquito exposure. While there is considerable variation between studies (ICC=0.12), using human IgG holds promise in complimenting more traditional mosquito surveillance methods as a proxy for individual and population exposure to Aedes spp. mosquitoes.

**50. ANTI-ZIKA & ANTI-DENGUE ANTIBODIES AMONG MOTHERS AND INFANTS IN HAITI**

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**Introduction:** Zika is a human arbovirus generally causing mild disease in humans, but if infection occurs during pregnancy, it can cause serious birth defects. Zika swept across the Americas in 2015 and 2016 bringing international attention and prompting increased research into the virus. Zika is closely related to dengue, which is endemic to Haiti. The interplay between both viruses and the antibody response they elicit is critical to understanding the ramifications of the emergence of Zika in areas where dengue is prevalent.

**Methods:** As part of the ZIKAction project, dried blood spot samples (DBS) were collected from infants at birth and at discrete time points up to 30 months of age, maternal sera were also collected during labor and though the enrollment period – beginning in 2019, concluding in 2021. The Plaque Reduction Neutralization Test (PRNT) is the gold standard serological test to detect the presence of neutralizing antibodies to a virus within a sample. A PRNT protocol optimized for dengue, with C6/36 (Aedes albopictus) cells and primate sera, was adapted for DBS elution and Zika virus, as well. The PRNT assays were conducted using a Zika virus isolate of Caribbean origin and a Haiti derived Dengue 4, independently.

**Results:** This study provides discrete-time data on the occurrence of anti-Zika and anti-dengue antibodies in infants and their mothers in Haiti. Optimal cutoffs for PRNT90 titers to determine positivity were determined using a mixture model. 74% (95% CI 69%, 78%) and 84% (95% CI 80%, 87%) of infants were seropositive for DENV4 and Zika, respectively. DENV4 and Zika PRNT titer was positively associated within infants, however, some infants were found to have positive PRNT90 titer to one virus and negative to another (10%). PRNT90 titers for both viruses were positively correlated with maternal PRNT90 titers (correlation coefficients of 86% for DENV4 and 90% for Zika). Longitudinal sampling available for a subset of infants showed declining titer over age with greater decay rates in Zika than DENV4.

**Conclusions:** This comparison of the infant DBS and the mothers' sera PRNT outcomes elucidates the contribution of cross immunity and passive immunity to infant neutralization while providing data on flavivirus infection rates. The outcomes provide evidence of ongoing endemic circulation of both dengue and Zika within Haiti. Enhancement assays using the infant DBS will be the next step in identifying if the short-term, passively acquired anti-zika antibodies are able to increase dengue virus uptake.

## 51. BENZALDEHYDE ANALOGS AS VAPOR ACTIVE REPELLENTS AND TOXICANTS

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Spatial repellents are useful tools in the control of public health vectors, as they are capable of preventing host/vector interactions. Unfortunately, few novel chemistries are being produced within this class of these urgently needed products. To date, the current spatial repellent market share is entirely comprised of pyrethroids and poorly effective natural products (e.g., citronella). Accordingly, chemical screening efforts in our labortaories recently found that 3-phenoxybenzaldehyde, a breakdown product of alpha-cyano pyrethroids, was a potent spatial repellent against Aedes aegypti mosquitoes in a test tube repellency assay. In order to identify other potential candidate analogs of this molecule, a set of benzaldehyde analogs was purchased and screened for their repellency and toxicity in vapor phase exposures. Of the ten analogs screened, four produced effective repellency, knockdown, and/or toxicity at the highest screening dose (100 µg/cm2). These were 3-vinylbenzaldehyde, isopthaldehyde, chlorobenzaldehyde, and tert-butyl-benzaldehyde. Individual dose-response analyses were performed for these select compounds in order to better characterize their repellency and toxicity compared to other commercially available toxicants. Chlorobenzaldehyde and tert-butyl-benzaldehyde were the most toxic compounds with LC50 values lower than 100 µg/cm2. Chlorobenzaldehyde, tert-butyl-benzaldehyde, and isopthaldehyde were also the most repellent of those screened with repellency EC50 values below 30 µg/cm2, which mnakes them about as active as DEET. These results demonstrate that benzaldehyde analogs are viable candidate repellent molecules, and may lead to the development of future repellent and vapor toxic vector control tools.

## 52. BLUETONGUE SEROTYPES FOUND IN DECEASED FLORIDA FARMED WHITE-TAILED DEER

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**Context:** Hemorrhagic Disease (HD), caused by bluetongue virus, is an important viral disease of domestic and wild ruminants worldwide. Bluetongue virus (BTV), a member of the Reoviridae family, accounts for the loss of billions of dollars in the agricultural industry and millions of animal lives. There are 26 identified serotypes with differing pathogenicity. In Florida's growing farmed cervid industry, mortality and morbidity from HD are serious concerns among deer farmers as white-tailed deer (Odocoileus virginianus) are extremely susceptible to HD infection, but infection is sub-clinical.

**Aims:** To determine the prevalence of BTV serotypes in white-tailed deer submitted to the University of Florida Institute of Food and Agricultural Sciences Cervidae Health Research Initiative (CHeRI) for post-mortem testing.

**Methods:** RNA from spleen and blood samples were extracted and HD status was characterized by RT-qPCR. BTV positive samples were sent for

cell culture and retested for BTV. Samples with Ct less than 20 were sent for Next Genome Sequencing (NGS).

**Key results:** From 668 tissues collected between 2016-2022 and tested for HD, 118 tested positive for BTV (17.7%). The amount of BTV positive deer varied by year, with 2018 having the largest amount (n=47). Most BTV positive cases occurred during late summer and fall, as expected from the peak in activity for the vector (Culicoides spp.). We have completely sequenced 6 of the BTV positive samples. BTV serotypes found circulating in deceased farmed white-tailed deer samples were BTV-18 (n=2), BTV-10 (n=1), BTV-1 (n=1), BTV-3 (n=1), and BTV-22(n=1).

**Conclusion:** Many serotypes are simultaneously circulating within Florida farmed white-tailed deer. Though BTV doesn't cause as high mortality as EHDV in Florida, it is still an economic burden to the growing deer farming industry. Understanding which BTV serotypes are circulating in Florida is essential for identifying research priorities for captive and wild cervids in Florida.

**Implications and next steps:** Understanding endemic versus exotic BTV serotypes will improve monitoring and response methods, mitigate financial loss and increase livestock productivity in both large-scale operations and emerging industries. We plan to retrospectively test all BTV positives using a new assay our lab is working on that will allow us to identify BTV types in a quicker and more cost-effective manner. Additionally, we plan to completely sequence all positive BTV samples from 2016-now to better understand what types of BTV are circulating across the state, and how they have evolved over time.

**53. CHARACTERIZING THE INSECTICIDAL PROPERTIES OF A COMPOUND ISOLATED FROM POGOSTEMON CABLIN OIL**

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New insecticides and insecticide synergists are needed to better control pestiferous arthropod pests, as insecticide resistance becomes an ever-growing concern. Moreover, the lack of new insecticidal chemistries for the control of public health vector suggests that the field is rapidly approaching product failure and a larger global burden of vector-borne disease. We recently identified a potent natural product from Pogostemon cablin oil (PC1) and characterized its toxicity against Aedes aegypti mosquitoes. PC1 was remarkably insecticidal for a natural product, with a KD50 value of approximately that of p,p'-dichloro-diphenyl-trichloroethane (DDT) and considerably lower than another potent natural product veratrine. We also assessed the ability of this molecule to be synergized by canonical synergists, and potentially synergize currently available insecticides. PC1 was synergized by DEM and TPP by approximately 5.6 and 9.7-fold, respectively. It was also capable of synergizing the effects of the pyrethroid permethrin and the neonicotinoid, dinotefuran, indicating its potential in future synergist and insecticide formulations. While not as toxic as any of the synthetic insecticides screened alongside it in this project, its unique ability to produce knockdown and comparable toxicity to other known insecticides provides compelling evidence for its potential use as a pest control agent.

**54. CHARACTERIZING THE ROLE OF KATP CHANNELS TO MOSQUITO CIRCULATORY HOMEOSTASIS AND ANTIVIRAL IMMUNITY**

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Arboviruses transmitted by mosquitoes pose significant threat to public health around the world. Synthetic insecticides remain the mainstay for mitigation of vector-borne diseases, yet the development of resistance to commercialized mosquitocides highlights the need to identify novel mechanisms to prevent horizontal transmission of arthropod-borne pathogens. Mechanisms to interrupt pathogen-insect interactions that prevent horizontal transmission is a logical approach for mosquito-borne disease control, yet druggable targets to interrupt these interactions remain enigmatic. Pathogens transmitted by mosquitoes must migrate from the midgut to the salivary gland in the hemocoel, during which pathogens are exposed to the hemolymph and directly contact with mosquito immune cells (hemocytes). More hemocytes aggregate toward the dorsal vessel during bacterial infection, which is believed to be a mechanism to increase cellular immunity. Therefore, we hypothesized that manipulation of mosquito cardiac contraction will increase antiviral immune responses driven by cellular immune cells to reduce viral replication in the hemocoel. However, little information exists regarding the functional coupling between cellular immunity and arboviruses within mosquitoes and further, mechanisms to modulate this interaction remains unknown. To bridge this gap in knowledge and provide insights to novel mechanisms for mitigation of mosquito-borne diseases, we injected chemical modulators of inward rectifier potassium (Kir) channels into Aedes aegypti mosquitoes and examined their influence on mosquito cardiac contraction and dengue virus replication. Agonists of ATP sensitive Kir (KATP) channels, pinacidil and VU063, significantly increased the contraction rate from 1.75 Hz for the control to 2.25 and 2.3 Hz respectively at the time point of 10 min, whereas the antagonist

tolbutamide reduced the rate by about 15% when compared to the control. Mosquitoes infected with dengue virus showed significantly reduced virus genomic RNA level from Day2 when provided with pinacidil dissolved in sucrose, whereas mosquitoes treated with tolbutamide had higher viral loads when compared to the control, suggesting Kir channel modulators regulated mosquito immune responses. Taken together, our data suggest that KATP channel modulators can alter mosquito cardiac contraction and dengue virus replication, indicating that KATP channels might serve as a potential intervention target to control arbovirus replication in mosquitoes and horizontal transmission of arboviruses.

**55. DEVELOPMENT OF AN EFFECTIVE STANDARDIZED METHOD TO SURVEY AND COLLECT SOFT TICKS (ORNITHODOROS SP.) IN GOPHER TORTOISE (GOPHERUS POLYPHEMUS) BURROWS**

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The ongoing global panzootic of African Swine Fever Virus has exposed the need for standardized protocols for the collection and screening of Ornithodoros ticks, which are potential vectors of this virus. In this project, our goal was to develop a standardized method for field collection and laboratory processing that was effective and reliable in the detection of Ornithodoros ticks infesting gopher tortoise (Gopherus polyphemus) burrows. First, we compared two field surveillance methods

previously described for O. turicata collection: burrow vacuuming and dry ice trapping. We repeatedly sampled 32 gopher tortoise burrows at Ordway Swisher Biological Station and evaluated reliability and efficiency of each method. Next, we developed two methods for separating and collecting ticks from the rest of the material that was vacuumed. The first method, manual separation, consisted of removing the debris from the sand and manually sifting through both using forceps, brushes, and a blacklight. The second method, sieving, used three sieves with decreasing mesh sizes to separate debris and ticks from the sand. A blacklight was then used on each sieve to find ticks that remained on the mesh. From the 32 burrows sampled, there was a total of 65 collection events – each burrow being sampled 1 to 3 times. We found that 21 of the 32 burrows sampled were infested with soft ticks. Our preliminary results comparing surveillance methods demonstrated that burrow vacuuming was more effective than dry ice traps. The vacuuming method detected soft ticks in infested burrows 74.6% of the time. Our evaluation of dry ice trapping showed it was inefficient in collecting ticks. No ticks were collected from the burrows using this method. The most successful method for tick collection from the vacuumed material was sieving. Not only was this method 35 to 45 minutes faster per sample than the manual separation method, but it also improved the reliability of detecting a soft tick in the collected material. Our preliminary data showed that manual separation had a detection probability of 53.1% while the sieve system had a detection probability of 80.0%. The creation of fast and effective methods to survey and collect Ornithodoros ticks will benefit researchers looking to study these vectors. In the likely event that an African Swine Fever Virus outbreak occurs in Florida, this standard operating procedure will expedite surveillance and research on the disease and its potential for spread.

**56. DEVELOPMENT OF ANTIFEEDANT APHICIDES TO PREVENT PLANT PATHOGEN TRANSMISSION**

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The cotton aphid, Aphis gossypii Glover (Hemiptera: Aphididae), is a destructive agricultural pest, due to photosynthate removal and plant virus transmission. Therefore, we aimed to test the antifeedant properties of small-molecule inhibitors of inward rectifier potassium (Kir) channels expressed in the aphid salivary glands. Two Kir channel inhibitors, VU041 and VU730, reduced the secretory activity of the aphid salivary gland by 3.3-fold when compared to control and importantly, foliar applications of VU041 and VU730 significantly ($P<0.05$) increased the time to first probe, total probe duration, and nearly eliminated ingestion of phloem. The elimination of phloem feeding by Kir channel inhibitors could potentially reduce persistent virus acquisition and transmission in plants. Although promising, foliar applications of chemicals have significant pitfalls including non-target toxicity and increased costs of application. Thus, we tested the capability of a novel natural product based solubilizer to facilitate systemic movement of VU041 and VU730 through evaluation of a novel natural product based solubilizer containing rubusoside that was isolated from Chinese sweet leaf (Rubus suavissimus) plants. Upper leaves were infested with aphids 60-72 hours after treatment with with Kir inhibitor soluble liquid (KI-SL) and systemic movement throughout the plant was verified via toxicity bioassays and changes to feeding behavior through the electrical penetration graph (EPG) technique. Trans-laminar and translocation of KI-SL was confirmed as we observed a significant ($P<0.05$) reduction of aphids able to reach E1 (phloem salivation) and E2 (phloem ingestion) waveforms when compared to the untreated control. Furthermore, VU730 mixed into the soluble liquid (SL) and treated on a single lower leaf

significantly ($P < 0.05$) reduced G phase (xylem ingestion) waveforms on upper (untreated) cotton leaves. These data further support hemipteran Kir channels as a target to prevent feeding and plant virus transmission through novel delivery mechanisms generate plant systemic movement of lipophilic insecticides.

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

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Insecticides remain an integral component of mosquito control operations but sustained use of a limited number of active ingredients (AI) has led to the widespread development of resistance. New types of insecticides will be necessary in maintaining control efficacy. Toxicity screening of metal nanoparticles were conducted via topical applications to assess their viability as potential insecticides Nanoparticles were synthesized from silver nitrate (AgNO3) using essential oils from different plants. Essential oils contain bioactive compounds and metabolites that can act as both a reducing and capping agent to stabilize the AgNP molecule. The resultant AgNP were characterized by spectrophotometer analysis.

**58. EXPERIMENTAL TRANSMISSION OF MAYARO VIRUS BY AEDES AEGYPTI MOSQUITOES**

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Mayaro virus (MAYV) is an emerging mosquito-borne arbovirus and public health concern. We evaluated the influence of temperature on Aedes aegypti responses to MAYV oral infection and transmission at two constant temperatures (20 °C and 30 °C). Infection of mosquito tissues (bodies and legs) and salivary secretions with MAYV was determined at 3, 9, 15, 21, and 27 days post ingestion. At both temperatures, we observed a trend of increase in progression of MAYV infection and replication kinetics over time, followed by a decline during later periods. Peaks of MAYV infection, titer, and dissemination from the midgut were detected at 15 and 21 days post ingestion at 30 °C and 20 °C, respectively. Mosquitoes were able to transmit MAYV as early as day 3 at 30 °C, but MAYV was not detectable in salivary secretions until day 15 at 20 °C. Low rates of MAYV in salivary secretions collected from infected mosquitoes provided evidence supporting the notion that a substantial salivary gland barrier(s) in Florida Ae. aegypti can limit the risk of MAYV transmission. Our results provide insights into the effects of temperature and time on the progression of infection and replication of MAYV in Ae. aegypti vectors.

## 59. EXPLORING THE IMPACT OF YELLOW FEVER IN AMERICA IN UNDERGRADUATE EDUCATION

**Nina Stoyan-Rosenzweig** - Health Science Center Library, Smathers Libraries, University of Florida; **Kimeta Grant** - College of Liberal Arts and Sciences, University of Florida

**Introduction:** This poster highlights the importance of studying the history of medicine in understanding the social and psychological response to epidemic disease outbreaks and particularly, appearance of new diseases where the manner of transmission, morbidity and mortality and treatment are not yet understood. As a study of response to infectious disease shows, there are repeat patterns of response, understanding of which can help human populations understand and perhaps even alter their response to avoid fear, panic and even loss of faith in authority that have consistently been part and parcel of response to epidemics.

**Methods:** An important part of developing this understanding and indeed response is to explore these patterns with undergraduates. In particular this poster highlights an undergraduate course, taught by an undergraduate and mentored by faculty, that explores centuries of American response to periodic yellow fever outbreaks on the North American continent.

**Results:** A description of results focuses on the course and its format and course material.

**Conclusions:** Creation of a successful undergraduate course shows how information on the basic framework of response to epidemic disease provides a means for lessening fear of the unknown that is part of disease outbreaks.

## 60. EXPOSURE OF DENGUE-1 VIRUS TO AEDES AEGYPTI AND SENSITIVITY TO ADULTICIDES

**Robert Aldridge** - USDA-ARS-CMAVE; **Barry W. Alto** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **C. Roxanne Connelly** - CDC - Fort Collins; **Bernard Okech** - College of Medicine, Uniformed Services University; **Blair Siegfried** - Pennsylvania State University; **Kenneth Linthicum** - USDA-ARS-CMAVE

Chemical control of adult vector mosquitoes relies on effective evaluation of active ingredients to determine their performance on healthy vector populations. However, chemical control is frequently used to quell potential disease outbreaks from infected adult mosquitoes. Evaluation of active ingredients of chemical controls to pathogen exposed mosquitoes has not been performed and

therefore the condition of mosquitoes utilized in bioassays evaluating active ingredients is flawed. Here we evaluate LD50 difference in permethrin and malathion to pathogen exposed Aedes aegypti and non-exposed Aedes aegypti mosquitoes to determine if prior pathogen exposure influences insecticide susceptibility.

## 61. HISTORY OF THE TROPICALIZATION OF MALARIA FOR A GLOBAL PUBLIC HEALTH

**Suvendu Ghatak** - Center for the Humanities and the Public Sphere, College of Liberal Arts and Sciences, University of Florida

Malaria is marked as the affliction of the "underdeveloped" tropical world. Drawing upon medical and administrative archives from mid-nineteenth century South Asia and Africa, this study focalizes the historical processes that shaped malaria's designation as a disease of the "backward" tropics. John MacCulloch's pioneering medical treatise on the disease, titled Malaria (1828), mapped the disease across the globe and included the conventionally temperate regions such as Italy, Britain, and Switzerland, as well as tropical places, like India, and the Congo basin. Malaria could mean a specific disease, with the emblematic symptoms of remittent and intermittent fevers, as well as a generalized cause of other diseases because of its ties with the so-called pathogenic exhalations (from its literal meaning of Mala-aria or "bad air") from swamps and marshes. He amalgamated the methodologies of natural history as well as political history in drawing out the medical topography of malaria. The fever was part of the geological process of the "deposition of alluvia" and marker of the "new land" (MacCulloch 1828, 201). In this regard, the tropic was a geologically new site of the planet, and hence more susceptible to malaria. But he also aligned it with a racially essentialized pathogeny: the "moral condition and habits of the people" (MacCulloch 1828, 363). MacCulloch's book became popular with colonial administrators in "locations as distant as Kentucky in the United States and Rajputana in India within two years of its publication", the historian Rohan Deb Roy suggests (Malarial Subjects, 86). In its colonial reception, the medical topography of malaria becomes increasingly embodied, and it becomes not only the disease of swamps or marshes but of putatively stagnant spaces and populaces, stuck in the swamp of time, as I show by referring to the work of colonial physicians, T. Wilson, and James Ranald Martin. Malaria becomes marked as an exclusively tropical disease as the tropics increasingly come under colonial control. This history becomes especially important for effective global action in mitigating malaria as

WHO classifies it as a Neglected Tropical Disease (NTD), and the socio-economic variable becomes an important component of public health.

**62. INVESTIGATING THE INTRODUCTIONS OF ZIKA VIRUS INTO FLORIDA**

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**Introduction:** The first Zika virus (ZIKV) autochthonous transmissions in the American continent were reported in Brazil in May 2015. Cases emerged in Florida in 2016, shortly followed by the report of local transmission.

**Methods:** We have sequenced a total of 63 full Zika virus genome sequences: 25 locally transmitted and 38 travel-related. Full genomes were sequenced with Illumina and aligned with publicly available genomes from Florida and the Americas. Following recombination analyses with RDP4, IQ-Tree and BEAST were used, respectively, for phylogenetics and phylodynamic analyses. Phylodynamics were run with best demographic parameters selected for each Florida clade.

**Results:** Florida epidemic originated from more than ten independent clusters reflecting multiple and simultaneous introductions from Mexico, Colombia, Brazil, Venezuela, French Guyana, Honduras, Nicaragua,

Dominican Republic, Cuba, Jamaica, Trinidad and Tobago. Five well supported clades were further examined through phylodynamics. The estimated times from the most recent ancestor (TMRCA) were different for the several Floridian subclades, with two independent local transmission cases diverging from their most closely related non-USA isolates as early as November 2015.

**Conclusions:** Multiple ZIKV introductions occurred at several points in time in Florida from North, Central, South America as well as the Caribbean. The TMRCA for locally acquired cases was estimated to be months before the first reported local transmission, indicating a period of undetected transmission. This highlights the need for models that can help direct resources for surveillance and early case detections. We are modeling the likelihood of a country to export ZIKV to Florida using the posterior probability support of the MCC individual subclades, number of introductions into the state and available travel data. We are also estimating the likelihood of a location in Florida to be a hotspot for local transmission based on Aedes aegypti and A. albopictus population size.

**63. MECHANISTIC PATHWAYS OF TICK EXPOSURE RISK IN NATIVE AND INVADED PLANT COMMUNITIES**

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**Introduction:** Invasive species are known to directly reduce biodiversity, alter ecosystem services, and shift disturbance regimes, but they may also indirectly influence human health by changing environmental conditions and interactions among species. Plant invasions may alter vector-borne disease risk by several mechanistic pathways, including modifying microclimates that influence vector survival or changing habitats to influence reservoir host use.

**Methods:** Here, we used a field experiment and observational data to evaluate multiple mechanistic pathways by which plant invasions may alter vector-borne disease risk using the common disease vector lone star tick (Amblyomma americanum) and the widespread invasive cogongrass (Imperata cylindrica) in the southeastern USA.

**Results:** In the field experiment, ticks survived over 50% longer in areas dominated by the invader compared to those with only native plant species. Invaded areas had lower temperatures and higher humidity that likely reduced tick desiccation. The observational study showed no differences in average tick abundance or wildlife host activity between native and invaded plant communities, although there was a positive relationship between tick abundance and host activity in native areas.

**Conclusions:** Together these results suggest that tick abundance in native-dominated areas is driven by host activity while microclimate conditions resulting in greater longevity is the dominant driver of tick abundance in invaded areas. Greater tick longevity due to favorable microclimate conditions in invaded areas potentially offset relatively lower host use in those areas. Our results demonstrate that plant invaders affect multiple, potentially off-setting mechanistic pathways contributing to tick exposure risk. The complexity of these relationships highlights the need for better understanding of how invasive species and other global change drivers influence abundance of disease vectors and, ultimately, disease transmission.

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

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

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

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

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

**65. POINT-OF-CARE DEVICES FOR DETECTING MOSQUITO-BORNE AND AIRBORNE VIRUSES**

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A new point-of-care (POC) device for detecting both mosquito-borne and airborne viruses has been developed. The device integrates 3D-printed sample preparation with laminated paper-based RNA amplification, using a ball-based valve to control the storage and delivery of reagents. The

device can detect viruses such as Chikungunya, Zika, Dengue, SARS-CoV-2, and influenza viruses in just 50 minutes. This new technology offers a quick, effective solution to the ongoing public health challenges caused by these viruses.

The device consists of three components: a buffer unit, a mixing unit, and a detection unit. The buffer unit holds reagents in 4 wells, each with its own ball-based valve for reagent storage and delivery. The mixing unit slides along the buffer unit, delivering reagents to the detection unit, where RNA is enriched and purified. The detection unit is made of a polycarbonate well layer and a paper pad laminated between two thermoplastic films.

The device under examination was assessed for its ability to detect both airborne viruses, such as SARS-CoV-2 and influenza A, and mosquito-borne viruses, such as ZIKV and DENV, simultaneously. The total time required for sample preparation was 25 minutes, followed by 25 minutes for RT-LAMP amplification. The limit of detection for ZIKV was found to be 0.5 PFU in human urine and saliva samples, and 0.1 PFU in water samples. The device was also successful in detecting 2 genome equivalents for SARS-CoV-2 and 6 genome equivalents for influenza A H1N1.

The accuracy and speed of the device's detection make it a valuable tool for healthcare professionals in detecting and controlling the spread of viruses. In addition, the ability to detect ZIKV in both human saliva and urine samples provides a convenient and non-invasive alternative to traditional diagnostic methods that require blood samples. This can be especially useful in resource-limited settings where access to equipment and trained personnel is limited.

In conclusion, the device's ability to detect both airborne and mosquito-borne viruses simultaneously with a high level of accuracy and in a timely manner demonstrates its potential as a valuable tool in the fight against the spread of viruses. The results of the test further support the use of this device for rapid and effective diagnosis of viral infections in real-world settings.

**66. PRELIMINARY CHARACTERIZATION OF UNUSUAL EPIZOOTIC HEMORRHAGIC DISEASE VARIANTS**

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We are characterizing unusual epizootic hemorrhagic disease virus (EHDV) variants that our laboratory isolated from dead farmed white-tailed deer (WTD) in Florida. The virus is transmitted by biting midges and is the causative agent of epizootic hemorrhagic disease, which is an acute and often fatal disease of wild ruminants including WTD. Until recently, eight EHDV types were identified using serologic methods (such as by use of neutralizing antibody directed against EHDV surface proteins). These viruses are now typed by RT-PCR directed at the cell surface protein genes. But it has become apparent that neither serology nor RT-PCR methods are entirely reliable for identifying EHDV types in clinical samples or for outbreak investigations. This is because each EHDV virion has 10 double-stranded RNA genomes that can reassort ("mix and match") as they are packaged into progeny virions when two or more different virus strains co-infect a host cell. Reassortment often results in new virus variants with altered biological properties, and this can confound vaccination efforts and epidemiological studies during EHDV outbreaks. The EHDV isolates we are characterizing are unusual in their host cell tropism in vitro and RT-PCR tests have generated weak signals,

suggesting mutations at the primer binding sites. Their genomes must be fully sequenced to identify the genetic basis of their altered biological properties. However, sufficient EHDV RNA must be purified from these viruses to obtain complete genome sequences, and to attain that goal, we must amplify the amount of virus by cultivating them in cell cultures. We are thus analyzing the growth patterns of the untyped variants in three cell lines that are typically used for EHDV isolation: Baby hamster kidney (BHK-21), African green monkey kidney (Vero E6), and Aedes albopictus mosquito (C6/36) cells. So far, we have observed virus-induced cytopathic effects (CPE) in BHK-21 and C6/36 cells. In BHK-21 cells, the CPE typically (but not always) consists of formation of cytoplasmic inclusions, followed by cell enlargement or formation of elongated, spindle-shaped cells, leading to cell death. Unlike other commonly studied EHDV strains, the unusual variants have not induced easily discerned CPE in Vero E6 cells. Full-genome sequencing of these untyped EHDV variants will help further our understanding of their origins and evolution. The results of this work will help guide vaccine production and the rationale for usage thereof in deer farms.

**67. REPURPOSING SYNTHETIC HPPD INHIBITOR HERBICIDES TO CONTROL HEMATOPHAGOUS ARTHROPODS**

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Identification of novel target sites and chemical scaffolds for arthropod vector control has been a focal point within the field of insecticide science yet repurposing of established herbicides for control of arthropod vectors has received relatively little attention. 4-Hydroxyphenyl pyruvate dioxygenase (HPPD) is a well-established target for herbicides where it is blocks photosynthetic electron transfer chains leading to leaf bleaching and plant death. Interestingly, HPPD is also highly relevant in arthropods where it is a primary enzyme within the tyrosine degradation pathway.

When hematophagous arthropods digest a blood meal, large quantities of tyrosine are produced and require metabolism to prevent tyrosine-mediated mortality of the insect, which provides support for repurposing HPPD-directed herbicides to control hematophagous arthropods. Thus, the objective of this study was to characterize the toxicity and phenotypic effects of HPPD herbicides to blood-fed arthropods to justify large-scale insecticidal development campaigns targeting enzymes in the tyrosine degradation pathway. Topical exposure of nitisinone to blood fed Aedes aegypti yielded high toxicity with an LD50 of 3.81 ng/insect (95% CI: 3.09 to 4.67 ng; Hillslope: 0.97, r2: 0.99) after blood feeding, yet was non-toxic to non-blood fed individuals. Additional HPPD inhibitor herbicides were tested against A. aegypti and rank toxicity was tembotrione > mesotrione > tebuconazole against blood fed female mosquitoes, but were approximately 30-fold less toxic when compared to nitisinone. In addition to mosquitoes, we assessed the toxicity of HPPD inhibiting herbicides to the Lone Star tick, Amblyomma americanum, through ingestion and contact exposure. Nitisinone was highly toxic to A. americanum with a lethal time to kill 50% of subjects (LT50) of 23 hours at 10 µM. However, topical exposure of nitisinone against blood fed adults yielded only 10% mortality at 1 µg of nitisinone/tick, indicating poor pharmacokinetics. RNA interference (RNAi) of the HPPD enzyme to A. aegypti and A. americanum produced knockdown of 85% and 98%, respectively. HPPD knocked down mosquitoes and ticks both yielded approximately 65% mortality after ingesting a blood meal. Toxicity of additional HPPD inhibitor herbicides to A. aegypti and A. americanum will be presented.

**68. SALINITY EFFECTS ON THE DISTRIBUTION OF CONTAINER BREEDING AEDES SPECIES IN SAINT JOHNS COUNTY, FLORIDA**

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There have been observations that the distribution of Aedes aegypti in St. Johns County (SJC), Florida is restricted to the coastal areas of the county. This led to the investigation of the effects of salinity in the distribution of container-breeding Aedes in SJC.

Weekly abundances of Ae. aegypti and Aedes albopictus at 7 different distances away from the coast were monitored for 10 weeks using Biogents (BG) Sentinel traps baited with BG lures and dry ice. Salinity levels of three potential outdoor Aedes breeding places were obtained bi-weekly at each distance. A laboratory test was conducted by releasing a known number of colonized mosquitoes to replicate cages with ovi-cups of six different salinity levels (0, 0.05, 0.5, 1, 5, 10 ppt) for preferential oviposition. Adult emergence from different salinity levels was monitored for 5 weeks and the cumulative adult number was used in the analysis.

Ae. aegypti abundance was significantly higher up to 3 km from the coast compared to the other distances ($P<0.05$ for all with $P<0.0005$ for most). Similarly, significantly higher salinity levels were red=corded up to 3 km. Ae. aegypti abundance was positively correlated with the salinity (ranged from 0.04-0.7 ppt), measured 1-week ($r=0.34$, PP<0.0005) and 2-weeks ($r=0.31$, $P<0.0005$) before the trap collection. In contrast, Ae. albopictus abundance was negatively correlated with salinity measured 1-week ($r=-0.24$, $P=0.001$) and 2-weeks before the trap collection ($r=-0.26$, $P=0.002$). In the laboratory test, the adult numbers of both species were similar across all salinity levels up to 5 ppt. and no adult emergence was observed at 10 ppt. Similar to the field test, the adult numbers of Ae. aegypti did not show any significant correlation with salinity while Ae. albopictus showed a strong and significant negative correlation ($r\geq-0.6$, $P<0.0005$).

The study confirmed the coastal distribution of Ae. aegypti and indicates a positive correlation of distribution with the breeding water salinity while confirming the broader and away coast distribution of Ae. albopictus in SJC. The results warrant further studies to determine other possible factors influencing distribution such as other physico-chemical properties of breeding waters, higher availability of breeding places, higher human population density, etc., or if the wild-type Ae. aegypti has preferentially adapted for higher salinity levels.

**69. SHIFTS IN THE SEASONALITY OF DENGUE ASSOCIATED WITH THE TRANSITION TO ENDEMICITY**

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**Introduction:** Many pathogens exhibit seasonality in the incidence of infection in humans due to abiotic and biotic factors associated with climate seasonality. However, the timing of peak incidence of emerging pathogens often differs starkly from post-emergence seasonal patterns (e.g. influenza in 1918 and 2009). Here, we examine the seasonality pattern of dengue in Brazil to determine whether shifts in dengue season have occurred and are consistent with effects mediated by changes in immunity over time.

**Objective:** Assess the seasonality of dengue in each province of Brazil over a 15 year period to determine if the timing of the dengue season has changed as immunity to dengue in the population accumulates.

**Hypothesis:** There is a shift in the seasonal timing of dengue due to the accumulation of immunity to dengue over time and a shift towards endemic transmission after the re-emergence of dengue in Brazil in 1986.

**Method:** Analyze monthly time series from 1999 to 2014 of reported cases of dengue from Brazil surveillance data using multiple metrics of the timing of annual increases in dengue (timing of peak, splines, wavelet spectra) and its association with secular time. Associate changes in the timing of the dengue season with climate (temperature, precipitation, humidity). Reconstruct age-specific rates of immunity over time using infectious disease transmission models. Simulate the potential impact of accumulation of immunity on the timing of seasonal peaks to understand mechanisms that may be driving the change.

**Result:** We found that 25 of 27 provinces across Brazil experienced a delay in the timing of seasonal peaks in dengue incidence with an average delay across all provinces of 2.5 days per year. Splines fit to the timing of peaks using generalized additive models show a non-linear delay in peaks consistent with the linear trend. Temperatures increased throughout Brazil, but only subtle changes in the timing of season have occurred. Mechanistic models incorporating changes in immunity show shifts in the timing of the dengue season consistent with observed data whereas increases in temperature shift the season earlier (inconsistent with observations).

**Conclusion:** We found evidence for a shift in dengue seasonality towards later times of year and simulation models suggest that this change is consistent with the slow accumulation of immunity in the Brazilian population over the last thirty years. Our findings may help us build intuitions for the changes in seasonality that we might expect for other emerging pathogens (Zika, influenza, SARS-CoV-2).

**70. SPATIOTEMPORAL INVESTIGATION OF HUMAN DENGUE VIRUS IN EL ORO, ECUADOR**

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**Introduction:** Dengue is a disease caused by dengue virus (DENV) infection transmitted primarily by Aedes spp. mosquitoes. Post-infection immunity for one dengue serotype does not protect against all four known, leaving previously infected individuals living in high-risk areas at continued risk for re-infection. Ecuador has been characterized by historically high DENV incidence, particularly in the southern lowlands including the coastal province of El Oro. Despite local elimination in the 1950s, decreased vector control in the 1970s led to difficulties in sustained elimination and pathogen reemergence. Continuing dengue activity in El Oro highlights a potential need for spatiotemporal analysis, to explore when and where to target intervention. Spatial scan statistics can detect disease clusters, which are statistically significant occurrences of a high number of events, occurring in space and/or time. Prospective analysis monitors forward-looking sequential changes in disease rates through continuous data collection to identify cluster emergence, while retrospective analysis incorporates evaluations of events in space-time to identify cluster locations and/or durations. This study examined spatiotemporal clustering of DENV cases in El Oro, Ecuador, using a long-term case data set.

**Methods:** Weekly DENV case data reported from 2003 to 2011 were provided by the Ecuadorian Ministry of Health for nine cantons (secondary administrative unit) in El Oro. These were digitized and geospatially joined to GIS layers for analyses. Prospective space-time permutation was performed in the software SaTScan, to test for significant emergent clusters of DENV cases in El Oro across the study period. This was repeated, using retrospective analysis to identify

significant spatiotemporal clusters, and results were compared between methods.

**Results:** Prospective analysis identified three emergent DENV clusters with the primary cluster persisting for 40 weeks, late in the study period (3/27/2011 – 12/25/2011) in the north central region of El Oro. Retrospective analysis identified four DENV clusters with the primary cluster persisting 70 weeks and occurring much earlier (5/11/2003 – 9/05/2004) in the western region of the province. Prospectively identified secondary clusters persisted longer than retrospective clusters. Nearly all clusters in both analyses began in February or March. The primary prospective cluster and secondary retrospective cluster coincided in terms of persistence, timing, and location.

**Conclusions:** Spatiotemporal analysis methods were successful in prospectively detecting cluster emergence and retrospectively identifying clusters of DENV cases in El Oro. Prospective analysis can be a useful tool in continued surveillance of DENV in El Oro and throughout Ecuador to detect emerging clusters. Identifying clusters can inform intervention, targeting strategies for vector control in both time and space. Overall, cluster analysis can be a powerful tool in furthering dengue research and surveillance to protect against increasing regional DENV incidence.

**71. TESTING ABILITY OF AUTOMATIC CAPTURE-MARK-RECAPTURE TRAPS TO PREDICT AEDES AEGYPTI ABUNDANCE IN THE FIELD**

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**Introduction:** Quantifying arbovirus disease risk requires assessing contact probabilities between humans and mosquito vectors. Yet, measuring real-time mosquito abundance in the field remains a challenge. Capture-Mark-Recapture (CMR) approaches have been successful in many animal systems for quantifying abundance in the field. In this approach, animals are captured, marked in a permanent or semi-permanent way, released, and then recaptured. During capture events, three quantities are measured: number of individuals marked (n), number of marked individuals recaptured (k), and total number of individuals recaptured (K). The Lincoln-Peterson Index is then used to provide an estimate for population size, NE, where NE = nK/k).

A major hindrance to using CMR to predict mosquito abundance is the challenge posed by marking captured mosquitos in the field in real-time. Members of our team (D.R-A., T.D., B.W.A., N.B-C., D.A.T.C) previously developed an automatic CMR unit that resolves this issue by efficiently marking mosquitos without human intervention. Mosquitos are attracted with bait, caught and recorded (for species ID, presence/color of marking), and marked with a powdered dye (one of 5+ colors available) before being released. The dye adheres to adults for the duration of their lifespan (30 days), and does not alter mating behavior, blood-meal acquisition, tethered flight, or survival. However, the trap still needs to be

tested for accuracy of field abundance estimates. We plan to test trap function in multiple areas where Aedes aegypti are present (Miami, Florida, San Juan and Ponce, Puerto Rico) to assess the trap's ability to measure mosquito movement between traps and feasibility of its use for monitoring adult abundances.

**Proposed Study:** We aim to test trap function under different climatic field conditions. We will deploy traps at corners of a pentagon with at least 150 meters between immediate neighbors. Each trap will use a different marking color to assess mosquito mobility between traps. A small number of destructive traps will be deployed to ground-truth CMR results.

**Simulations:** Experimental design will be informed by predictions of individual-based model simulations. We simulate mosquito agents that move in three-dimensional space and, upon arriving at a trap, are caught, counted, marked with a color specific to that trap, and released. Parameters are from the literature.

By interfacing experimental field deployments and individual-based modeling simulations, we hope to demonstrate the strengths, and explore the limitations of, the automatic CMR traps for estimating Aedes aegypti abundance in a field setting.

**72. THE BITING RATE OF AEDES AEGYPTI AND ITS VARIABILITY: A SYSTEMATIC REVIEW (1970 - 2022)**

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**Background:** Transmission models have a long history in the study of mosquito-borne disease dynamics. The mosquito biting rate (MBR) is an important parameter in these models, however, estimating its value empirically is complex. Modeling studies obtain biting rate values from various types of studies, each of them having its strengths and limitations. Thus, understanding these study designs and the factors that contribute to MBR estimates and their variability is an important step towards standardizing these estimates. We do this for an important arbovirus vector Aedes aegypti.

**Methodology/Principal Findings:** We perform a systematic review using search terms such as 'biting rate' and 'biting frequency' combined with 'Aedes aegypti' ('Ae. aegypti' or 'A. aegypti'). We screened 3,201 articles from PubMed and ProQuest databases, of which 21 met our inclusion criteria. Two broader types of studies are identified: human landing catch (HLC) studies and multiple feeding studies. We analyze the biting data provided as well as the methodologies used in these studies to characterize the variability of these estimates across temporal, spatial, and environmental factors and to identify the strengths and limitations of existing methodologies. Based on these analyses, we present two approaches to estimate population mean per mosquito biting rate: one that combines studies estimating the number of bites taken per gonotrophic cycle and the gonotrophic cycle duration, and a second that uses data from histological studies. Based on one histological study

dataset, we estimate biting rates of Ae. aegypti (0.60 and 0.56 bite/mosquito-day in Thailand and Puerto Rico, respectively).

**Conclusions/Significance:** Our review reinforces the importance of engaging with vector biology when using mosquito biting data in transmission modeling studies. For Ae. aegypti, this includes understanding the variation of the gonotrophic cycle duration and the number of bites per gonotrophic cycle, as well as recognizing the potential for spatial and temporal variability. To address these variabilities, we advocate for site-specific data and the development of a standardized approach to estimate the biting rate.

## 73. TRANSMEMBRANE PROTEIN MICROTRANSPLANTATION OF ARTHROPOD TISSUES IN XENOPUS LAEVIS OOCYTE: AN EMERGING TOOL FOR DEVELOPING NOVEL INSECTICIDES

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The identification and development of novel insecticide targets has been of consistent interest to the field of insecticide science, yet only a few insecticides that act at novel biochemical targets have been commercialized over the past two decades. Thus, additional tools for the development of novel molecules or identification of novel targets are needed for continued control of arthropod vectors. The Xenopus laevis oocyte as proven to be an efficient system for the heterologous functional expression and two-electrode voltage clamp electrophysiology of ligand-gated and voltage-gated ion channels, yet the previous utility of this method relied on cloning and injection of cloned RNA into X. laevis oocytes. Here, we aimed to expand the utility of TEVC in oocytes by developing methods for testing native insect receptors through microtransplantation of purified insect membranes from neuronal tissues into Xenopus oocytes, which is an established method for vertebrates but understudied in insect systems. This application enabled characterization

of native nicotinic acetylcholine receptors expressed in the synganglion of I. Ricinus. Here, the pharmacological profile of nicotinic acetylcholine receptors expressed in the synganglion of the tick, Ixodes Ricinus were described. Currents induced by acetylcholine and nicotine and the neonicotinoid insecticides were recorded using the two-electrode voltage-clamp electrophysiology technique and data verify that microtransplantation of membranes enables electrophysiological screening of native channels and receptors. The utilization of this technique for testing of novel chemistry in a moderate-throughput manner for identification of potent pharmacophores against insect receptors in the central nervous system or salivary gland will be discussed.

**74. TWEET TWEET TICK: A QUANTITATIVE CONTENT ANALYSIS OF RISK COMMUNICATION ABOUT TICKS ON TWITTER**

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**Introduction:** Cases of vector-borne diseases in the United States have doubled in recent years (Rosenberg et al., 2018). These human illnesses are caused by infected arthropods, such as mosquitoes and ticks, that carry parasites, viruses, and bacteria (World Health Organization, 2020). The tick is one of the leading causes of a large number of infectious diseases (Petersen et al., 2019; Rosenberg et al., 2018). Understanding current messaging related to tick risks and their prevention can contribute to important practical implications and the design of effective communication strategies to improve agricultural health and safety.

**Method:** This study selected Twitter as a representative communication channel because it is one of the most used and well-established social

media platforms, and it is widely used to communicate public emergencies and natural disasters (Kostkova et al., 2014; Sleigh et al., 2021). With quantitative content analysis and analysis of variance, this study aimed to analyze the framing of posts related to tickborne diseases to understand the current discussions on ticks, their diseases, and their prevention on Twitter. This study also examined the visual and textual information that Twitter users are more likely to interact with to ascertain how communication efforts and frames changed throughout the year.

**Results:** The results showed when communicating tick risks on Twitter, both photographs and illustrations/rendered pictures were the most frequently used visual information, and individual persons, news, and health/governmental organizations were the main tweeters. Because of the character limit and the characteristics of social media users, it is difficult for communicators to describe complicated scientific information succinctly on Twitter, causing the majority of tweets to use situational awareness, tool acquisition, and research frames to present tick risks, which are easier for the public to understand. About half of the tweets present tick risks in loss-frame. Tweets with visual aids have higher engagement rates, while those with URLs are not. Lastly, when tweets present the situational awareness frame, most of them are used to presenting high-risk content, while using the research frame is mostly low-risk.

**Conclusions:** This study is the first to analyze visual information and frames of tick risk communication on Twitter to our knowledge, exploring how vector-borne diseases are presented on social media. It is important to understand how social media present emerging diseases, which helps communicators modify existing strategies and communicate the disease and risk prevention.

**75. USING HIERARCHICAL SPECIES OCCUPANCY MODELS TO UNDERSTAND THE DISTRIBUTION OF ORNITHODOROS TURICATA, A SOFT TICK OF EPIDEMIOLOGICAL CONCERN**

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The soft tick Ornithodoros turicata (Dugés), 1876 is a vector of Tick-Borne Relapsing Fever spirochetes, which are pathogens that infect humans worldwide, and a potential vector for African Swine Fever Virus, a globally spreading virus affecting wild and domestic swine. Soft ticks are cryptic and nidicolous which makes surveillance difficult. Thus, despite its epidemiological importance, there are large gaps in our understanding of the distribution and ecology of O. turicata in Florida, where the only described microhabitat for this tick are gopher tortoise burrows. To contribute filling this gap, our aim is to assess habitat suitability and distribution of O.turicata at a landscape scale through Florida. We will sample the occurrence of O. turicata within10x10 km cells gridded over Florida to better understand the climatic and host presence variables that drive the species distribution. Given the cryptic nature of this species, we will use a hierarchical occupancy framework approach in which the detection and occurrence process are modeled simultaneously. In this poster, we present rationale for our methodological approach and sampling design. As occupancy modelling requires repeated surveys of the sampling units, and multiple temporal surveys of our study area is hindered by logistical constraints, we modeled the trade-offs of replacing time for space and the optimum number of replicates per site. We simulated landscapes with plausible occupancy and detection probabilities estimated from preliminary surveys, and randomly sampled

these landscapes with different combinations of sites and burrows per site. We assessed the power and accuracy of each combination to estimate occupancy, detectability, and the influence of covariates over these parameters. Our preliminary survey indicated that ticks can infest a large proportion of burrows within a landscape (84%), and when present, there was a 54% probability of detecting them with the burrow vacuum method we employed. The simulations informed by these data suggest that visiting 110 cells and surveying five gopher tortoise burrows from each cell will provide enough data to obtain a robust estimate of prevalence in the landscape, and the influence of environmental variables on O. turicata occurrence. With this approach, we expect to generate new insights into the ecology of O.turicata, enhancing our capacity to predict its current and future distribution for epidemiological survey purposes.

**76. UTILIZING ANTIBODIES AND FLUORESCENT MICROSCOPY TO EVALUATE SINDBIS VIRUS INFECTION IN THE MOSQUITO AEDES AEGYPTI**

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Prior to transmission by mosquito, Sindbis virus (SINV) must first infect the mosquito's midgut. Due to the small size of viruses, visualization of viral infection can present a hurdle in research. As an alternative to the traditional method of electron microscopy to image viruses, immunohistochemistry can be utilized to locate viral infections and produce images using a confocal microscope. SINV-TaV-eGFP produces green fluorescent protein upon replication. While this can be viewed without any staining, it provides a limited perspective of the arbovirus infection, only indicating where the virus was, not necessarily where it currently is. Utilizing fluorescent antibody staining, a more complete story can be told. A polyclonal primary antibody against SINV and a secondary antibody conjugated with the fluorochrome TX-Red was employed to stain viral proteins in dissected midguts of Aedes aegypti. The colocalization of GFP and TX-Red results in yellow when images are merged. It is shown by the results that TX-Red generally has a larger spread than GFP, indicating that there is virus present, but that the reporter gene for GFP has not yet been expressed. The concentration of

TX-Red around the edges of the foci could also be viral proteins that are moving toward the membrane of the cell before they egress for cell-to-cell spread. In conclusion, while GFP by itself can allow us to locate an infection, it is the combination with immunolabeling that allows for a more precise view of the infection. This detailed view can be used in future studies to better understand the characteristics, spread, and persistence of SINV in mosquitoes.

### 77. BIOCHEMICAL AND STRUCTURAL CHARACTERIZATION OF HAEMOPHILUS INFLUENZAE NITROREDUCTASE IN METABOLIZING NITROIMIDAZOLES

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**Introduction:** Nitroheterocycle antibiotics, particularly 5-nitroimidazoles, are among the most effective choices for combating infections by many anaerobic pathogens, and ongoing efforts include their use for treating parasitic diseases and tuberculosis. The antimicrobial activities of these nitro-containing compounds heavily rely on in vivo bioactivation, mainly by nitroreductases (NTRs) that are widely distributed in bacteria. However, nitro bioactivation can also lead to unwanted outcomes in the long-term clinical usage of 5-nitroimidazoles, including severe toxicities and drug resistance. In this regard, mechanistic studies of nitroreductase-mediated metabolism of 5-nitroimidazoles can offer new insights for addressing these critical issues.

**Method:** We analyzed the distribution of thousands of NTR homologs by sequence similarity network (SSN). We then selected the enzyme from a human pathogen Haemophilus influenzae (HiNfsB) for further biochemical characterizations as well as X-ray structural analysis and site-directed mutagenesis. We finally performed a bacterial susceptibility test

with an E. coli mutant expressing Hinfsb gene against nitroimidazoles under both aerobic and anaerobic conditions.

**Results:** NTRs are widely distributed among bacterial species and HiNfsB represents a group of unexplored oxygen-insensitive NTRs. HiNfsB was able to effectively metabolize six clinically used 5-nitroimidazole antibiotics as well as three nitro-containing anti-TB and immunosuppressive drugs. Besides nitroreduction metabolites, HiNfsB also produced stable, novel dimeric products from three nitroimidazoles. Structural analysis coupled with mutagenesis studies identified four active site residues important to the catalysis and broad substrate scope of HiNfsB, including R20, W71, K119 and Y120. Finally, transient expression of HiNfsB could sensitize the E. coli Δnfsa/b mutant to 5-nitroimidazoles under anaerobic conditions.

**Conclusions:** Our biochemical and structural characterization of HiNfsB demonstrate that it effectively metabolizes a series of structurally diverse nitroimidazoles used in clinical. These results indicate that HiNfsB and its homologs, none of which have been biochemically characterized yet, can contribute to the activation and/or resistance of many nitro-containing drug molecules. This work advances our fundamental understanding of the metabolism, resistance, and toxicity of an important family of antibiotics, guiding their future use and development. Furthermore, the role and mechanistic base of HiNfsB in metabolizing 5-nitroimidazoles uncovered in our work suggest the exploration of the vast majority of unstudied NTRs from different bacteria species for biotechnological and biomedical applications.

## 78. CHARACTERIZING THE ASSOCIATION BETWEEN PSEUDOMONAS AERUGINOSA AND PROTEOSTASIS DISRUPTION IN A CAENORHABDITIS ELEGANS MODEL OF NEURODEGENERATIVE DISEASE

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Neurodegenerative protein conformational diseases (PCDs) are characterized by poor protein folding environments and toxic protein aggregation which lead to neuronal cell death. They encompass a variety of conditions including Alzheimer's, Parkinson's, and Huntington's disease, causing considerable patient suffering and economic burden. Despite these considerations, disease etiology remains largely unknown. Correlational evidence has established a link between bacteria within the human gut microbiota (HGM) and PCD occurrence, but how these bacteria affect PCDs is not fully understood, presumably due to the complexity of the microbiota. Previous research in our laboratory simplified the complexity of the HGM using a single bacterial strain approach using the nematode Caenorhabditis elegans, a bacterivore that can be colonized by a single bacterial species. Our model harbors aggregation-prone polyglutamine (polyQ) tracts that serve as sensors for the protein folding environment and aggregate in response to factors that stress protein folding networks. Among 19 bacterial strains tested, we previously found that P. aeruginosa was the strongest inducer of polyQ

aggregation in C. elegans. To elucidate the role of P. aeruginosa in host protein folding, we have undertaken a whole-genome knockout screen to identify genes in P. aeruginosa responsible for the observed induction of polyQ aggregation in C. elegans. Knockouts which induce significantly fewer aggregates in C. elegans relative to wild type control will be subjected to further rounds of screening. This study then seeks to characterize the effect of the HGM on neurodegenerative PCDs and lay the foundation for mitigating treatments.

**79. DISTRIBUTED COMPACT PLASMA REACTOR STERILIZATION FOR PLANETARY PROTECTION IN SPACE MISSIONS**

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This work presents a proof-of-concept study performed to establish the effectiveness of the Active Plasma Sterilizer (APS) and evaluates it for sterilization applications pertaining to planetary protection. The APS is based on surface dielectric barrier discharge (SDBD), a type of non-thermal plasma which can generate and distribute reactive oxygen and nitrogen species (RONS), mainly ozone, and utilize them for decontamination. SDBD is produced using miniature reactors, Compact Portable Plasma Reactors (CPPRs), which are compact, lightweight and energy efficient. The CPPRs use the Fan and Comb SDBD configurations which have contrasting flow actuation capabilities. They are strategically integrated in the APS to achieve uniform spatial sterilization. Design and fabrication of the first APS prototype (APS.V0) is discussed. Sterilization tests were performed with pathogenic bacteria - Escherichia coli and Bacillus subtilis, and materials - Aluminum, Polycarbonate, Kevlar and Orthofabric, relevant to space missions. Results show that the APS can achieve 4 to 5 log reductions of the two species on four selected materials, simultaneously at 11 points inside the APS, within 30 minutes using power of 13.2 + 2.22 W. Spatial distribution of the sterilization data at the central plane of the APS established that it can uniformly sterilize

several areas of a contaminated surface within 30 minutes. Successful ozone penetration through Kevlar and Orthofabric layers was established using the CPPR in an enclosure with a maximum reduction of 16.17% in ozone concentrations through the layers without using an external agent to assist penetration. Further, preliminary material compatibility tests with SEM analysis of the above-mentioned materials exposed in the APS showed no significant material damage. Thus, this study shows the potential of the APS as a sterilization technology for planetary protection applications with advantages of uniform spatial decontamination, low processing temperatures, low exposure times, lightweight design with no moving parts, ability to decontaminate porous surfaces and compatibility with space mission relevant materials.

**80. DYNAMIC NETWORKS OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN COMMUNITIES DRIVE HOSPITAL TRANSMISSION AS REVEALED BY WHOLE-GENOME SEQUENCING**

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**Background:** Modern methicillin-resistant Staphylococcus aureus (MRSA) transmission pathways have been characterized separately in hospital and community settings using whole-genome sequencing approaches. Given the blurring of the traditional location-based distinctions of MRSA, the genomic epidemiology and networks between these interfaces must be further investigated.

**Methods:** Serial cross-sectional community and hospital sampling were performed at a large tertiary teaching hospital between 2010 and 2019. For community-based sampling, pediatric and adult patients presenting to the emergency department at a large tertiary care teaching hospital with evidence of a skin or soft tissue infection (SSTI) were prospectively enrolled between August 2015 to January 2017. Nasal and SSTI specimens were collected, as well as patient-level geographic, social, and medical epidemiological data via a self-reported survey. For hospital-based sampling, specimens from hospital-acquired MRSA infections collected as part of routine standard-of-care procedures were sampled before (2010), concurrently (2015-2017), and after (2019) the community sampling period. Whole-genome sequencing was conducted on cultured S. aureus isolates. 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. To assess patterns in community MRSA transmission, bivariate analyses were used to test associations of geographic, medical, and social determinants of health with community MRSA microbiology.

**Results:** After whole-genome sequencing on selected community (n=42) and hospital (n=37) isolates, phylogenetic analysis revealed two major clades distinguished by t008 and t002 spa types, where 73.8% (31/42) community samples and 35.1% (13/37) hospital samples were t008. Multiple independent introductions of MRSA lineages from the community to the hospital setting were observed. Community MRSA transmission was sustained without influence from hospital-based isolates. Kernel density estimation models identified high-density geographic clustering of community MRSA isolates beyond the urban center compared to methicillin-susceptible 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.

**Conclusions:** MRSA transmission in hospital settings was introduced from MRSA species with ancestral origins in the community settings. Nosocomial MRSA outbreak prevention strategies should target unique aspects of the community rather than focusing solely on the hospital, with particular attention given to identification of community hot spots, risk behaviors, and possible strain reservoirs.

**81. EVALUATING LIVESTOCK COMMINGLING IN A BRUCELLOSIS RISK ZONE IN KAZAKHSTAN**

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Brucellosis, caused by species of Brucella bacteria, is one of the most widespread bacterial zoonoses (diseases affecting animals and humans) globally, with >500,000 human cases reported annually. Continued surveillance of brucellosis is necessary due to economic impacts and burden from disease in livestock, wildlife, and humans. Kazakhstan, in Central Asia, suffers a heavy brucellosis burden with high human incidence and $45 million spent annually on animal testing and compensation for slaughtered seropositive animals. The ability of this disease to maintained in Kazakh wildlife is currently unknown. This data would be vital to determine best intervention strategies to reduce livestock burden This research project aims to ascertain the frequency/degree of commingling between species of livestock and wildlife in order to contribute to current knowledge on the ecology of brucellosis in Kazakhstan to better inform intervention strategy

development. This study was conducted on several privately owned farms in Kazakhstan, where 12 cameras were deployed from 2018 to 2021 to automatically capture images when motion is detected, resulting in images that were tagged with data concerning species present and behavior. These data were used to create histograms and kernel density estimates of species activity by hour of day and plots of the percentage of commingling between livestock and wildlife species. We found direct commingling occurred most often between cows and horses, sheep and domestic cattle, and sheep, cattle, and horses. Sheep and domestic cattle interactions are the most likely for brucellosis transmission at shared grazing patches; such transmission has not been confirmed in this study. Additionally, while livestock were not seen directly commingling with wild cervids, such as the roe deer, there was evidence of shared patch use between domestic cattle and horses with deer in more forested farms within the study area. The information gained by this study will aid policy makers in the identification of effective and economically efficient intervention strategies to lessen the burden of brucellosis to Kazakhstan and other endemic foci of this disease.

**82. EXAMINATION OF ENTEROBACTER CLOACAE PHYSIOLOGY AND PROTEOME CHANGES DURING GROWTH IN LOW-SHEAR MODELED MICROGRAVITY (LSMMG)**

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While Gram-negative pathogens like Salmonella are known to have increased virulence upon spaceflight and simulated microgravity conditions, the effect of these conditions on the gut microbiota, which can modulate host metabolic homeostasis and immunity, is mostly unknown. E. cloacae, a commensal member of the human gut microbiota, are typically benign but can sometimes cause infections, even causing bacteremia. Given that microgravity and the space flight environment can

have negative impacts on astronauts' immune systems as well as promote increased virulence potential of bacteria, we hypothesize that simulated microgravity will alter the physiology or virulence potential of E. cloacae, which will be reflected by changes to its cellular proteome when grown in simulated microgravity. To this end, E. cloacae were grown in High Aspect Rotary Vessels (HARVs) using a rotary cell culture system (RCCS) in control (normal gravity), inverted normal gravity, and LSMMG (simulated microgravity) orientations. The HARV cultures were removed from the rotating bioreactor at the late exponential growth phase and subjected to growth analysis (optical density (OD), CFU/ml, and pH measurements) and storage of cell pellets for protein analysis. Growth properties of E. cloacae were most similar between LSMMG and the inverted normal gravity orientations. In contrast, the normal gravity orientation yielded higher growth results. Assessment of cytosolic protein profiles under each growth condition by SDS-PAGE suggested that proteins migrating at 150 kDa and 75 kDa in the normal gravity samples were more pronounced compared to the inverted normal gravity and LSMMG samples. Current mass-spectrometry-based proteomic analyses are ongoing. Data collected from this research has the potential for developing a more comprehensive understanding of the effects of simulated microgravity on commensal bacteria, which will help design future studies that assess how the interaction of commensal bacteria and the host immune system changes in this environment.

## 83. HIDDEN INFECTION SOURCES IN A VIETNAMESE ANTHRAX OUTBREAK REVEALED THROUGH COMBINED SPATIAL AND PHYLOGENETIC ANALYSES

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Bacillus anthracis, the bacterial cause of anthrax, is a zoonosis affecting herbivores (livestock and wildlife) and often spilling over into humans. Anthrax has been nationally reportable in Vietnam since 2015 with most cases occur in northern Vietnam. In April 2022, an outbreak was reported in Son La province following the butchering of a water buffalo, Bubalus bubalis. A total of 137 humans from three villages were likely exposed to contaminated meat. Epidemiological investigations suggested a single animal was involved in all exposures. Five isolates were recovered from clinical cases along with one from the buffalo hide, another from associated maggots, and also from soil at the carcass site. Whole- genome sequencing of these isolates was performed, and phylogenetic analysis placed the strains globally, regionally, and locally. All strains from the outbreak belong to the canSNP lineage A.Br.001/002, not previously identified in Vietnam, a lineage that has been identified in nearby China, Thailand, India, Indonesia, and Northern Australia. At the local level, a 25

loci multi-locus variable number tandem repeat analysis (MLVA-25) was used to investigate the relationship between human cases, the soil, and buffalo samples. Locally, four MLVA-25 genotypes were identified from the 8 isolates. This level of genetic diversity is unusual for the limited geography and timing of cases and has not been reported in past literature. The coupled spatial and phylogenetic data suggest this outbreak originated from multiple, likely undetected, animal sources.

**84. INFORMING ONE HEALTH ANTHRAX SURVEILLANCE AND VACCINATION STRATEGY FROM SPATIAL ANALYSIS OF ANTHRAX IN HUMANS AND LIVESTOCK IN HA GIANG PROVINCE, VIETNAM (1999-2020)**

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**Background:** Anthrax, caused by Bacillus anthracis, has a nearly global distribution but is understudied in Southeast Asia, including Vietnam. Here we used historical data from 1999-2020 in Ha Giang, a province in northern Vietnam. The objectives were to describe the spatio-temporal patterns and epidemiology of human and livestock anthrax in the province and compare livestock vaccine coverage to human and livestock anthrax incidence.

**Methods:** Annual incidence rates for humans, buffalo/cattle, and goat (per 10,000) were used to explore anthrax patterns and to compare with livestock annual vaccine variation. A data subset describes anthrax epidemiology in humans by gender, age, source of infection, type of anthrax, admission site, and season. Zonal statistics and SaTScan were employed to identify spatial and space-time clusters of human anthrax.

**Results:** SaTScan revealed space-time clusters in 1999, 2004, and 2007-2008 in areas including the northeast, east, and west of the province. Most human anthrax was reported between July and October. Most human patients were male, aged 15-59 years, handled sick animals, and/or consumed contaminated meat. High case-fatality rates were reported with gastrointestinal or respiratory cases. Our data suggest vaccination in buffalos and cattle reduce the disease burden in humans and vaccinated animals but did not reduce the incidence in unvaccinated animals (goat).

**Conclusions:** This study identified spatial areas of high-risk for anthrax and informs the one health surveillance and livestock vaccination planning in settings of similar context to the province.

## 85. JOINT APPLICATION OF THE TARGET TRIAL CAUSAL FRAMEWORK AND MACHINE LEARNING MODELING TO OPTIMIZE ANTIBIOTIC THERAPY: USE CASE ON ACUTE BACTERIAL SKIN AND SKIN STRUCTURE INFECTIONS DUE TO METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS

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**Introduction:** Acute bacterial skin and skin structure infection (ABSSSI) due to methicillin-resistant Staphylococcus aureus (MRSA) is a challenging condition with reduced treatment options—vancomycin is the preferred choice, but it has non-negligible side effects. We leverage large-scale, real-world electronic health record data collected from United States southern academic clinics to emulate a clinical trial (i.e., 'target trial') and develop a machine learning model of mortality prediction and individual treatment effects (ITE) estimation for patients diagnosed with ABSSSI-MRSA.

**Method:** First, we use propensity score matching to emulate the trial, and create a treatment-randomized (vancomycin vs. other antibiotic) dataset. Next, we use this data to train various machine learning methods - boosted/LASSO logistic regression, support vector machines (SVM), and random forest (RF)- and choose the best-performing ones in terms of area under the receiver characteristic (AUC) through bootstrap validation. Lastly, we use the models to calculate ITE and identify possible averted deaths by therapy change.

**Results:** The out-of-bag tests indicate that SVM and RF are the most accurate, with AUC of 81% and 78%, respectively, but BLR/LASSO is not far behind (76%). By calculating the counterfactuals using the BLR/LASSO, vancomycin increases the risk of death, but it shows a large variation (odds ratio 1.2, 95% range 0.4-3.8), and the contribution to outcome probability is modest. Instead, the RF exhibits stronger changes in ITE, suggesting more complex treatment heterogeneity.

**Conclusion:** This study shows that our causal AI approach can provide models with high prediction accuracy and causality with respect to an intervention of interest, i.e., antibiotic treatment choice.

## 86. MUTATIONS IN A NOVEL TRANSCRIPTIONAL REGULATOR OF FRANCISELLA VIRULENCE, ARAC, RESULTS IN RESISTANCE TO TOLFENPYRAD

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Francisella are highly infectious Gram-negative bacterial pathogens capable of being transmitted from animals to humans. Due to their pathogenicity, ease of transmission, and potential for major public health impact, Francisella are classified as high-priority pathogens by the CDC. The development of Francisella as a bioweapon by multiple countries has spurred interest in the discovery of novel antibiotics that target this and other bacterial pathogens. To identify drugs that inhibit Francisella growth, we screened a library of 1,000 compounds with F. novicida, a surrogate for human virulent species Francisella. Among the most potent inhibitors of F. novicida growth is Tolfenpyrad, a drug previously characterized as an insecticide. To find the molecular targets of Tolfenpyrad, we selected Tolfenpyrad-resistant F. novicida strains and sequenced their genomic DNA. Comparing the genomes of these mutants to wild type revealed point mutations in two genes: araC, an uncharacterized transcriptional regulator, and nuoM, a component of the respiratory electron transport chain. Expression of wild type nuoM did not complement resistance to Tolfenpyrad, making interpretation of the requirement for nuoM unclear. Remarkably, we found that mutating three amino acid residues in araC resulted in resistance to Tolfenpyrad, and expression of araC in these mutants' complemented sensitivity to

wild type levels. Interestingly, these point mutants of araC displayed attenuated growth in interferon-gamma-stimulated macrophages but not in untreated macrophages, suggesting that AraC may control evasion of the host immune response in vivo. This research has identified a novel antimicrobial drug, the bacterial pathways it targets, and how one of these targets controls Francisella virulence.

**87. OF DRUMS, HEROIN, AND ANTHRAX: PATHOGENIC CHANGES AMONG ANTHROSE NEGATIVE BACILLUS ANTHRACIS**

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**Introduction:** Bacillus anthracis causes anthrax. Anthrax spores infect ungulate mammals through environmental reservoirs in soil. Human anthrax is commonly contracted from infected animals or their contaminated agricultural byproducts causing inhalational, cutaneous, and gastrointestinal anthrax. In recent history, export-related anthrax caused high-profile public health incidents. Our study initially focused on anthrose negative signatures in West African B. anthracis (Nigeria, Chad, Mali, and Cameroon). Anthrose is a spore surface oligosaccharide the removal of which could modify vaccine response, change bacterial pathophysiology, and impact disease severity.

**Method:** Genome sequencing and whole genome SNP analysis for genome characterization was used to identify anthrose negative B. anthracis. Anthrose negative strains were returned to anthrose positivity to analyze toxin secretion and capsule production. Luminescent promoter fusion strains were produced to allow dynamic expression analysis of important virulence factors in anthrose negative strains. Virulence of fully pathogenic B. anthracis was assessed by LD50 studies in the Galleria mellonella larva injection and intranasal guinea pig challenge models. Guinea pig LD50 data were modeled using pure-death simulation processes. Efficacy of the Sterne veterinary vaccine against these strains was assessed.

**Results:** Anthrose negative strains were found in the US and Turkey. Transmission incidents involving djembe drums and heroin were linked to human cultural practices and not agriculture, although anthrose negative livestock-related strains were found in Chile. Anthrose in vegetatively growing B. anthracis is likely an important metabolic mediator whose absence can result in high levels of bacterial chaining and increased secretion of toxins. Anthrose negative spores have decreased exosporium nap density and modified immunoreactivity. Anthrose negative strains express high levels of virulence factors associated with anthrax. Strains had lower LD50‘s than Ames type strains in all models and was dependent on genetic mechanisms at play. The vaccine was partially effective against anthrose negative strains tested and its efficacy will be determined in the mouse model.

**Conclusions:** Additional anthrose negative strains that caused human anthrax cases across Europe were linked to djembe drumskins and the illicit heroin trade over a decade. Anthrose negative strains are from diverse B. anthracis lineages and are linked to high profile export related anthrax outbreaks. Anthrose can act as an intracellular, extracellular, and intercellular signal that can modify physiology of the spore and vegetatively growing cell. Loss of the spore surface sugar can change the immunoreactivity of the spore, impact toxin secretion, capsule production, and vaccine efficacy and warrants further characterization.

**88. POPULATION DYNAMICS AND GENOMICS OF XANTHOMONAS EUVESICATORIA OF PEPPER IN FLORIDA**

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Florida ranks second nationally in the production of bell pepper. However, bacterial leaf spot caused by Xanthomonas spp. has emerged as one of the significant diseases challenging Florida pepper growers. In hot, humid conditions, the disease can result in yield losses of up to 44%, and management is a challenge. Understanding the population biology of the pathogen is critical for sustainable disease management. The goal of this study was to characterize the population diversity of Xanthomonas euvesicatoria of pepper in Florida. From a survey of four farms in two counties in Southwestern Florida between 2019-2021, a total of 506 Xanthomonas euvesicatoria strains were isolated. Six races were identified of which the majority of strains were race 1 (33%), race 3 (28%), or 6 (28%). Sixty-five and ninety-nine percent of strains were sensitive to copper sulfate and streptomycin, respectively. One farm that did not use copper to manage the disease contained only copper-sensitive strains. Strains were assayed for amylolytic activity of which a third of strains were positive, previously reported to be atypical of Xanthomonas euvesicatoria. Whole-genome sequencing and phylogenetic analysis of core genomes for 96 representative Xe strains revealed two distinct genetic lineages that corresponded to amylase activity. Copper tolerant strains had the copLAB gene cluster. This knowledge on the diversity of Xanthomonas euvesicatoria in Florida will be used to determine changes in population composition and help pepper growers to make informed decisions on disease management.

**89. SILVER NANOPARTICLES ENHANCE THE EFFICACY OF AMINOGLYCOSIDES AGAINST ANTIBIOTIC-RESISTANT BACTERIA**

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Antimicrobial resistance is one of the biggest threats to modern healthcare that compromises the safety of standard medical procedures. With the increasing resistance and nearly ceased development of novel antibiotics, alternative approaches are desperately needed to address this dire problem. Silver (Ag) has been known to have antibacterial properties for centuries and developments in Ag-based nanoparticles (AgNPs) have gained traction as potential antimicrobials. As the antibacterial efficacy of Ag varies with structure, size, and concentration, in the present study we examined different formulas of AgNPs for their antimicrobial activity and safety. These commercially available AgNPs were tested against gram-negative Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, and gram-positive Staphylococcus aureus methicillin-resistant and susceptible strains. The most effective formula of AgNPs tested had single-digit (μg/mL) minimum inhibitory concentrations to gram-negative multidrug-resistant clinical bacterial isolates with novel and emerging

mechanisms of resistance. Consistent with previous studies using other AgNP formulas, this formula had a bactericidal mode of killing against E. coli. Using the Caenorhabditis elegans to measure cytotoxicity through a physiological readout we found that motility, but not the lifespan was affected by AgNP exposure. The AgNPs were non-cytotoxic to macrophages, stem cells, and epithelial cells at their antibacterial concentrations. More interestingly, we found that a non-toxic and non-effective concentration of AgNPs synergized with clinically relevant aminoglycoside antibiotics, lowering the minimum inhibitory concentration by approximately 22-fold. Since both aminoglycosides and Ag can target the bacterial ribosome, we tested their effects on the eukaryotic ribosome to ensure no cross-reactivity. We found no effect on the rate of mistranslation at the bactericidal concentration indicating that AgNPs are not proteotoxic to the host at the tested concentrations. Our results indicate that AgNPs have a potential clinical application as an antibiotic alternative or adjuvant.

**90. SPATIAL INVESTIGATION OF ANTHRAX EPIZOOTIC CLIMACTIC CONDITIONS IN THE TEXAS ANTHRAX TRIANGLE WITH THE ENHANCED VEGETATION INDEX**

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**Background:** Past studies have shown certain climactic conditions, as measured by vegetation indices from satellite data, are associated with anthrax outbreak intensity in the West Texas Anthrax Triangle. Here enhanced vegetation index (EVI) values represent these conditions. This study was performed on a ranch in Texas that has documented anthrax

outbreaks in its white-tailed deer population from 2000 to 2019. Hematophagous flies are potential vectors of anthrax and have been included in this study for 2005, the largest recorded outbreak during the period. Previous work measured EVI overtime to define seasonality, here we measure local spatial patterns of those signals.

**Methods:** Local spatial autocorrelation analysis in GeoDA was used to assess clusters of vegetation growth. For this study, EVI was integrated from Day 0 to the first day of greenup and Local Moran's I used to measure patterns of those values across the landscape for each year, and to compare years with high anthrax case counts, like 2005. Spatial regression will determine if vegetation growth predicts anthrax deer cases and fly counts. ArcGIS Pro was used for data preparation and visualization of results. SPSS and GeoDA were used for statistical analyses.

**Results:** According to statistical analyses (Mann-Whitney U), vegetation growth is significantly different in sites with anthrax-positive deer carcasses than non-carcass sites. Local Moran's I confirmed high-high clusters of higher EVI values where cases have been reported and low-low clusters where they have not. Spatial regression confirmed fly counts can predict cases and will be further used to determine if EVI values can predict fly counts or anthrax-positive carcass sites.

**Conclusions:** These results confirm past studies, identifying outbreak intensity increases in years that green up early. We extend those studies to show areas with more intense vegetation growth are localized on the landscape where deer anthrax cases occurred. This can help inform future surveillance by habitat types associated with these conditions.

**91. TAL EFFECTOR MUTATION CONTRIBUTES TO HOST ADAPTATION**

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Transcription activation-like effectors (TALE) are important virulence factors for bacterial plant pathogens from genus Xanthomonas. The pthA family of TALEs are essential for complete virulence of citrus canker (CC) pathogen, Xanthomonas citri subsp. citri (Xcc), and serve as host range determinants within Citrus. Our study aims to demonstrate the potential of TALE evolution in host adaptation and to estimate the mutation rate of TALE repeats. A Xanthomonas euvesicatoria gene, avrbs3, which encodes a pthA4-like TALE was expressed in a pthA-deficient Xcc, which does not produce typical CC pustule. The bacterium was incubated from a low concentration starter suspension (~$10^2$ colony forming units per milliliters) and upon infiltration of the overnight culture into Citrus leaves, typical CC pustules were observed at a low frequency ($1.25\times10^{-4}$), indicating complementation of pthA4 through alteration of avrbs3 repeats which led to upregulation of pthA4 target, CsLOB1. To quantify TALE mutation, a kanamycin resistance gene (aph) was fused to C-terminus of designer avrbs3 with stop codon in one of the repeats, preventing translation of aph. Kanamycin resistance clones arose at the rate of ~$1.2\times10^{-8}$ and ~$5\times10^{-9}$ per cell division by alterations involving third or thirteenth repeats, respectively, in Luria–Delbrück fluctuation

test. Thus, evolution of TALE repeat structures can play a role in remodeling host adaptation of Xanthomonas.

## 92. TRUEPERELLA PYOGENES, A LETHAL PATHOGEN FOR FARMED WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) IN FLORIDA

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White-tailed deer (Odocoileus virginianus) farming is a growing industry in Florida. Bacterial infections and viral hemorrhagic diseases are significant sources of mortality in farmed white-tailed deer in Florida, causing important production loss. The coinfection of bacterial pathogens and hemorrhagic disease viruses may increase the pathogenesis, and promote immune dysfunction, leading to difficulty in treatments. However, the characteristics of bacterial pathogens in white-tailed deer in Florida have barely been studied. This study aims to identify the prevalence, possible transmission routes, and antimicrobial resistant genes of Trueperella pyogenes, one of the most common bacterial pathogens in farmed white-tailed deer in Florida. The University of Florida Cervidae Health Research Initiative (CHeRI) provides a diagnostic service to Florida deer farmers to determine and monitor the cause 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. Our data suggested that

Escherichia coli and Trueperella pyogenes are the most frequently isolated bacteria in farmed deer. T. pyogenes cases have been increasing since 2018, and showed a much higher prevalence in north Florida. Whole genome sequence of the T. pyogenes in Florida farmed white-tailed deer grouped into two clades, one is close to T. pyogenes from pigs, and the second is close to T. pyogenes from cattle. The high diversity of the T. pyogenes in Florida farmed white-tailed deer compared to the sequences from other hosts and regions indicates the possibility of multiple origins and transmission routes. Likewise, most isolates have numerous antimicrobial resistant genes, such as tetracycline resistance genes, suggesting that antimicrobial resistance could play a significant role in the high mortality of Florida farmed white-tailed deer by T. pyogenes. Future works aim to identify the different T. pyogenes serotypes affecting white-tailed deer, their antimicrobial susceptibility, and special heterogeneity. Our results will provide valuable information to improve preventative health measures and clinical management of Florida white-tailed deer, improve herd health, and reduce mortality.

**93. TWO ARE BETTER THAN ONE: ZCCR AND SMU1790C CONTROL THE EXPRESSION OF THE ZCCE METAL EXPORTER IN STREPTOCOCCUS MUTANS**

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A resident of dental biofilms, Streptococcus mutans is a keystone pathogen of dental caries, one of the most prevalent and overlooked diseases globally. Zinc is an essential trace metal to all forms of life that becomes poisonous at high concentrations. Because of its antimicrobial nature, anti-inflammatory properties, and relatively low toxicity to mammalian cells, zinc is used therapeutically for various infectious and noninfectious conditions, including through incorporation in toothpastes and mouthwashes. Zinc efficacy in preventing dental caries remains controversial and the mechanisms allowing zinc survival in oral bacteria like S. mutans are largely unknown. Recently, we discovered S. mutans

tolerates higher zinc concentrations compared to other streptococci because of a metal-translocating P1B-type ATPase (termed ZccE) unique to the species. Additionally, zccE is positively regulated by a MerR-type regulator immediately upstream, named ZccR (zccE regulator). Gel mobility shift assays revealed that ZccR directly and specifically binds the zccE-zccR intergenic region (IGR) in a zinc-dependent manner. While ΔzccE and ΔzccR strains are hyper-sensitive to zinc salts, stable suppressor strains can arise in the ΔzccR background. qRT-PCR analysis revealed basal zccE transcription is much higher in the ΔzccR suppressors than the parent strain. Whole genome sequencing identified a second MerR-type regulator, smu1790, with an early truncation all suppressor strains shared. Deletion or truncation of smu1790 partially restored zinc sensitivity in ΔzccR. Thus, zccE is regulated by two MerR regulators that likely interfere with each other's capacity to bind the zccE-zccR IGR. Studies investigating this and defining the scope of each regulon are ongoing.

**94. A REVERSE GENETICS SYSTEM TO FACILITATE STUDIES OF CANINE CORONAVIRUS Z19**

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Coronaviruses are RNA viruses that are significant pathogens of vertebrates. Various coronaviruses are known to infect humans, including alphacoronaviruses NL63 and 229E, and betacoronaviruses OC43 and SARS-CoV-2. Canine coronavirus (CCoV) is an alphacoronavirus that has only recently been found in humans in two studies: CCoV-HuPn-2018, found by another research group in a hospitalized patient with pneumonia, and CCoV-Z19, found by our group in a visitor to Haiti who developed fever and malaise. Little is known about the cell and host tropisms of CCoV-Z19, and since we are at the initial phases of investigation, we are exploring the types of cells that are permissive and susceptible to the virus. Viruses can only produce progeny virions in permissive cells, and virions can only attach to susceptible cells. Fortuitously, coronaviruses have positive-sense genomes, and if their genomic RNA is internalized by permissive cells, progeny virions can be produced. It is thus possible to insert coronavirus RNA into cells that may not be susceptible to the virus, but are permissive, and form progeny virions, and that information is useful during initial studies to determine

host tropism. Such an approach is facilitated by the use of reverse genetics, wherein virus RNA is transcribed off DNA plasmid templates, the RNA subsequently purified, then inserted into test cells via lipofection or electroporation (and other methods).

The 29.6 rnt genome of CCoV-Z19 was chemically synthesized as six separate DNA fragments and cloned into plasmid vectors. Gibson synthesis was used to produce a full-length cDNA copy of CCoV-Z19, and virus genomic RNA (vRNA) transcribed off the cDNA template. vRNA was lipofected into A72 cells (canine tumor fibroblast), and virions produced in the cells. CCoV-Z19 virions were then inoculated onto A549 cells (human lung alveolar carcinoma), Caco-2 (human colorectal adenocarcinoma), and Vero E6 (African green monkey kidney) cells.

Virus-induced cytopathic effects (CPE) were observed in all cell lines after inoculation with CCoV-Z19. These initial findings suggest that cells from various species can be susceptible and permissive to CCoV-Z19.

**95. ASSOCIATIONS BETWEEN TRAUMATIC EXPERIENCES AND SELF-REPORTED VIRAL SUPPRESSION AMONG PEOPLE LIVING WITH HIV: RESULTS FROM THE FLORIDA COHORT STUDY.**

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**Introduction:** People with HIV (PWH) are more likely than the general population to be exposed to violence. Trauma is linked to decreased adherence to antiretroviral medication, making viral suppression more challenging to achieve and maintain in PWH. Research on the association of traumatic events and their timing - with HIV treatment is scarce. Traumatic experiences are common and associated with poor outcomes,

but it is not clear whether specific types of traumas or timing of experience would be more strongly associated with successful HIV treatment. The aim of this study was to describe the prevalence of specific types of traumatic events (i.e., abuse, and hate crimes) across different times of exposure and to determine the association between traumatic experiences and self-reported viral load suppression among PWH.

**Methods:** We conducted secondary data analysis of the Florida Cohort wave III data (n=475), Sample characteristics included males (60%), more than 50 years (57%), and heterosexuals (50%). These participants were Black (41%), White (35%), Hispanic (19%), and African or Caribbean (6%). Self-reported viral suppression was measured by participants' responses to questions about whether they were told that their viral load was detectable in the past 12 months. Traumatic events were measured by participants' responses to questions about ever experiencing physical, emotional, or sexual abuse, and hate crimes before 18 years and in the past 12 months. SAS V9.4 software was used for Chi-square, Fischer's exact test, and Logistic regression analysis.

**Results:** Close to 58% of the participants reported ever experiencing emotional abuse, 53% physical abuse, 26% sexual abuse, and 22% hate crimes. A total of 25% reported detectable viral load in the past year. Of those whose reported viral load was detectable, more than half experienced recent sexual abuse (53%), followed by physical abuse (37%), emotional abuse (31%), and hate crimes (36%) in the past 12 months. We did not find a significant association between lifetime abuse and viral load. However, recent sexual abuse was significantly associated with the detectable viral load. Respondents who reported experiencing sexual abuse had 3.5 times the odds of having a detectable viral load (OR=3.58, 95% CI 1.27, 10.12).

**Conclusion:** Experiencing recent sexual abuse (in the last 12 months) was associated with more viral non-suppression. Interventions focusing on recent abuse should be considered during screening in HIV clinical care.

**96. CHARACTERIZATION OF BLUETONGUE VIRUS SEROTYPE 1 STRAINS ISOLATED FROM FARMED WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) IN FLORIDA, USA**

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Bluetongue virus (BTV) particularly concerns farmed white-tailed deer (WTD) in Florida, given its severe clinical signs and wide distribution. In this study, we reported the clinical findings, ancillary diagnostics, and complete coding sequence of two new BTV serotype 1 strains in farmed WTD in Florida. A 2-year-old doe (OV1049) in 2019 and a 3-month-old buck fawn (OV1706) in 2022 presented with neurological signs, and the necropsy report revealed lung pneumonic lesions. RNA extract from spleen fragments were screened for BTV, epizootic hemorrhagic disease virus, eastern equine encephalitis virus, and West Nile virus using a multiplex RT-qPCR assay. Both animals were positive for BTV and negative for the other viruses. Next-generation sequencing was performed on cDNA libraries generated from the RNA extracts of virus isolation in Vero E6 cell cultures displaying cytopathic effects. The 5’ end of the coding sequence of segment 1 of sample OV1049 was determined using a 5’ Rapid Amplification for cDNA End (RACE) PCR Kit, followed by Sanger sequencing. Nucleotide identity and phylogenetic analyses supported both isolates as BTV serotype 1. All BTV sequences that showed a high degree of identity to OV1409 and OV1706 strains were classified as either established or reported BTV serotypes in the U.S. Sequence identities, phylogenetic analyses, and recombination analysis indicated BTV-1 strains

OV1409 and OV1706 were reassortants. Continued surveillance efforts are needed to determine the prevalence and potential threat of new BTV strains that may pose to domestic and wild ungulates.

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

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White-tailed deer (Odocoileus virginianus) farming is a growing industry in Florida. Viral hemorrhagic diseases and bacterial infections are known to be significant sources of mortality in farmed white-tailed deer in Florida, causing important production loss. However, little is known about other emerging viral pathogens in white-tailed deer in Florida that might also cause production loss. This study aims to identify the special heterogeneity, possible origins, and transmission routes for two viruses, bovine viral diarrhea virus (BVDV) and deerpox virus, two emerging viral pathogens in the Florida deer farming industry. The University of Florida Cervidae Health Research Initiative (CHeRI) provides a diagnostic service to Florida deer farmers to determine and monitor the cause of death. From 2016 to date, participating Florida ranches provided recently deceased farmed white-tailed deer for necropsy or shipped tissues for

analysis by the CHeRI diagnostic program. We detected three cases of BVDV1 on one farm and one case of BVDV2 on another farm in Florida white-tailed deer in 2018. The full genome sequence data has shown that BVDV2 from Florida white-tailed deer is highly similar to BVDV2a found in beef cattle in other states in the US. Similarly, we have found deerpox virus cases throughout Florida since 2017, with the highest mortality cases seen among animals 2 to 12 weeks old. Our results show that the deerpox virus can be detected through conventional PCR not only from skin scabs and tongue lesions but from most internal organ tissue collected during necropsy such as lung, heart, and spleen. Full genome data has shown that the deerpox virus in this study is 99 percent identical to the first detected deerpox virus in Florida in 2016. We have confirmed that this deerpox virus is very conservative in time. Future works aim to better understand the viral ecology by identifying wild and farm hosts and vectors in Florida. Our results will provide valuable information to improve preventative health measures and clinical management of Florida white-tailed deer, improving herd health and reducing mortality.

**98. ESTIMATING RISK OF DENGUE IN TRAVELERS FROM UNITED STATES TO ENDEMIC COUNTRIES**

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**Introduction:** An understanding of the risk of dengue to a traveler from a non-endemic country can inform public health preparedness Sentinel surveillance systems can be used to report notified cases. However, since about 80% of dengue infections are asymptomatic, and most symptomatic cases are not reported, surveillance systems cannot prove a robust estimate of the risk of infection. In this case, understanding the burden of infection among returning travelers is important in non-

endemic countries with suitable vectors as returning travelers with active infection can spark local outbreaks. The purpose of this study is to provide supporting evidence for vaccine recommendations for travelers to endemic countries.

**Method:** In this cross-sectional study, we fit a model using travel data, case data, and serological data to estimate travel-associated dengue cases and force of infection (FOI) among travelers from the United States to 87 dengue-endemic destination countries from 2010 to 2019. We got the maximum likelihood from the Bayesian MCMC method and used the leave-one-out information criteria (LOOIC) to assess the goodness of fit.

**Results:** From 2010 to 2019, the range of reporting rates of travelers from the United States was between 0 and 24.73% (95% CrI, 11.07-29.33). The highest reporting rate was Cuba (24.73%), followed by Central African Republic (22.97%) and Paraguay (22.57%). The estimated average number of dengue infections by country ranged from 0 to over 13000 (95% CrI, 12264.68-13941.38). Almost one-half of the estimated infections came from Mexico (13148), Dominican Republic (10019), Puerto Rico (9828), and Brazil (6513).

**Conclusions:** The study shows that surveillance systems cannot fully report travel-associated dengue cases. Furthermore, Dengue vaccines should be considered for travelers going to epidemic countries.

**99. HIV STATUS DISCLOSURE AND ANTIRETROVIRAL THERAPY CONCEALMENT, ADHERENCE, AND TREATMENT PREFERENCES AMONG PEOPLE LIVING WITH HIV**

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**Introduction:** Antiretroviral therapy (ART) concealment among people living with HIV (PLWH) to prevent inadvertent disclosure of one's HIV status is understudied, and the impact of these behaviors on ART adherence is unclear. The aims of this research are to characterize ART concealment behaviors and associated factors among a cohort of PLWH and assess whether ART concealment is associated with ART adherence or interest in long-acting injectable (LAI) ART.

**Methods:** The ongoing Florida Cohort Study has been enrolling adult PLWH around the state since October 2020. Participants were asked

about ART adherence (optimal ≥85%) in the past 30 days, engagement in at least one of five ART concealment behaviors to hide their HIV status in the past year (hiding ART bottles, removing prescription labels, moving ART to another bottle, changing their pharmacy, and traveling ≥30 miles to obtain ART), and preference for LAI. Disclosure of HIV status to a close social network was categorized as having no social network to disclose to, incomplete disclosure of status to social network, and complete disclosure. Simple and, if significant, multivariable logistic regression models (adjusting for age, alcohol use, injection drug use, depression, and anxiety) were used to discern differences between those with and without ART concealment and associations with ART adherence and LAI preference.

**Results:** Of 406 participants (62% aged 50+, 60% male, 42% non-Hispanic Black), 47% reported at least one ART concealment behavior, 93% reported optimal adherence, and 64% of the 308 respondents to the question on treatment type preferred LAI. ART concealment was associated with at-risk drinking (aOR 2.5 95% CI 1.4, 4.6), depressive symptoms (aOR 2.2 95% CI 1.1, 4.2), incomplete disclosure to close social network (aOR 3.1 95% CI 1.9, 5.0) or not having a social network (aOR 3.3 95% CI 1.4, 7.8), and optimal ART adherence (aOR 0.3 95% CI 0.1, 0.9). ART concealment was not significantly associated with LAI preference in simple regression (OR 1.3 95% CI 0.8, 2.3).

**Conclusions:** Concealing ART to hide one's HIV status was common and associated with reduced likelihood of optimal ART adherence. PLWH may prefer receiving ART through mechanisms that ensure privacy. This research can inform care models and intervention delivery for PLWH concerned with disclosure of their HIV status. With the emergence of LAI, which is administered in a healthcare setting rather than at home, future research can examine its effect on privacy concerns, viral suppression, and Ending the HIV Epidemic.

**100. INTERLABORATORY REPRODUCIBILITY OF A TAQMAN RT-QPCR ASSAY FOR DETECTION OF TILAPIA LAKE VIRUS**

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Tilapia is the second most important aquaculture species globally and a primary source of protein in many developing countries. Although tilapia are known for rapid growth and general hardiness, they are susceptible to common finfish pathogens such as viruses, bacteria, fungi, water molds, and parasites when reared intensively. Tilapia lake virus (TiLV) is the causative agent of an emerging viral disease associated with high morbidity and mortality in cultured tilapia worldwide. Since the first outbreak in 2012 in Ecuador, TiLV has spread globally, causing variable mortality in all ages of tilapia species in Asia, Africa, and the Americas. Although diagnostic assays to detect TiLV (or exposure to TiLV) have been developed, these assays have not been fully validated. The University of Florida Wildlife and Aquatic Disease Veterinary Laboratory has developed and partially validated (analytic and diagnostic performance) a TaqMan RT-qPCR assay to detect TiLV. In the current study, the reproducibility of the TiLV TaqMan RT-qPCR assay was evaluated through a collaborative effort involving six laboratories. All participating laboratories received a standard operating protocol for the RT-qPCR assay and a blinded test panel consisting of 31 positive and 19 negative RNA samples. Seventeen positive RNA samples were extracted from striped snakehead (SSN-1; E11 clone) cell culture supernatant (n=14) and fish tissues (n=3) infected with the same TiLV isolate. Seven positive RNA samples were generated from a single in vitro transcription event by preparing 7 aliquots of 106 copies in vitro standards from a single 107 copies tube; each was then diluted

separately down to 104 copies. Furthermore, each of these 7 samples was duplicated within the panel (two vials aliquoted from the same tube) for a total of 14 samples. Nineteen negative RNA samples were extracted from fish tissues unexposed to TiLV. Performance measures, including variation between- and within-laboratory, were evaluated for cell culture supernatant and tissue RNA extracts. For the RNA samples generated by in vitro transcription, variations between-laboratory and within-laboratory were evaluated, including the variation within-vial and between-vial for the latter. All laboratories reliably detected both positive and negative samples, except for one laboratory reporting a negative sample as a suspect. For cell culture supernatant and tissue RNA extracts, the estimated standard deviation (SD) of mean Ct values between-laboratory was nearly double (0.39) that of the within-laboratory (0.20). However, the magnitude of this variation is relatively small, with both SDs being less than a full cycle threshold. The estimated SD of mean Ct values between-laboratory was 0.70 for in vitro transcript samples. For within-laboratory, the estimated SD of mean Ct values between-vial was nearly double (1.24) that of the within-vial (0.65); note that the former is an artifact of the sample preparation rather than the testing process. Thus, standard deviations reflecting the testing process all fell below a single cycle threshold for each sample type. This interlaboratory validation trial provided data to support the reproducibility (stage 3) of the post-extraction component of the TiLV TaqMan RT-qPCR assay as outlined by the World Organisation for Animal Health (OIE) for diagnostic assay validation.

**101. MACHINE LEARNING PREDICTION AND PHYLOANATOMIC MODELING OF VIRAL NEUROADAPTIVE SIGNATURES IN THE MACAQUE MODEL OF HIV-MEDIATED NEUROPATHOLOGY**

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In human immunodeficiency virus (HIV) infection, virus replication in and adaptation to the central nervous system (CNS) can result in neurocognitive deficits in approximately 25% of patients with unsuppressed viremia. While no single mutation can be agreed upon as distinguishing the neuroadapted population, earlier studies have demonstrated that a machine learning (ML) approach could be applied to identify a collection of mutational signatures within the envelope glycoprotein (Gp120) predictive of disease. The S[imian]IV-infected macaque is a widely used animal model of HIV neuropathology, allowing in-depth tissue sampling infeasible for human patients. Yet, translational impact of the ML approach within the context of the macaque model has not been tested, much less the capacity for early prediction in other, non-invasive tissues. We applied the previously described ML approach to prediction of SIV-mediated encephalitis (SIVE) using gp120 sequences obtained from the CNS of animals with and without SIVE with 97% accuracy. The presence of SIVE signatures at earlier time points of infection in non-CNS tissues indicated these signatures cannot be used in a clinical setting; however, combined with protein structural mapping and statistical phylogenetic inference, results revealed common denominators associated with these signatures, including 2-acetamido-2-deoxy-beta-D-

glucopyranose structural interactions and the infection of alveolar macrophages (AMs). AMs were also determined to be the phyloanatomic source of cranial virus in SIVE (but not SIVnoE) animals, implicating a role for these cells in the evolution of the signatures identified as predictive of both HIV and SIV neuropathology.

**102. PHYLOGENOMIC CHARACTERIZATION OF RANAVIRUS ISOLATED FROM WILD SMALLMOUTH BASS (MICROPTERUS DOLOMIEU)**

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**Introduction:** Largemouth bass virus (LMBV; species Santee-Cooper ranavirus, SCRV; genus Ranavirus, family Iridoviridae) is associated with disease of North American black bass species (Micropterus spp.). The first LMBV mortality event occurred in 1995 at the Santee Cooper Reservoir, South Carolina, where at least 1000 largemouth bass (M. salmoides) died. Since then, LMBV outbreaks have been recorded throughout the Midwestern and Southern United States and Asia. Clinical signs of LMBV disease include ulcerated skin lesions and over-inflation of the swim bladder, altering the equilibrium of the infected host. Since 2005, LMBV has been reported from an increasing number of smallmouth bass (SMB; M. dolomieu) in Pennsylvania, Michigan, and Wisconsin.

**Method:** In September 2021, 14 wild SMB with ulcerated skin lesions were collected from the waters surrounding Door County, Wisconsin, and submitted for diagnostic evaluation. All samples tested positive for LMBV by conventional PCR. A homogenized skin sample was inoculated into

Epithelioma papulosum cyprini cells, and cytopathic effects characterized by enlarged and refractile cells detaching from the monolayer were observed 24 hours post-inoculation at 25℃. The infected cell culture media was then clarified by centrifugation prior to DNA extraction, DNA library generation, and sequencing using an Illumina MiSeq sequencer. The de novo assembly of paired-end reads using SPAdes v3.15.3 resulted in a 99,354 bp LMBV genome.

**Results:** Maximum Likelihood (ML) phylogenetic analysis based on the 21 core iridovirus genes supported the LMBV isolated from SMB (21117) as a member of the species SCRV. A separate ML phylogenetic tree, based on the complete major capsid protein gene (MCP) alignment, grouped LMBV isolate 21117 with other LMBV isolates reported from the United States and China, as well as doctorfish virus (DFV) and guppy virus 6 (GV6). In addition, pairwise nucleotide comparison of the MCP gene showed that LMBV isolate 21117 is identical to LMBV reported from the United States and nearly identical to DFV and GV6 (99.2%), and LMBV from China (99.1%).

**Conclusions:** Thus, the LMBV isolate generated in this study represents a different strain within the species SCRV and is closely related to previously isolated strains from the United States and Asia.

**103. RABIES EXPOSURE CASE INVESTIGATIONS IN POLK COUNTY, FLORIDA 2015-2022: POTENTIAL FACTORS ASSOCIATED WITH A GREATER THAN TWO-FOLD INCREASE IN ANIMAL BITE REPORTING DURING 2021-2022**

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**Background:** The Florida Department of Health in Polk County (FDOH-Polk) receives and records reports of animal bites involving local residents from healthcare providers, county animal control, and self-reporting. Reports are investigated to identify risk factors for rabies where post-exposure prophylaxis (PEP) may be indicated, such as animal type, rabies vaccinations status, and availability for observation or testing.

During 2015-2020, FDOH-Polk investigated an annual average of 723 reported animal bites. Beginning in 2021 however, the annual average increased to over 1600. A descriptive data analysis was conducted to identify factors contributing to the increase (e.g., increased reports of animals testing positive for rabies, reporting of vaccinated and/or owned animals, or possible under-reporting in prior years), and to determine whether the increase affected PEP recommendations.

**Methods:** Cases among Polk County residents that met the definition of "Rabies, Possible Exposure" (i.e., cases where PEP was indicated) were reviewed using the FDOH web-based reportable disease surveillance system Merlin, along with laboratory results from animal rabies testing. Data were also reviewed from the FDOH-Polk animal bite database maintained using Microsoft Access Version 2202.

**Results:** During 2015-2020, an annual average of 269 cases where PEP was indicated were identified. By comparison, PEP was indicated for 289 during 2021, and 295 in 2022.

Between 2015 and 2022, lab-confirmed animal rabies reported in Polk County were identified in raccoons (n=15), bats (n= 13), and foxes (n=4);

in 2021 alone, those included bats (n=2), foxes (n=2), and raccoons (n=1), and in 2022, just one bat (n=1).

Bite reports from county animal control increased from an annual average of 584 during 2015-2020, to 789 in 2021 and 1340 in 2022. During 2015-2020, dogs accounted for most reported animal bites (64%) among Polk residents, followed by cats (24%), bats (5%), and raccoons (4%). The number of dog bites reported in the years 2021 and 2022 increased to over 70%. Among cats (19%) and raccoons (1%) there was a decrease in the overall percentages reported from the period of 2015-2020 and 2022.

**Conclusions:** Beginning in 2021 there was an increased reporting of bite cases, where reports from county animal control accounted for the majority, and which were overwhelmingly attributable to domesticated animals. However, there was no notable corresponding increase among cases where PEP was indicated. Methods to improve the current bite reporting and investigation process and reduce the amount of reporting for cases not meeting definition are being explored.

## 104. USING MACHINE LEARNING ALGORITHMS TO DETERMINE RISK FACTORS ASSOCIATED WITH BOVINE LEUKEMIA VIRUS INFECTION IN BEEF CATTLE IN FLORIDA

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**Introduction:** Supervised machine learning (ML) algorithms are potentially powerful tools for identifying risk factors associated with infectious diseases in cattle. Here, we compared different ML models to identify risk factors for bovine leukemia virus (BLV) infection in beef cattle in Florida.

**Methods:** The analyses were performed on 2,046 blood samples submitted to Bronson Animal Disease Diagnostic Laboratory from 2012 to 2022 for BLV antibody testing. Multiple logistic regression (MLR), classification and regression tree (CART), random forest (RF), and gradient boosting (GB) algorithms were used.

**Results:** A total of 383 (15.8%, 95% CI 14.3 to 17.2) serum samples were positive for BLV. The highest prevalence of BLV infection was reported in female (12.4%) crossbred cattle (12.1%) aged ≥5 years (4.0%) and raised in south Florida (6.5%). The CART model showed the highest determination power (AUC=0.91), compared to GB (AUC=0.86), RF (AUC=0.83), and MLR (AUC=0.79). In the CART model, age (importance score=6.5) and breed (4.1) are the most important risk factors for BLV infection in beef cattle in Florida.

**Conclusions:** Utilization of the CART modeling may identify the risk factors associated with BLV infection in cattle with high determination and predictive capabilities, allowing for the implementation of effective preventive strategies.

**105. A COMPREHENSIVE ANALYSIS OF SNAKE BITE ENVENOMATIONS IN NORTH CENTRAL FLORIDA**

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**Introduction:** Venomous snakes are among the most lethal animals worldwide, and envenomation is a major neglected tropical disease. Florida hospitals experience hundreds of snake envenomation every year due to the presence of six naturally occurring venomous snakes and the popular snake hobby industry in our state. We aim to improve snake envenomation medical knowledge via a comprehensive epidemiological analysis of treatment methods and clinical outcomes among patients with envenomations presenting to our academic medical center in Florida.

**Methods:** Utilizing specific ICD-codes in the electronic medical records, study personnel conducted a retrospective analysis for patients who presented to University of Florida Health Shands Hospital located in Gainesville, Florida, with suspected snake envenomation between 2002-2022.

**Results:** A total of 829 encounters were reviewed and 546 venomous snake bites were confirmed. Among these 546 encounters, our preliminary results of 121 encounters will be presented. Most bites were of accidental origin (26% caused by accidentally grabbing or stepping on a snake and 26% caused by walking/passing by a snake), but an additional 29% were a result of a patient purposefully handling/provoking a snake in the wild. Most patients were bitten on their hand/finger (59%), and other bite locations included the foot/toe (32%) and elsewhere on an extremity

(9%); no patients were bitten on the torso, neck, or head. The most frequent symptoms of envenomation were edema (73% of patients), paresthesia (21%), and nausea (18%). However, more serious symptoms were seen after bites from the eastern diamondback rattlesnake (Crotalus adamanteus), with 31% experiencing respiratory distress and 23% having acute encephalopathy. From the time of initial snake bite, it took an average of 131 +/- 34 (SE) minutes for patients to first encounter a healthcare provider. Prior to seeing a provider, 5% of patients attempted at-home venom removal methods (i.e., incision and/or suction). Once in professional care, only 73% of patients received antivenom and initial dosing was not administered until 277 +/- 75 (SE) minutes after envenomation. Of the patients who did receive antivenom, 11% had a suspected or confirmed anaphylactic reaction to antivenom treatment.

**Conclusions:** Our preliminary findings show a significant delay in the administration of antivenom and indicate that anaphylactic reactions to antivenom are not uncommon. Broader public education on local venomous snake species, not provoking/handling wild snakes, and proper methods for pre-hospital self-treatment after a bite could further reduce the prevalence of envenomation events altogether.

**106. A NEW PARTNERSHIP ACADEMY FOR SCIENCE AND CAREERS TO ADVANCE HEALTH SCIENCE EDUCATION**

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The UF Health Science Center (HSC) is partnering with the Center for Precollegiate Education and Training (CPET) to establish a UF Health Academy for Science and Careers (ASC) that will promote K-16 student and teacher health science education and outreach throughout the state of Florida. ASC programs are designed to promote a love of learning, equip teachers with cutting-edge knowledge for their classrooms, and engage people at all levels in health science education and research. Faculty from the Emerging Pathogens Institute have been particularly instrumental in the success of teacher development programs, including CATALySES and Mini Medical School. ASC harnesses the over 60 years of experience at CPET to partner with UF HSC colleges, institutes, faculty, and trainees to build and implement exciting new educational programs, expand and develop new partnerships, and highlight and disseminate the wonderful programs and research at the HSC.

CATALySES: Entering its final year, CATALySES is a professional development program for secondary STEM teachers in collaboration with the EPI and funded by an NIH SEPA grant. The two-week summer institute focuses on infectious disease and translational research through lectures, discussions, laboratory experiments, and workshops. The participating teachers work with UF researchers to develop action proposals to translate concepts covered in the program into lessons and lab activities to implement in their classrooms, and report how they will collect, analyze, and interpret data on student outcomes from their action proposal. The teachers receive continued support in implementing their proposals through the fall and spring semesters via regular meetings with the CATALySES team and UF researchers. Thanks to enthusiastic

participation from EPI faculty, we had 56 teachers participate from 24 counties in the first 3 years of the program!

Mini Medical School: The Mini Medical School is an initiative supported by the UF Medical Guild to offer teachers a one-day, in-service program to advance STEM education in health science topics. The program has expanded to include both fall and spring semester symposiums on current and cutting-edge topics in biomedical research and medicine. The teachers hear lectures from UF researchers, have discussions focused on research and health profession careers, and participate in hands-on lessons they can take back to their classrooms. EPI faculty have been instrumental in delivering content lectures and developing active learning experiences for Mini Medical School teachers, especially in previous programs that focused on interdisciplinary approaches to Epidemiology research, the SARS-CoV 2 pandemic, and antibiotic resistance.

**107. ANTIMICROBIAL RESISTANCE PROFILE OF MULTIDRUG RESISTANCE E. COLI FROM A BRAZILIAN BEEF, PORK AND CHICKEN PRODUCTION CHAIN**

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**Introduction:** Antimicrobial resistance is a worldwide concern, and the food production chain plays an important role in spreading this hazard. Antibiotics are usually employed in poultry, pork, and beef production as therapeutic and preventive treatments for some specific diseases. However, the abusive use of these substances can contribute to accelerating the development of resistant bacteria, especially multidrug

resistant (MDR) bacteria, allowing their distribution in the whole production chain.

**Methods:** Escherichia coli (n = 110) were previously isolated from pork, beef, and poultry production chains in Brazil based on conventional plating and screened for resistance to amoxicillin (8 μ/mL), ceftiofur (8 μ/mL), ciprofloxacin (1 μ/mL), chloramphenicol (32 μ/mL), trimethoprim-sulfamethoxazole (4/76 μ /mL) and tetracycline (16 μ/mL). Considering the resistance profiles, MDR isolates were selected (pork: 57, beef: 25, poultry: 28) and subjected to the whole genome sequencing. The raw sequencing data (short reads) were trimmed, quality evaluated, and assembled using bioinformatics trimmommetics, FastQC and spades software. The predicted resistance genes were obtained from The Comprehensive Antibiotic Resistance Database (CARD) platform and all information was stored and analyzed.

**Results:** A large genetic cluster of resistance genes was found among the three different chains. The beef was the group with more diverse antibiotic resistance genes found (n = 159), followed by pork (n = 112) and poultry (n = 105). Ten antibiotic classes (b-lactams, diaminopyrimidine, macrolide, phenicol, phosphonic acid, sulfonamide, fluoroquinolone, tetracycline, aminoglycoside, and an efflux pump gene responsible for multidrug resistance) were predicted in the production chains, being fluoroquinolone, tetracycline, aminoglycoside, and a general efflux bomb presented in 95% of isolates. At least, one predicted gene of the antibiotic tested in the phenotypical assay was found in the isolates, showing the association between phenotypical and genotypical data. Most of all genes predicted in the isolates were related to the MDR efflux pump. After an individual analysis, isolates from beef seem to carry a lower number of antibiotic resistances related genes when compared to isolates from pork and poultry. Only a single from beef was considered as an outlier due to the presence of more than one-hundred resistance related genes.

**Conclusions:** Beef, pork, and poultry production chains in Brazil are carrying many resistance genes, playing an important role in the maintenance and dissemination of

**108. CAENORHABDITIS ELEGANS MODELS OF PROTEIN CONFORMATIONAL DISEASES REVEAL THAT BACTERIA GENERALLY AFFECT HOST PROTEOSTASIS RATHER THAN A SPECIFIC DISEASE**

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Different neurodegenerative protein conformational diseases (PCDs) are hallmarked by proteotoxicity associated with the aggregation of metastable proteins encoded within the proteome of the affected individuals. For example, Alzheimer's disease is predominantly associated with amyloid beta 1-42 (Aβ1-42) and Tau, while Parkinson's and Huntington's diseases are associated with α-synuclein (α-syn) and polyglutamine (polyQ) expansions, respectively. Recently, bacteria were found to affect the pathology of these diseases; however, the microbial role remains elusive. To better understand the contribution of bacteria on PCDs, we employed Caenorhabditis elegans PolyQ models that allowed us to monitor changes in the folding environment upon intestinal colonization. We characterized the effect of all culturable isolates from the Human Microbiome Project, and from among over 240 unique

species, we identified those that are proteotoxic and those that enhance proteostasis and suppress aggregation. We tested the most robust bacterial isolates using C. elegans models expressing Aβ1-42, α-syn, and a novel tauopathy model. Our results reveal that bacteria do not discriminate between different metastable proteins but rather generally affect the stability of these culprit proteins. While we are only beginning to address the protective mechanisms, we have already screened over 6,000 mutations in bacterial genes to determine microbial factors that affect host proteostasis. The initial analysis reveals specific bacterial genes involved in the regulation of secretion, adhesion, membrane modification, and motility. Collectively, our results will be used to predict the effect of the human microbiome on the pathogenicity of PCDs and aid in the development of potential prophylactic and therapeutic strategies.

**109. CAUSE OF DEATH IN FLORIDA FARMED WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) FROM 2016 TO 2022**

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**Introduction:** White-tailed deer (Odocoileus virginianus) farming is a growing industry in Florida. Viral hemorrhagic diseases and bacterial infections provoke high mortalities in fawns and yearlings deer in Florida farms. To implement disease prevention and management strategies, causes of death must be determined. The University of Florida Cervidae Health Research Initiative (CHeRI) provides a free diagnostic service to Florida deer farmers identifying the causes of death throughout Florida.

**Methods:** From 2016 to 2022, diagnostic testing was performed to determine causes of death for 718 farmed White-tailed deer. Tissue samples were tested for hemorrhagic disease 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.

**Results:** Of the 237 deceased farmed white-tailed deer aged 1-90 days sampled from 2016 to 2022, 76% of deaths were caused by bacterial infection, and only 26% were due to viral hemorrhagic disease. Of the 221 animals aged 4-12 months sampled from 2016 to 2022, 70% of deaths were attributed to hemorrhagic disease viruses and 66% to bacterial infection. Lastly, of the 260 animals aged 13 months and older sampled from 2016 to 2022, hemorrhagic disease viruses caused 54% of deaths, and 67% 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 87% 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 from 2016 to 2022, 62% of infections were attributed to EHDV-2, 27% of infections to EHDV-6, and 11% of infections to EHDV-1. Of all the EHDV cases seen during this period, 26% could not be typed. Additionally, bacterial infections significantly increase from late May to September during fawn season.

**Conclusion:** 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.

## 110. COMPARISON OF TRANSCRIPTOME CHANGES OF STREPTOCOCCUS MUTANS CULTURED USING TWO DIFFERENT GROUND-BASED SIMULATED MICROGRAVITY MODELS

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Streptococcus mutans is a facultative anaerobic, Gram-positive bacterium commonly found in the human oral cavity and a primary contributing factor to dental caries. Our lab previously determined that S. mutans undergoes a significant change in its early stationary phase global gene expression profile when cultured under low-shear modeled microgravity (LSMMG) using the rotary cell culture system (RCCS), relative to the RCCS normal gravity control. Some of these gene expression changes appeared to correlate with the altered cell clumping and oxidative stress sensitivity phenotypes observed under simulated microgravity. As a follow-up, we recently compared growth of S. mutans in High Aspect Rotating Vessels (HARVs) using two different simulated microgravity models: the RCCS system, and the random positioning machine (RPM). A RCCS HARV normal gravity control was used for both experiments. All cultures (n=3 per growth condition per time point) grew comparably and cells were harvested at mid-exponential (~3 hours) and late exponential (~6 hours) growth phase. Isolated RNA had RIN values within the range of 8.9-9.6. Ribodepletion, library construction, and Illumina sequencing was performed by the UF Institute for Biotechnology Research. Raw data was analyzed using Qiagen CLC Genomics Workbench. PCA analysis revealed that all the mid-exponential phase (~3 hour) samples clustered together, while strong separation was observed between each late-exponential phase (~6 hour) sample set (RCCS microgravity, RPM, RCCS normal gravity). The exception to this was one outlier each in the 6 hour RCCS microgravity and RCCS normal gravity sets, which may have been mixed up in sample processing pipeline post-RNA isolation. Differential expression analysis and follow-up bioinformatic analysis of the data is in

progress. This study will have a broad impact on understanding S. mutans physiology and genetic responses under two simulated microgravity models, which may help us better predict how this bacterium responds to space flight conditions.

**111. DECAPOD FISHERIES AND DISEASE DIVERSITY: A SYNTHESIS APPROACH EXPLORING HOST TRAITS AND PARASITIC INFLUENCE**

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Some hosts harbor more parasites than others. Overdispersion of parasitism indicates that coevolution with parasites may be more important to the biology and ecology of certain species. We examined patterns of parasitism and host traits in fished decapod crustaceans, which are economically and ecologically important worldwide. Using a meta-analysis approach, we determine that host life history, including habitat, longevity, sociality, invasion history, and fisheries involvement, correlate with the number and type of parasite species harbored. Indicator species analysis revealed close relationships between decapods and certain parasite groups, including crabs with rhizocephalans and dinoflagellates; crayfish with mesomycetozoans, oomycetes, branchiobdellids, and fungi; lobsters with copepods and amoebae; and shrimp with viruses. Decapods that are commercially fished, aquacultured, invasive, exhibit parental care, or live in freshwater, tend to have higher parasite species diversity. Parasite diversity also increases with how well-studied a host group is. Identifying patterns such as these increases our broad understanding of decapod disease ecology but also enabled us to develop a series of recommendations on how to focus future research, management, and aquaculture development efforts.

## 112. DESIGN AND PRINTING OF PHYSIOLOGICALLY RELEVANT ALVEOLAR MODEL FOR COVID-19 APPLICATIONS

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Since the coronavirus disease 2019 (COVID-19) outbreak, more than 100 million confirmed cases and about 2.3 million deaths have been reported with increasing numbers everyday globally. Despite the availability of animal models and clinical specimens, novel 3D models that recapitulate the human alveolar blood-air barrier are needed to gain mechanistic insights into SARS-Co-2 infection and to facilitate the development of therapeutics. Using embedded three-dimensional (3D) printing technology, we aim to design and fabricate a physiologically relevant human alveolar model, which can serve as a platform to study viral pathogenesis and candidate therapeutics. The designed 3D alveolar model has two perfusable channels embedded in a thick tissue construct: a helical fluid channel and a straight air channel surrounded by the fluid channel, resulting in a blood-air barrier with diffusional permeability between the two channels. The two perfusable channels are fabricated by directly extruding a sacrificial ink through a printing nozzle in a gelatin-based yield-stress support matrix, which locally liquefies upon the traverse of the printing nozzle and resolidifies in the wake of the nozzle, immobilizing the printed template in place. This embedded printing fashion mitigates the effects of gravity by providing omnidirectional support on extruded filaments, allowing freeform printing of complex channel structures in a 3D matrix. After the printing is complete, the matrix is enzymatically cross-linked, followed by the removal of the sacrificial template to allow the formation of void channels. The created fluid and air channels are separately seeded with endothelial cells and

alveolar epithelial cells, and then perfused with cell medium and oxygenated air, respectively, to preserve the dynamic flow conditions and key cellular components that viruses target. For identification of new therapeutics, alveolar epithelial cells allow infection of SARS-CoV-2 and evaluation of drug candidates perfused through the fluid channel. In comparison to conventional 2D cultures, this model recapitulates the key morphological and functional characteristics of the alveolar blood-air barrier at structural and cellular levels. The 3D alveolar model may enhance the understanding of the SARS-CoV-2 infection and facilitate the development of new treatments.

**113. DISCOVERING VIRULENCE INHIBITORS IN HIGHLY PATHOGENIC BACTERIA**

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Francisella tularensis is a deadly  bacterium that causes a zoonotic disease called tularemia. Due to its low infectious dose and potent virulence, it is a potential agent of bio-terrorism. The chief virulence factor driving pathogenicity in these bacteria is a toxin secretion apparatus called the type VI secretion system (T6SS). An essential component of the T6SS apparatus is a contractile sheath that is composed of two proteins, IglA and IglB. In this study, we will discover novel therapeutics to block the secretion nanomachinary and attenuate virulence. To this end, we have developed a high-throughput method to detect the interaction of IglA with IglB by using a split Renilla luciferase assay. As a proof of principle, we screened a library of one thousand drugs composed of natural and synthetic compounds. Two of these compounds block sheath assembly, secretion of toxins, and bacterial virulence. Since these drugs block virulence but do not affect bacterial viability, they represent a new class of antibiotic that has the promising property of avoiding microbial resistance.

## 114. EMPLOYING CHEMICAL GENETICS TO DEVELOP INSECT GLIA AS A CELLULAR TARGET FOR INSECTICIDES

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**Introduction:** The development of novel insecticide targets has been of consistent interest to the field of insecticide science, yet few new biochemical targets have emerged over the past two decades. Insect neurons are the target tissue for >85% of commercialized insecticides, thus it is surprising the functional roles of insect glial cells remain poorly understood and there are no glia-directed insecticides. Recent work has shown expression of various ion channels in insect glia cells, raising the intriguing possibility that glial cells contribute to neuronal function of insects and may represent a cellular target for insecticide design. Considering this, we tested the hypothesis that inwardly rectifying potassium (Kir) channels expressed in glia cells contribute to nerve firing and Kir inhibition will have deleterious consequences to neuronal function.

**Methods and Results:** To test this hypothesis, we built Kir2 promoter drive GAL4 fly line, combined with UAS and LexA/LexAop binary system, we showed Kir2 channel subunits are primarily expressed in perineural, subperineural, and astrocyte-like glia in the Drosophila melangoster CNS. Patch clamp studies confirmed membrane expression in spg and astrocyte-like glia with an average of 131.9 ± 72.9 pA and 182.3 ± 40.9 pA of barium inhibitable current, respectively. These data led us to speculate Kir channels constitute a mechanism for rapid clearance of K+ ions from the extracellular space during neuronal activity and thus, inhibition of glial Kir channels would result in membrane depolarization and increased firing. Therefore, we performed extracellular recordings of Drosophila descending neurons and found pharmacological inhibition of Kir channels significantly ($P<0.05$) increased the firing rate and lead to nerve death (IC50: 23 μM). Importantly, inhibition of glial cell function with Kir specific

pharmacophores resulted in acute lethality that highlights the potential for developing novel mode- and mechanism- insecticides targeting glial cell function.

**Conclusions:** Kirs express in glia cells and are responsible for the potassium movement in neuronal functions. Further, insect glial Kirs could be potentially insecticide target site.

**115. IMPACT OF BETA-LACTAM EXPOSURE ON GRAM-NEGATIVE BACTERIAL RESISTANCE**

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**Introduction:** Beta-lactam (BL) antibiotics are commonly used to treat Gram-negative infections. The purpose of this study is to evaluate beta-lactam pharmacokinetic/pharmacodynamic (PK/PD) parameters associated with Gram-negative bacterial resistance.

**Methods:** Retrospective data from adult patients between 2016-2019 at UF Health-Shands Hospital (Gainesville, FL) who received cefepime, meropenem, or piperacillin (administered with tazobactam) and had two separate cultures with minimum inhibitory concentration (MIC) were included. Beta-lactam exposure was generated and free drug concentrations exceeding minimum inhibitory concentration (ƒT>MIC) and four multiples of MIC (ƒt>4xMIC) were calculated for the first 24 hours, 7 days, and duration of therapy. Classification and regression tree (CART) analysis was used to determine a breakpoint for average daily area under the time concentration curve over MIC (AUC/MIC) of 494mcg*hr/mL. Multiple regression analysis was performed as a final step to evaluate PK/PD impact on bacterial resistance (defined as any increase in MIC within 30 days of antibiotic therapy completion).

**Results:** A total of 258 patients with 316 bacterial isolates were included. The median (IQR) age was 58 years (42-68.3), weight 73.6 kg (60.9-94.3), and 152 (59%) patients were male. The most common cultures were sourced from lung (n=121) and blood (n=121), and Pseudomonas aeruginosa was the most common bacteria (n=135). Sixty-two (20%) isolates developed bacterial resistance. Renal replacement therapy, time on antibiotics (days), mechanical ventilation, hospital length of stay (days), and ICU length of stay (days) were associated with increased resistance and controlled for in the final analysis. In the multiple regression analysis, failure to attain an average daily AUC/MIC ratio of 494 (mcg*hr/ml) was associated with an increased risk of resistance (OR: 3.06, 95% CI 1.35-6.90).

**Conclusion:** PK/PD target attainment impact on Gram-negative bacterial resistance require additional investigation, but AUC/MIC may be a potential target.

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

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As of 2019, there are an estimated 4.95 million deaths worldwide every year associated with antimicrobial resistance (AMR). In the United States alone, AMR is responsible for nearly 35,000 deaths and an estimated $55 billion in financial costs, with these numbers expected to increase ten-fold by 2050 if no effective alternatives are found. Bacteriophages (phages), viruses that target and infect bacteria, provide a promising tool for the treatment of antibiotic-resistant bacterial infections. Despite recent advancements, selecting phages that exhibit specificity for Pseudomonas aeruginosa—a multidrug-resistant, opportunistic pathogen that commonly infects ill, hospitalized patients—has been challenging partly because of the knowledge gaps in virus-host interactions. In this study, we aim to increase our understanding of bacterial factors that affect phage infectivity. First, we isolated and characterized seven unique bacteriophages that target 101 of 146 diverse clinical, animal, and environmental isolates of P. aeruginosa, each with a unique antibiotic resistance profile and a sequenced genome. Second, we tested our isolated phages in combination with antibiotics and found potential synergistic effects, particularly with colistin. Finally, utilizing a knockdown library of all non-essential P. aeruginosa genes, we are beginning to reveal bacterial genes that are involved in phage infection. Recognized as one of the largest threats to global healthcare, antibacterial resistance continues to press the need for alternative therapies. Our bacteriophages have shown strong specificity towards diverse clinical, animal, and environmental isolates of P. aeruginosa and have shown increased efficacy in combination with antibiotics. Furthermore, the discovery of genes vital for bacteriophage infection will reveal a new mechanistic understanding of phage-bacteria interactions, which will lead to improved therapy from a personalized One-Health perspective.

## 117. KNOWLEDGE ATTITUDE AND PRACTICE OF ANTIBIOTIC USE AMONG MEDICAL STUDENTS IN BANGLADESH

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Antibiotics have been the foremost weapon wielded by humankind in our war against pathogenic microorganisms. Antibiotic abuse affects people all across the world. One of the major health issues in the modern world is the abuse or overuse of antibiotics. Antibiotics are not generally thought of as drugs for prevention. Numerous unskilled and marginally qualified doctors believed that antibiotics could both treat and prevent infections. Nevertheless, because of the growing resistance to life-saving antimicrobial medications, which has significant consequences on both individual and societal health, the world is on the verge of returning to the "pre-antibiotic age." Clinically significant and commensal bacteria are developing alarming rates of antibiotic resistance globally, posing a danger to the successful management of infectious illnesses. The objective of this study is to assess the KAP level of medical students. A cross sectional study will be conducted on 456 medical students to assess the KAP of antibiotic use. Frequency, percentage, and chi square test will be done to evaluate the association. Young people in Bangladesh are very important to a variety of societal initiatives. As doctors' knowledge plays an important role in prescribing antibiotics, medical students' knowledge needs to be assessed for future recommendations. However, research on KAP on antibiotic usage and resistance in this population, particularly among medical students, is still lacking. Therefore, in order to understand KAP with regard to antibiotic use and resistance, we set out to perform a cross-sectional study among medical students. We expect to find out if students in medical schools had higher KAP scores than other students. As future policymakers curb inappropriate uses of antibiotics, young healthcare professionals will have an impact on national recommendations.

## 118. LEVERAGING AN ONLINE PLATFORM FOR REGISTERING BIOHAZARDOUS RESEARCH

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In order to ensure regulatory compliance and adherence to appropriate safety standards, it is essential for research entities to gain a coherent understanding of newly proposed and ongoing research studies. Historically at the University of Florida (UF), avenues of research involving infectious/toxic biohazardous materials, federal/state permits associated with regulated biological products, as well as recombinant/synthetic nucleic acid experiments were registered with the Division of Environmental Health and Safety (EH&S) and/or the Institutional Biosafety Committee (IBC) through electronic form submissions and a combination of paper-based and electronic database recordkeeping. This strategy had the following major limitations: 1) from both an administrative oversight as well as investigative personnel perspectives, it was challenging to keep track of various amendments as a given study evolved over time; 2) University oversight lacked assurance that all research personnel listed on a given study had access to the project data and accompanying approval conditions; 3) maintaining records and regular updates was administratively taxing both in terms of physical space demands as well as dedicated processing efforts. At the start of 2020, as hosted by SafetyStratus, UF EH&S/IBC rolled out an online module for Biohazard Project Registrations. In addition to addressing the

aforementioned issues associated with the former registration system, the SafetyStratus module also has the following advantages: 1) streamlines the review/approval workflow; 2) enables integration of formerly independent avenues of research; 3) affords a unified platform that is readily queried. As of January 2023, approximately 34% (175 out of 515) investigators with registered projects are fully integrated in the SafetyStratus module. However, 22% (114) investigators have projects that are both registered within the SafetyStratus module as well as in the former system. Yet 44% (226) investigators remain solely registered in the former system. We are continuing to make strides to improve the online registration process as well as phase out the former paper-based system.

**119. META-ANALYSIS REVEALS COMPOSITIONAL AND FUNCTIONAL MICROBIAL CHANGES ASSOCIATED WITH OSTEOPOROSIS**

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**Introduction:** Over the past decade, the role of the gut microbiota (GM) in many disease states has gained massive attention. Mounting evidence from different case control and observational studies have linked changes in gut microbiome compositions and functions to the pathophysiology of osteoporosis(OP). However, they have presented discrepancies in results leaving the literature without a consensus on osteoporosis associated microbial signature and features. Here, we conducted a comprehensive pooled re-analysis of five publicly available 16srRNA osteoporosis datasets to identify gut bacteria consistently associated with osteoporosis across different Asian cohorts.

**Methods:** Demultiplexed raw DNA sequences from the stool of osteoporosis and healthy subjects from different studies were downloaded from the NCBI Sequence Reads Archive (SRA). Due to the technical variation in the datasets included in the metanalysis(DNA extraction kits, primers, sequencing, platform). Each dataset was

separately denoised and processed into amplicon sequence variants(ASVs) using DADA2. Taxonomy assignment of each (ASVs) was performed using the Bayesian RDP classifier trained with the RDP_train_set_18 database. Batch effect associated with technical variation and heterogeneity of studies involved in the metanalysis was adjusted using the MMUPHin Package. Downstream analysis was done using the phyloseq package on R.

**Results:** We observed a significant shift in the microbiome composition in the osteoporosis group. Increase in the relative abundance of opportunistic pathogens like Clostridium sensu stricto, Bacteroides and Intestimonas was observed in the OP group. Moreover, short chain fatty acids producers including members of genus Collinsella, Megasphaera Agathobaculum, Mediterraneibacter, Clostridium XIV was depleted in the OP group relative to the HC group. Lactic acid producing bacteria including Limosilactobacillus, was significantly increased in the OP group. Random forest algorithm further confirm these group of bacteria to be important to differentiate between the two group at 55% prevalence. Furthermore, functional prediction revealed depletion of SCFA biosynthesis pathway (Glycolysis, TCA, Wood-Ljungdahi pathway) and amino acid biosynthesis pathway(Methionine, Histidine and Arginine) in the OP group relative to the HC group.

## 120. O-ANTIGEN MUTATIONS AFFECT THE TUMOR TARGETING ABILITY OF SALMONELLA

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As a gram-negative bacterium, Salmonella has been proven to be an effective immunotherapy agent that induces tumor regression against cancers through multiple mechanisms in mice. Despite this conducted research, clinical trials showed only 3 out of 25 patients had Salmonella colonization at the tumor sites after intravenous injection. Due to this low percentage of colonization, targeting efficiency of the live bacterium needs to be increased. A strain that displays an OmpA fused with PLZ4 peptide on the surface of Salmonella was developed to increase the targeting ability of bladder cancer cells. A single copy of this insertion on a chromosome has proven to be ineffective, however multiple copies were able to partially overcome this issue. This suggests that the cell surface modification of Salmonella with a greater amount of ompA3Ωplz4 could positively impact the effectiveness against bladder cancer cells. Due to these results, this research focuses on further surface modification to potentially increase Salmonella tumor targeting. Several mutations were introduced into this specific strain and the attachment to and invasion of bladder cells were evaluated via tissue culture cell assay. More specifically, the OmpA porin is located on the outer membrane of the surface of Salmonella to assist with peptidoglycan linking and is covered by LPS, a molecule composed of lipid A, an inner core, an outer core, and an O-antigen. While the deletion of lipid A is lethal, reducing the LPS-O antigen and cores has the potential to strengthen the exposure of OmpA to the cell. Thus, this research compares the ompA3Ωplz4 strain with various core and O-antigen mutations to test this hypothesis. The mutations introduced into the Salmonella ompA3Ωplz4 strain included ΔwaaL, ΔwaaC, and ΔwaaG and were compared to the parent strain. The attachment to and invasion of human carcinoma urinary bladder cells

(5637), mouse bladder tumor cells with different genetic complexities (BBN963), and urothelial carcinoma bladder fibroblast cells (MB49) in culture were all observed to further determine the tumor targeting efficiency. As a result, some mutations and surface modifications resulted in a more significant effect on cell attachment and invasion than others and an increase in tumor targeting efficiency was seen. This research is significant to increasing the effectiveness of immunotherapy agents in tumor regression and future animal studies will be performed.

**121. RECIPE FOR HEART HEALTH: A PLANT-BASED CULINARY DIET INTERVENTION TO TEST INDEPENDENT BENEFITS OF OLIVE OIL**

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Cardiovascular diseases (CVD) remain the leading cause of mortality worldwide with poor diet as a major contributor in the development of CVD risk factors. Multiple cohort studies have reported extra virgin olive oil (EVOO) as a promising source of anti-inflammatory benefits, associated with reduced CVD risk. Research has yet to elucidate the exact role of the gut microbiome in the mediation of beneficial cooking oils and subsequent impacts on clinical markers of CVD risk in individuals already consuming heart healthful plant-based diets. The ubiquitous use of oil in food preparation supports the need for research of specific plant-based oils. No study to date (to our knowledge) investigated this relationship in ASCVD patients. This pilot study is a prospective randomized cross-over clinical trial investigating the association of high and low EVOO consumption and gut microbiome changes in ASCVD patients. 43 primary prevention outpatient adults (male and female) with intermediate to high ASCVD risk factors (as defined by the ASCVD risk calculator as > 7.5%) of

all races/ethnicities ≥18 years who previously followed a standard Western diet and provide consent were enrolled. Participants were randomized into 2 groups, (4 tablespoons) or low (< ~1 teaspoon) in raw extra virgin olive oil on a standardized PBD. Stool samples were collected at start and end of the first intervention (baseline 1 and endpoint 1), with a two-week washout period prior to starting second intervention (baseline 2 and endpoint 2). MiSeq 16s rRNA sequencing data was used to determine microbial taxonomic and relative abundance changes between intervention groups. Statistical testing on compositional similarity between samples was performed with QIIME2 to produce alpha and beta diversity metrics. Study findings displayed shifts in microbial richness, phylogenetic diversity, and differential abundance indicating the need for additional research on the gut microbiome in the presence of EVOO.

**122. SENTINEL GARDEN DATA MANAGEMENT**

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This study investigates the use of modern data management platforms to improve data collection and management in sentinel gardens. Sentinel gardens serve as a way of monitoring for invasive insect pests, which can have devastating consequences for ecosystems and economies when introduced to new environments. However, the success of sentinel gardens as early warning systems relies on effective data management and communication. Conventional methods, such as spreadsheets and email, have been insufficient in efficiently and accurately collecting and managing heterogenous data collected in sentinel gardens.

The study utilized a widely used commercial data management and collection platform called Device Magic to collect data from sentinel gardens in the United States, China and South Korea. The data included images, field samples, and observation data. The results showed that incorporating modern technologies into data management can

significantly improve the accuracy and efficiency of data collection in scientific research.

The findings highlight the importance of modern data management platforms in the monitoring and early detection of invasive insect pests. The use of configurable data management software, such as Device Magic, enables the collection, storage, sharing, and standardization of heterogenous data in a more efficient and accurate manner than traditional methods. Improving data collection and management in sentinel gardens is crucial for the preservation and protection of local as well as global ecosystems and economies and should be a priority for researchers and policymakers.

**123. THE COLLABORATION COMPONENT OF RESILIENCE OF OFFICIAL VETERINARY SERVICES DURING THE COVID-19 PANDEMIC: CASE STUDIES FROM CHILE, NEPAL, SWITZERLAND, AND TUNISIA**

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Official veterinary services provide a variety of functions that contribute directly and indirectly to human and animal wellbeing. The COVID-19 pandemic, by interrupting or otherwise affecting Veterinary service functioning, has had potentially negative impacts on the quality or extent of service delivery, including on the ability of veterinary services to collaborate with others . In this mixed methods study, data was gathered

through a self-administered questionnaire and interviews with members of the official veterinary services of Chile, Nepal, Switzerland, and Tunisia to study the patterns of social networks of collaboration underlying the delivery of their services. The official veterinary service of all four participating countries assisted the human health sector with COVID-19-related activities, ranging from sharing knowledge and expertise to vaccinating humans. Collaboration was described as important and requiring institutional structure, clear roles for collaborating partners, and legal frameworks. Trust, leadership, shared goals, and understanding were competencies useful for collaboration. Of the four official veterinary services, those of Nepal, Switzerland, and Tunisia appear to have been able to recover from the initial disruption created by the pandemic, while the services of Chile were still recovering at the time of data collection. Only the services of Tunisia adapted its operations by enhancing its digitalization. None of the services formed new collaborations since the beginning of the pandemic until the time of the interviews.

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