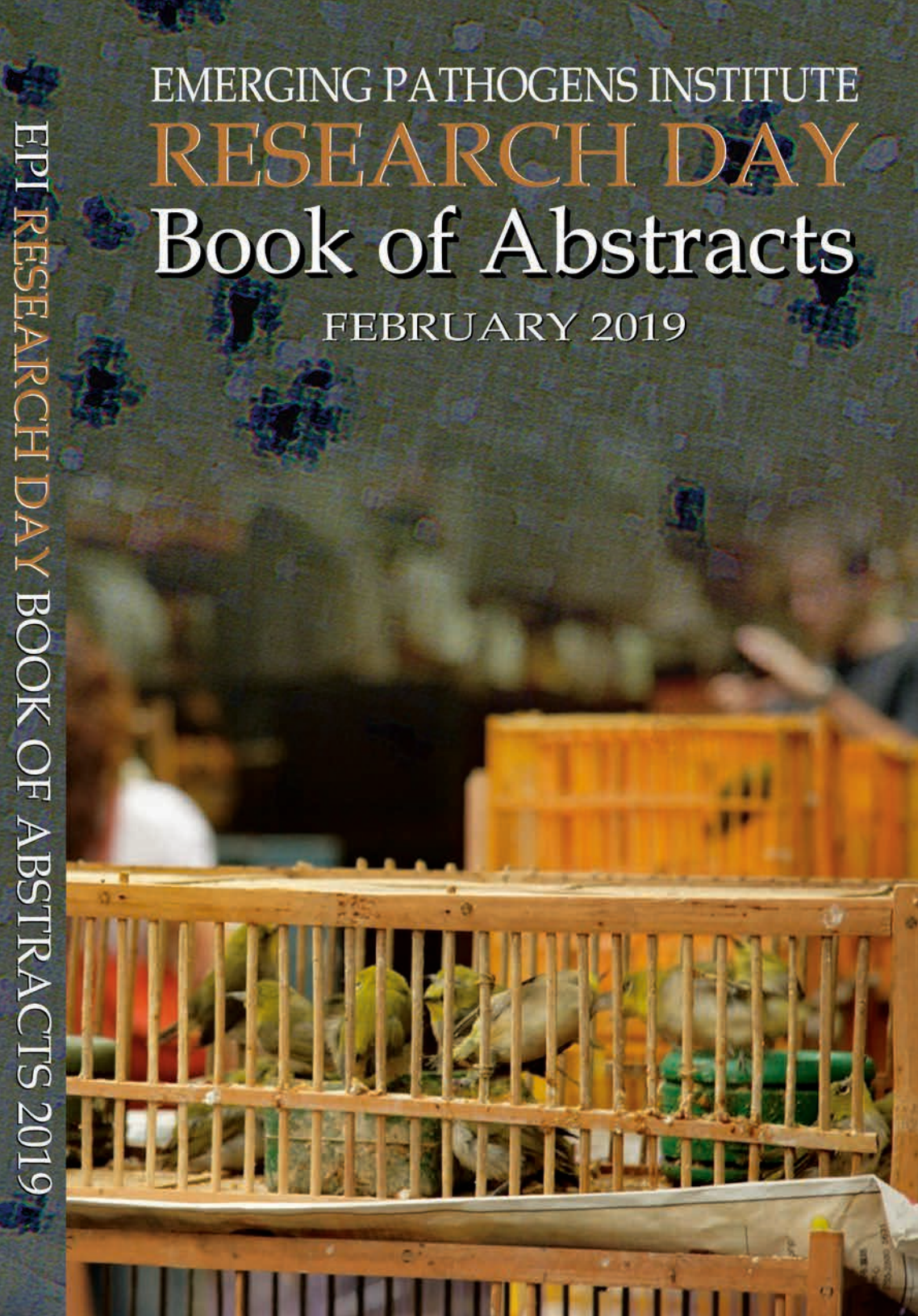
EMERGING PATHOGENS INSTITUTE

RESEARCH DAY

Book of Abstracts

FEBRUARY 2019



EPI RESEARCH DAY BOOK OF ABSTRACTS 2019

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Letter from the Director

Welcome to the twelfth annual EPI Research Day! As you look through the abstracts in this book, and view the correlating posters, you should get a feel for the wide range of emerging pathogens-related research conducted by EPI members and collaborators. We are particularly pleased to welcome investigators from outside of UF: authors on abstracts are affiliated with institutions in over a dozen different countries, over 20 other U.S. universities, and over a dozen different local, state, and federal agencies.

This year we have the honor of introducing you to two outstanding investigators who will be providing keynote talks during our afternoon session:
Dr. Michael Jeger is an Emeritus Professor at Imperial College London, UK. He received a PhD from the University College of Wales in 1979 and worked subsequently at Texas A&M University, USA; Natural Resources Institute, UK; and Wageningen University, the Netherlands; joining Imperial College in 1999. He is internationally recognized for his work on plant pathogens, and pathogen epidemiology and modelling. He is a Fellow of the American Phytopathological Society, the UK Royal Society of Biology, and the Linnean Society of London, and a past President of the British Society for Plant Pathology and of the Association of Applied Biologists.

He is joined by Dr. Maria Elena Bottazzi, Associate Dean, National School of Tropical Medicine, and Professor of Pediatrics and Molecular Virology and Microbiology, Baylor College of Medicine. Her research has focused on parasitic diseases such as hookworm and other soil-transmitted helminths, Chagas disease, amoebiasis, schistosomiasis, and leishmaniasis. She will be discussing advances in the development and testing of the human hookworm and schistosomiasis vaccines.

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

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

Schedule of Events

9:00 AM - 10:00 AM	Registration, Breakfast, and Poster Setup *Reitz Union – Rion Ballroom*
10:00 AM - 1:00 PM	Poster Session *Presenters, please stand by your posters*
12:00 PM - 12:45 PM	Lunch *Reitz Union – Rion Ballroom*
12:45 PM - 1:00 PM	Keynote Assembly *Reitz Union – Rion Ballroom*
1:00 PM - 1:10 PM	Welcome *Dr. J. Glenn Morris, Jr., M.D., M.P.H. & T.M.* *EPI Director and Professor of Medicine* Introductions *Dr. J. Glenn Morris, Director, EPI*
1:10 PM - 3:15 PM	Keynote Speeches
3:15 PM - 4:00 PM	Poster Removal

(1:10-2:10)
Professor Mike Jeger

Emeritus Professor and Senior Research Investigator, Centre for Environmental Policy, Imperial College London, Silwood Park, UK.

"Emerging Risks to Plant Health: Limits to Predictability"

(2:10-3:10)
Dr. Maria Elena Bottazzi

Associate Dean, Professor and Co-Director of Texas Children's Hospital Center for Vaccine Development at National School of Tropical Medicine, Baylor College of Medicine

"Global Health Technologies to Address Health Disparities and Neglected Tropical Diseases in the U.S. and Abroad"

01. ANALYSIS AND INTERACTIVE MAPPING APPLICATION FOR FLORIDA COMPLAINTS AND OUTBREAK REPORTING SYSTEM (FL-CORS) DATABASE

Amanda Sapp - Emerging Pathogens Institute, University of Florida; **Laura Matthias**; **Jamie DeMent**; **Arie Havelaar** - Emerging Pathogens Institute, University of Florida

Background: Florida Department of Health's Food and Waterborne Disease Program (FWDP) updated their method of data collection by implementing the Florida Complaint and Outbreak Reporting System (FL-CORS), a web-based system, in 2012. From 2013 to 2018, Florida residents reported complaints most frequently by phone, reporting to regional health departments in their area and by use of an online form located on the FL-CORS website. In 2017, FWDP heard the state of New York was using social media mechanisms for food and waterborne complaint reporting. FWDP began considering social media as a source for capturing complains in the state of Florida. Potential shifting trends of reporting may be occurring in the state's target audience. While the state actively follows up on Florida resident's Twitter posts that may imply foodborne illness, response rates to an invitation to report the illness on the website are low. Our objective is to plan a systematic evaluation of complaints over years, county and reporting sources to better understand the drivers of reporting. By working with social scientists, we aim to provide interactive enhancements on the FL-DOH website to capture the maximum number of complaints

Methods: FL-CORS complaints for 2013 – 2017 were extracted. County level population data for the state of Florida was obtained from Floridahealthcharts.com. Shapefiles for interactive mapping of Florida were obtained from myflorida.com. Seasonal trends and incidence rates of complaints were analyzed. The mapping application was written in R software using the Shiny package.

Results: From 2013 – 2017 complaints increased by 22%. On average, incidence rates of foodborne complaints in the state of Florida increased every year, with 2017 (mean = 100/yr.) being the

highest of all five years analyzed. August had the highest incidence rate of foodborne complaints across all years (mean = 1.2 /yr.) and of the 67 counties in Florida, Bay county, having a smaller county population, as compared to larger county populations, such as Miami – Dade, had the highest incidence rate of complaints across all years (mean = 230/yr.)

Conclusion: The incidence of foodborne complaints in the state of Florida has increased with every year between 2013 and 2017. Providing an interactive mapping enhancement on the Florida DOH website may help highlight seasonal trends of potential foodborne illnesses in the state for years to come.

02. ANTIVIRAL EFFICACY OF CHITOSAN MICROPARTICLES ON MS2 BACTERIOPHAGE, A HUMAN NOROVIRUS SURROGATE

Candace Barnes - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; **Rebecca Barber** - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; **Anita Wright** - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; **Kwang Cheol Jeong** - Department of Animal Sciences, College of Agricultural and Life Sciences, University of Florida; **Naim Montazeri** - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida

Chitosan, derived from natural, abundant chitin in the environment, has been shown to exhibit antimicrobial effects against a range of bacteria and has sparked interest as an alternative to traditional antiviral treatment against human norovirus, the leading cause of food-borne illnesses worldwide. Since human norovirus is not easily culturable in vitro or in vivo, viral surrogates which are morphologically similar and culturable are essential in shedding light on the virus response to the antiviral treatments. In this study, we investigate the antiviral efficacy of chitosan microparticles (CM) against male-specific bacteriophage (MS2), which is also an RNA non-human pathogen virus with a size and shape similar to that of the

norovirus. CM was produced by ion gelation, involving cross-linking with sodium sulfate, sonication, and washing with de-ionized water. A 10 log10 PFU/ml stock of MS2 was added to the 0.3% CM in PBS (w/v) for a final titer of 7 log10 PFU/ml, and incubated for 0, 1, 2, 3 and 20 hours at room temperature while gently shaking. The infectious titer of MS2 was quantified by plaque assay using the host E. coli. Similarly, RT-qPCR was performed on RNA extracted from the MS2 following the treatment with 0.3% CM. A calibration curve established from the serially diluted MS2 genome obtained was then utilized to determine the titer of the viral genome in the treated samples. In the presence of 0.3% CM, the infectious titer of MS2 was immediately decreased by 3.5±0.6 log10 PFU up to 3 hours. After 20 hours, the reduction reached 4.5±0.0 log10 PFU. RT-qPCR data indicated an immediate reduction in viral genome of 4.0±1.1 log10 RT-qPCR units up to 3 hours and appears to remain constant after 20 hours. These findings reveal CM as a potential antiviral treatment against human norovirus. Further investigation into the mechanism of action and effects on contact surfaces may add more insight into the use of CM against human norovirus in water and on environmental surfaces.

03. ATTENUATED SALMONELLA ENTERICA SEROVAR TYPHIMURIUM STRAINS DELIVERING SHIGELLA FLEXNERI 2A O-ANTIGEN POLYSACCHARIDE INDUCE ROBUST CD4+ T-CELL AND HUMORAL IMMUNE RESPONSES

Huali Su - 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; **Matthew Bellefleur** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Roy Curtiss III** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Qingke Kong** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida

Shigella flexneri 2a (Sf2a) is one of the most frequently isolated Shigella strains that causes the endemic shigellosis in developing countries. In this study, we used recombinant attenuated Salmonella vaccine (RASV) strains to deliver Sf2a O-antigen and characterized the immune responses induced by the vectored O-antigen. A plasmid containing genes identified to be sufficient for biosynthesis of Sf2a O-antigen was introduced into the RASV strains. The strains were manipulated to produce only the heterologous O-antigen and a modified lipid A. After oral immunization of mice, intracellular cytokine staining and flow cytometry were performed to detect splenic lymphocyte cytokine expression and IgG+ B220low/int memory B (BM) cells against the Sf2a O-antigen. ELISA, complement deposition, and serum bactericidal antibody assay (SBA) were used to measure the anti-Sf2a antibody responses. We demonstrated that RASV strains could induce potent humoral immune responses as well as robust CD4+ T-cell responses against Sf2a Lipopolysaccharide (LPS) and protect mice against virulent Sf2a challenge. The induced serum antibodies mediated high levels of Shigella-specific serum bactericidal activity and C3 deposition. Moreover, the IgG+ B220low/int BM cell and T follicular helper (Tfh) cell responses could also be triggered effectively. These findings underscore the potential

of RASV delivered Sf2a O-antigen for induction of robust CD4+ T-cell and IgG responses and warrant further studies toward the development of Shigella vaccine candidates with RASV strains.

04. BACTERIOPHAGES REDUCE PATHOGENIC E. COLI COUNTS IN MICE WITHOUT DISTORTING GUT MICROBIOTA

Upuli Dissanayake - Department of Epidemiology, Emerging Pathogens Institute, College of Medicine, University of Florida; **Maria Ukhanova** - Emerging Pathogens Institute, University of Florida; **Zachary Moye** - Intralytix, Inc.; **Alexander Sulakvelidze** - Intralytix, Inc.; **Volker Mai** - Department of Epidemiology, Emerging Pathogens Institute, College of Medicine, University of Florida

Background: Treatment or prevention of enteric pathogens through the appropriate bacteriophage therapy may provide viable alternatives to the current use of antibiotics. During elimination of disease-causing pathogens antibiotic treatment disrupts the normal symbiotic gastrointestinal microbiota. Our previous studies focused on efficacy of bacteriophage therapy designed against single targets (Shigella spp., Listeria Monocytogenes). The purpose of this study was to (i) investigate the efficacy of Intralytix's E. coli/Salmonella/Listeria bacteriophage cocktail (FOP) to reduce the pathogenic E. coli in experimentally infected mice and (ii) determine whether bacteriophages preserve the normal gut microbiota when compared with antibiotic therapy.

Methods: A total of 75 mice were inoculated with pathogenic E. coli O157:H7 strain 231, which is nalidixic acid resistant (NalAcR) via oral gavage. They were divided into four treatment groups; 1st group received PBS as a control, 2nd group received FOP therapy, 3rd group received FOP at 1:10 dilution, and 4th group received ampicillin, with all treatments administered twice daily for four consecutive days. Stool samples collected at days 0, 1, 2, 3, 5, 10, were homogenized, and plated on LB plates containing NalAc to determine viable pathogenic E. coli counts. Mice weight for every group was monitored for each animal at every stool sample collection timepoint for trend analysis. We performed qPCR with

specific E. coli primers to quantify the number of E. coli genome copies. We also analyzed microbiota community profiles before and during treatment using DGGE (Denature Gradient Gel Electrophoresis).

Results: FOP bacteriophage treatment significantly ($P \leq 0.05$) reduced E. coli pathogen concentration by ≥ 55%, and this reduction was the same as that observed with the antibiotic therapy. However, greater initial weight-loss occurred in mice treated with antibiotic therapy group (-5.44%) compared to both control and FOP bacteriophage groups (-3.56% and -2.24%, respectively). DGGE displayed no changes in gut microbiota composition in the control and the bacteriophage therapy groups after therapy. In contrast, the antibiotic group displayed noticeable distortion of the gut microbiota composition, only partially returning to normal by day 10.

Conclusions: This study found that FOP administration was effective in reducing the levels of pathogenic E. coli in infected mice at a similar rate to ampicillin therapy. However, the FOP bacteriophage preparation had a milder impact on the gut microbiota compared to ampicillin: i.e., it did not trigger any distortion of the normal gastrointestinal microbiota; whereas, treatment with the antibiotic resulted in noticeable gastrointestinal microbiota distortion in mice.

05. CHILDHOOD STUNTING IS ASSOCIATED WITH CAMPYLOBACTER COLONIZATION IN RURAL ETHIOPIA

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

Background: Globally, it is estimated that nearly one in four children under five-years of age is stunted, which has far-reaching and substantial negative impacts on both childhood and adult life. Studies indicate that animal source foods (ASFs) may reduce the risk of stunting. Poultry farming is one possible mechanism by which small holder farmers globally may meet their household need for ASFs. However, exposure of young children to poultry and the contaminated environments of associated with small scale poultry production may increase Campylobacter exposure and colonization, which has been associated with Environmental Enteric Dysfunction (EED) and stunting. The formative research of the Campylobacter Genomics and Environmental Enteric Dysfunction (CAGED) project includes an investigation of the burden of Campylobacter exposure in young children and its association with stunting in young children.

Methods: A cross-sectional study was conducted in Haramaya woreda in rural eastern Ethiopia. 100 children, ranging in age from 360 and 500 days, were randomly selected for participation. Anthropometric measurements, Campylobacter colonization, and questionnaire-based information on demographics, household wealth, hygiene and sanitation, and nutrition were collected from September through December 2018. Descriptive data analysis was conducted to characterize the stunting and wasting prevalence in the population. We also explored the association between impaired growth and Campylobacter colonization. This project is a

collaboration between UF, Haramaya University, Ohio State University and Washington University.

Results: Anthropometric measurements suggested that the prevalence for stunting and wasting is 42% (LAZ < -2, 95% CI: (33% - 52%)) and 5% (WLZ <-2, 95% CI: (2% - 11 %)), respectively. The prevalence of Campylobacter colonization is 59% (95% CI: (49% - 68%)). Crosstabulation by Cochran-Armitage test for trend suggested a significant positive association between stunting and Campylobacter presence (p = 0.04).

Conclusion: The formative research quantifies the burden of Campylobacter and chronic malnutrition in young children in a rural region in Ethiopia. The association between these two factors opens a window for further investigation to evaluate a possible causal relationship and will be refined by including other putative environmental and behavioral determinants contributing to stunting. The results will be used to inform the design of a Randomized Controlled Trial, which aims to examine the benefits of coupling increased egg consumption with the elimination of contact between young children and chickens and their excreta on linear growth.

06. CHITOSAN MICROPARTICLES EFFECTIVELY REDUCE SALMONELLA ENTERICA AND GENERIC ESCHERICHIA COLI IN NATURAL WATER SAMPLES

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Water that is used for irrigating or washing produce is a food safety concern because it can become contaminated with foodborne pathogens. This study was conducted in order to determine the effectiveness of chitosan microparticles (CM) against the growth and survival of Salmonella enterica and Escherichia coli bacteria in natural water systems. CM is derived by sonicating chitosan (a solubilized form of chitin) and shows increased antimicrobial activity compared to unprocessed chitosan. CM activity was assessed for stationary phase Salmonella inoculated into various water sources, as well for natural populations of both Salmonella and E. coli. CM concentrations between 0.01 and 0.005% wt:vol consistently reduced inoculated Salmonella (3.7 log CFU/mL) to non-detectable levels within 24 h post-treatment for autoclaved pond water or in isotonic glucose. These effects were consistent for different chitosan formulations, and all CM preparations were relatively stable at 4° C for over 2 months, but activity was lost upon frozen storage. In addition, CM treatment effectively eliminated inoculated and natural populations of Salmonella for intact pond water that was obtained from multiple sources at different time points. Fecal indicator

bacteria (i.e. generic E. coli) were also reduced to non-detectable levels following CM treatment. One exception was an urban creek that showed reduced anti-Salmonella efficacy at one time point; however, this water source also had the highest level of fecal indicator bacteria. The research herein presents the first comprehensive examination of CM activity in the complex medium of natural water collected from pond, creek or lake systems. These data demonstrated that CM presents a relatively safe and biodegradable alternative for antimicrobial treatment of natural water systems contaminated with potential human pathogens.

07. DETECTION OF DIVERSE SEROTYPES OF VIBRIO CHOLERAE O1 IN AQUATIC RESERVOIR IN HAITI

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Cholera, a secretory diarrheal disease caused by toxigenic strains of Vibrio cholerae O1, was absent in Haiti for 100 years. However, Nepalese Peace keeping troops have accidentally brought the disease in that country on October 2010. Despite cholera is a water-borne disease, some previous investigators reported that there was no environmental and/or water contamination of V. cholerae in Haiti. In contrast, using carefully designed environmental sentinel monitoring, we have previously reported that, toxigenic V. cholerae

O1 strains in deed persisting in aquatic reservoirs in Haiti with rainfall and temperature contributing the environmental bloom of toxigenic V. cholerae O1 strains with subsequent spill over to humans. Although cholera has significantly declined in Haiti between 2016 and 2018 (over 58,213 suspected cases with 642 deaths as reported by PAHO and MSPP), we have isolated a total of seventeen V. cholerae O1 strains from three distinct environmental monitoring stations. These stations include Gressier, Jacmel and Port-au-Prince (PAP) with each station has multiple water sampling sites. We collect water sample from each site monthly for the isolation of toxigenic V. cholerae O1 strains. Of 17 isolates, 12 (71%), 1 (5.9%) and 4 (23.5) came from Gressier, Jacmel and PAP stations, respectively. On serotyping we identified that only 2 (11.8%) exhibited Ogawa serotype while 9 (52.9%) typed as Inaba serotype. Interestingly, 6 (35.3%) typed with only V. cholerae O1 polyvalent serum with no reaction was noted either with Ogawa or Inaba serum. The polyvalent positive strains were isolated both from Gressier (rural) and PAP (urban) sites, suggesting their potential distribution in aquatic reservoirs in greater Haiti. PCR-based genetic analysis revealed that 5 (29.4%) strains have ctxA, ctxB, and tcpA genes while 12 (70.6) isolates have lost both these critical virulence genes. Interestingly, all non-toxigenic V. cholerae O1 strains also lack ideA gene that inhibits the chitin-based natural transformation. We predict that these non-toxigenic V. cholerae O1 strains may acquire toxigenic genes through chitin-induced transformation and thereby potentially could promote cholera transmission. Our data clearly underscore the importance of regular environmental survey for the monitoring of prevalence of V. cholerae O1 strains in cholera endemic setting as in Haiti. Environmental monitoring of toxigenic V. cholerae O1 can lead to predict impending cholera at population level and alert authorities at national level to undertake appropriate cholera intervention/mitigation strategies.

08. DETECTION OF SALMONELLA SPP., LISTERIA MONOCYTOGENES, ESCHERICHIA COLI O157:H7, AND TOTAL COLIFORM COUNT IN NORTH CENTRAL FLORIDA RAW MILK

Priscilla Sinclair - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida

Unpasteurized milk (raw milk) hosts a wide array of microorganisms that are killed off during pasteurization. The sale of raw milk is illegal under the federal government but, it can be legally sold on the state level in Florida for animal consumption. Raw milk is commonly found in many grocery and pet stores as well as farmers markets. The presence of human pathogens in raw milk that is sold in Florida is unknown. Pathogens contaminate raw milk through contact with feces and contaminants in the farm environment. In the past raw milk has been the cause of Listeria monocytogenes, E. coli O157:H7 and Salmonella spp. outbreaks, causing serious illnesses. This study delves into the occurrence of major foodborne pathogens as well as the total coliform and E. coli count in raw milk sold on farms and in retail stores in North Central Florida. Milk samples were bought from retail stores and directly from the farmer (on the farm and at farmers markets). Raw milk samples were then analyzed for the presence of Listeria monocytogenes, E. coli O157:H7 and Salmonella spp. Analysis was carried out using commercial quantitative PCR (qPCR) assay kits to identify the presence of each respective pathogen. Five samples were surveyed with no occurrence of Salmonella spp. and E. coli O157:H7 however, four out of the five samples contained Listeria monocytogenes. Samples were also analyzed using petrifilm for total coliform and E. coli count. Two out of the five samples contained coliforms. The two samples from the farm had average total coliform counts of eighteen and thirty-four colonies per milliliter. One of these samples also had a total E. coli count of twenty-seven colonies per milliliter. These results could support future legislation to outlaw the sale of raw milk in Florida to protect the population from future outbreaks.

09. DEVELOPMENT OF ANTIBODY-CONJUGATED CHITOSAN NANOPARTICLES TARGETING SHIGA TOXIN PRODUCING ESCHERICHIA COLI IN GASTROINTESTINAL TRACT

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The objective of this study was to develop anti-Shiga toxin-producing Escherichia coli (STEC) antibody conjugated chitosan nanoparticles (CN) to control STEC at the pre-harvest level. STEC are important foodborne pathogens, and cattle are the major reservoirs. Several methods have been tried to reduce these pathogens at the pre-harvest level. However, the trials have not been successful. CN, derived from chitosan, have shown great broad-spectrum antimicrobial activity. Here, we report that conjugation of CN and anti-STEC IgY antibodies kill STEC selectively. Lipopolysaccharides (LPS) of seven STEC serotypes (O26, O45, O103, O111, O121, O145, and O157) were extracted and purified. Five laying hens were immunized with each LPS respectively, and IgY antibodies were purified from egg yolk. The sensitivity and specificity of IgY were tested by ELISA. The detection limit of IgY ranged between 2-3 log CFU/well. Five out of 7 types of anti-STEC IgY were able to recognize the corresponding STEC serotype selectively. CN and the antibodies were linked by stable covalent amide bonds at 10:1, 10:2, and 10:4 ratios respectively. The activity of anti-O157 CN-IgY and CN at 0.05% were examined against E. coli O157:H7, Salmonella enterica, Lactobacillus plantarum and their combination. Compared with CN, CN-IgY (10:2) showed significantly stronger activity against E. coli

O157 ($P < 0.05$), indicating that IgY enhanced the specificity of CN. In a mixed culture of bacteria, CN-IgY conjugates decreased the concentration of E. coli O157 significantly compared with CN ($P < 0.05$), while did not affect the concentration of S. enterica or L. plantarum. The same selective activity was maintained in synthetic intestinal fluid, even after digestion of synthetic gastric fluid. The activity of CN-IgY against STEC in an in vivo model, Caenorhabditis elegans, was also proved. These results suggested that the CN-IgY conjugates have strong specific antimicrobial activity, and are great candidates to eliminate pathogens selectively in the gastrointestinal tract of animals. This CN-antibody conjugation model can also be used to generate antimicrobials against other pathogens.

10. EXOSOMES DERIVED FROM SALMONELLA-INFECTED MACROPHAGES CARGO ANTIGENS LEADING TO THE STIMULATION OF A PROINFLAMMATORY RESPONSE FROM TYPE 1 T-HELPER CELLS

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Salmonella enterica serovar Typhimurium (S. Typhimurium) is a non-typhoidal, gram-negative, intracellular bacterium which invades macrophages and leads to the production of pro-inflammatory exosomes. S. Typhimurium is the causative agent of Salmonellosis affecting 1.2 million people annually in the United States. There are currently no vaccines against non-typhoidal Salmonella infections for human or animal reservoirs thus showing a significant limitation in current prevention methods. Exosomes are a subclass of

extracellular vesicles characterized by their size, morphology and biogenesis. The cargo, including protein, nucleic acids and metabolites, carried by exosomes vary depending on the physiological conditions present and the cell from which it arise. We hypothesize that during Salmonella infections, exosomes transport Salmonella antigen to alert neighboring cells which can lead to the stimulation of naïve T lymphocytes. Here, we focus on the release of exosomes by S. Typhimurium-infected macrophages and their function in stimulating an adaptive immune response in vivo. To determine if exosomes have any effect on the adaptive immune response, mice were given several doses of exosomes derived from S. Typhimurium infected macrophages for a 2-month period. Fluorescent activated cell sorting (FACS) was used to monitor T-lymphocyte response. In vivo, exosomes stimulate a distinct cytokine secretion pattern among CD4+ T lymphocytes. The cytokines milieu, including interferon gamma, tumor necrosis factor alpha and Interleukin 2, expression by T-lymphocytes suggest that the CD4 T-lymphocytes differentiated in to Type 1 T-helper (Th1) subset producing pro-inflammatory cytokines. Additionally, mouse serum was taken to analyze for antibody production against Salmonella in which we observe exosomes derived from Salmonella infected cells provide a similar antibody production to the live vaccine. Based on our proteomics study, we identify Salmonella antigens in exosomes isolated from Salmonella infected-macrophages from 24 and 48 hour infections therefore cargo can play a critical role in intercellular communication in response to infection as naïve macrophages treated with these exosomes result in M1 polarization. Overall, our data supports the hypothesis that exosomes isolated from Salmonella infected macrophages carry Salmonella antigens as a cargo and stimulates the activation of Type 1 effector T lymphocytes.

11. EXPLORING MOLECULAR EPIDEMIOLOGY OF SALMONELLA IN FLORIDA

Nitya Singh - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Xiaolong Li** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Elizabeth M Beshears** - Division of Disease Control and Health Protection, Florida Department of Health; **Jamie DeMent** - Division of Disease Control and Health Protection, Florida Department of Health; **Arie Havelaar** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Each year in the United States, it is estimated that non-typhoidal Salmonella enterica causes approximately 1 million illnesses, making it the second leading cause of domestically acquired foodborne illness. In 2017, Florida reported 6,557 cases of salmonellosis, with a higher incidence rate of 31.9 per 100,000 than the national average of 16.7 per 100,000. In October 2016 Florida rules were updated to require the submission of Salmonella specimens to the Florida Department of Health for further epidemiological analysis. Current funding, staffing and equipment capacity allow the department to sequence approximately 1,500-2,000 per year, which requires a systematic process of selection to obtain an unbiased sample set. A collaborative effort within the Florida Integrated Center of Excellence of Food Safety (CoE) between the Florida Department of Health and University of Florida is aimed at providing a more comprehensive understanding of the epidemiology of salmonellosis, including the distribution of different serotypes in Florida. To accomplish this, the Florida Department of Health will provide surveillance metadata by merging the laboratory data from Bionumerics and case information from Merlin databases. Initially, descriptive statistics will be created describing the distribution of different Salmonella serovars among age groups and sex at the resolution of county level. Further objectives are to understand the phylogenetic relationships of different circulating Sequence Types

(ST) and trace their spatio-temporal trends over recent years in the state. Currently, we are working with a limited data set of 1285 samples provided by the department from 2016-2018 to develop methods for data sorting and designing pipeline and methods to achieve this overall goal. With current dataset, we first sorted out the top 20 FL serotypes while remaining STs were aggregated into two categories by applying a statistical ranking based on the coefficient of variation (CV) described in Boore et al. (2015). Data was organized and visualized using in-house R-scripts. For understanding the phylogenetic relationship among the isolates, we have generated minimum spanning trees based on 7 gene MLST types, rMLST and eBG groups using the Enterobase web-based system and GrapeTree for visualization. Future steps include developing R-based pipelines for discerning the spatio-temporal trends of overall and ST type specific cases of Salmonella. This research will support Florida state Lab transition from PFGE to whole genome-based Salmonella surveillance and outbreak detection.

12. LASER CARBONIZED APTASENSORS FOR THE POINT OF USE DETECTION OF LISTERIA MONOCYTOGENES

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Bacterial foodborne illnesses currently affect approximately 9.4 million people annually in the United States. One of the most prevalent foodborne pathogens is Listeria monocytogenes, which can propagate in temperatures from -1.5 to 50°C and pH 4.3 to 9.6. Listeria has been associated with recalls in fresh produce to frozen foods. Aptamer-based biosensors are emerging as viable alternatives to traditional, time-consuming detection methods. The goal of this research is to develop rapid, low-cost L. monocytogenes biosensors based on composites of nanometal and stimuli-responsive polymer nanobrushes on flexible, laser carbonized electrodes. Laser scribed graphene (LSG) electrodes were engineered through carbonization of polyimide films using a low-cost UV laser. Laser pulse width was optimized via measurements of electroactive surface area, heterogenous electron transfer kinetics, and charge transfer resistance. Based on electrochemical performance, the optimal laser pulse width was found to be 40 ms. LSG was further analysed using electron microscopy, Raman/XRD spectroscopy, and hydrophobicity/porosity measurements. LSG electrodes were metallized and biofunctionalized with a L. monocytogenes specific DNA aptamer or further enhanced with a stimulus-responsive nanobrush (chitosan) terminated with an aptamer. Optimal chitosan deposition was at 2V for 5 min and demonstrated similar stimulus-responsive behaviour to our previous work with commercial electrodes. The current limit of detection (LOD) for L. monocytogenes is 10 CFU/mL in 20 minutes using electrochemical impedance spectroscopy with no nanobrush actuation. This work demonstrates the first low cost flexible biosensor for detection of L. monocytogenes in food samples. We are working toward improving

LOD using a combination of nanobrush actuation, sample pre-treatment, vacuum driven fluidics, and post hoc machine learning analysis. The LSG biosensors shown here are easily reproducible with a high degree of customizability, demonstrating a new analytical platform for rapid food safety monitoring as well as potential use in applications such as hospitals, disease tracking, and ecosystem health.

13. LECTIN-BASED BIOSENSOR FOR DETECTION OF E. COLI IN DRINKING WATER

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663 million people lack access to safe drinking water, and more than a billion people have to travel at least 30 minutes to a water source. As a result, 2.3 million people die each year from dysentery caused by contaminated drinking water. The aim of this study was to create an affordable, facile and field deployable biosensor that would allow these individuals to test the quality of their own drinking water quickly and efficiently. We chose concanavalin A (ConA), a lectin found in the jackbean plant, as our biorecognition element because it is selective for mannose, a complex sugar found in abundance on the surface of E. coli cells. In this study, we laser scribed a polyimide film (Kapton tape) which was adhered to the emulsion side of photopaper to create our laser scribed graphene (LSG) electrodes. We then placed the electrodes in a solution containing a mixture of cupric sulfate and copper nanoparticles extracted from reclaimed e-waste. The particles were plated using gravity-assisted electrodeposition, followed by the addition of ConA bound via electrostatic interaction to the copper on the electrode surface.

These electrodes were used for the detection of E. coli K-12 (ATCC 8739) as an initial stand in for pathogenic strains of bacteria. We conducted electrochemical impedance spectroscopy to measure the resistance on the working area of the electrode with just the cupric sulfate nanoparticles, with the lectin added, and finally with bacteria added. The lectin-bacteria complex causes agglutination, a phenomenon in which the bacteria, having a multitude of sugars on their surface, are able to bind to multiple lectins. We expected the bacteria to agglutinate on the surface of the electrode, causing the measured impedance (resistance) to increase for the bacterial run. In our preliminary studies we have been able to obtain a discernable signal change in impedance with bacterial concentrations as low as 40 CFU/mL. This leads us to conclude that a lectin-based sensor is feasible for detecting pathogenic bacteria in drinking water. Our sensor is easy to use and made of readily obtained and affordable materials. Future work will include using this sensor on 'real' samples from the environment, streamlining and improving our sensitivity and selectivity and eventually adding more lectins to use as a sort of 'lectin library' on the surface of our electrode.

14. OPTIMIZATION OF VIABILITY DETECTION METHOD FOR SHIGA TOXIN-PRODUCING E. COLI (STEC) USING DNA PHOTO-LABELING WITH PCR

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The CDC estimates Shiga Toxin-Producing Escherichia coli (STEC) causes over 265,000 infections and 30 deaths in the U.S. each year. While only viable STEC can cause illness, most rapid detection methods cannot differentiate between live and dead bacteria. DNA photo-labeling prevents PCR amplification of DNA from dead cells and thus selectively amplifies DNA from live cells. The purpose of this study was to develop and optimize a rapid, PCR-based detection method combined with DNA photo-labeling able to differentiate live and dead STEC. Live and dead STEC cells, of various cell mixtures/concentrations and photo-labeling conditions, were treated with or without DNA photo-labeling dye ethidium monoazide (EMA) and then exposed to LED light. Inhibition of PCR amplification of dead cells' DNA was confirmed using end-point PCR. With photo-labeling, various concentrations of live E. coli O157:H7 mixed with dead cells at high cell concentration were selectively detected using end-point PCR by inhibiting amplification of dead cell DNA. Live E. coli O121 and O145 (1x10^5 and 1.0x10^8 CFU/mL) were successfully differentiated from dead cells with modified PCR parameters. Limit of detection was 1.0x10^3 CFU/mL. With 12-h enrichment of E. coli O157:H7, as low as 1.0x10^2 CFU/mL live cells were detected in presence of 1.0x10^8 CFU/mL dead cells. Results suggest that DNA photo-labeling combined with PCR-based detection methods can potentially differentiate live and dead STEC.

15. PGE2 AUGMENTS MACROPHAGE POLARIZATION AND INFLAMMASOME ACTIVATION DURING INFECTION WITH ENTERIC PATHOGENS SALMONELLA ENTERICA TYPHIMURIUM AND YERSINIA ENTEROCOLITICA

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Prostaglandin E2 (PGE2) is an important immunomodulatory molecule that is part of a larger group of bioactive lipids known as eicosanoids. PGE2 has long been reported to have conflicting roles in the regulation of inflammation. For example, some effects are primarily anti-inflammatory and other roles that mediate inflammation. During intracellular infection with Gram-negative bacteria, host macrophages form complexes called inflammasomes. Inflammasomes drive activation and secretion of inflammatory cytokines such as IL-1β that signal nearby cells in the process of inflammatory cell death called pyroptosis. PGE2 is reported to inhibit NLRP3 inflammasome activation and enhance NLRC4 inflammasome activation. Since both NLRP3 and NLRC4 inflammasomes mediate IL-1β secretion, the PGE2's function in the inflammatory processes does not seem to be clear. Previous studies relied on LPS stimulation of macrophages in place of bacterial infection. In this study, we used an infection model of THP-1 human macrophages with Salmonella enterica Typhimurium (St) and Yersinia enterocolitica (Ye) that suggests PGE2 plays a vital role in enhancing the transcription and secretion of IL-1β upon inflammasome activation. Interestingly, distinct virulence strategies between St and Ye are reflected in the alteration of COX-2 and resulting PGE2 biosynthesis by these

pathogens. Ye virulence factors inhibit COX-2 expression and thus decrease the amount of PGE2 secreted during infection. St, in contrast, activates COX-2 and enhances PGE2 production. In our studies, PGE2 enhances bacterial load of St inside macrophages 48 hours post infection, but it decreases intracellular Ye. Finally, our results suggest that PGE2 primarily enhances the transcription of pro-inflammatory cytokines while repressing anti-inflammatory processes such as resource scavenging and matrix remodeling.

16. PROTECTIVE ABILITY OF BIOGENIC ANTIMICROBIAL PEPTIDE MICROCIN J25 AGAINST ENTEROTOXIGENIC ESCHERICHIA COLI-INDUCED INTESTINAL EPITHELIAL DYSFUNCTION AND INFLAMMATORY RESPONSES IN IPEC-J2 CELLS

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Poison of intestinal induce severe health problems in human infants and young animals due to contaminating foods and feedstuffs. With the emergence of public health concerns and high-speed diffuse of drug-opposition of bacteria, the adoption of antimicrobial peptides as potential candidates in treating pathogen infections raised up. Nature Microcin J25 (MccJ25), a class of lasso peptides separated from a fecal strain of E. coli, has been replied to display powerful antimicrobial behavior. Herein, the study was to assess the usefulness of biogenic MccJ25 in the prophylaxis of ETEC K88 infection in IPEC-J2 cells. In vitro antimicrobial activity against ETEC K88 and cytotoxicity of biogenic MccJ25 were determined first. To further understand how biogenic MccJ25 mediates its impact, ETEC K88 adhesion in cells, membrane permeability [as indicated by reduced release of lactate dehydrogenase (LDH)], transepithelial electrical resistance (TEER), barrier function, and proinflammatory

cytokines levels were determined in IPEC-J2 cells after treatment with biogenic MccJ25 and challenge with ETEC K88. Biogenic MccJ25 had a minimum inhibitory concentration of 0.25μg/mL against ETEC K88, decreased ETEC K88 adhesion in cells and did not cause cytotoxicity toward cells. Furthermore, biogenic MccJ25 protects against ETEC-induced barrier dysfunction by increasing the TEER, decreasing the LDH and promoting tight junction proteins (TJPs) by promoting the assembly of occludin and claudin-1 in the tight junction complex. Biogenic MccJ25 was further found to relieve inflammation responses through modulation of interleukine-6, IL-8 and tumor necrosis factor-α levels via inhibition of mitogen-activatedprotein kinase (MAPK) and nuclear factor κB activation. In summary, biogenic MccJ25 can protects against ETEC K88-induced intestinal damage and inflammatory response, recommend the hidden adoption of biogenic MccJ25 as a novel prophylactic agent to reduce pathogen infection in animals, food or humans.

17. RAPID DETECTION OF SALMONELLA USING SIMPLE LIQUID CRYSTAL-BASED IMMUNOASSAY

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Salmonella is one of major foodborne pathogens of great public health concern. A rapid and sensitive detection for this pathogen is critical to ensure food safety. Liquid crystal-based immunoassay uses unique optical characteristics of liquid crystals to perform a rapid and sensitive detection method of pathogens. The aim of this study is to evaluate a novel liquid crystal-based assay for detection of Salmonella. The liquid crystal-based immunoassay was developed using immunomagnetic beads (IMB) and liquid crystal. When Salmonella is present, formation of Salmonella-IMB aggregates distorts liquid crystal matrix, which creates rapid light transmission, resulting in detectable signal. The assay was tested for its sensitivity

and specificity was tested with various concentrations of Salmonella and common pathogens. The developed immunoassay was able to detect Salmonella with detection limits of 104 CFU/mL without any enrichment. The total assay was completed within 30 min. With 12-hr enrichment step, Salmonella as low as 1 CFU/mL was detected. The developed assay was highly specific to all tested Salmonella serotypes, and did not show any cross-reactivity with other common foodborne pathogens. Its sensitivity and specificity was comparable to ELISA, but the positive result was confirmed in shorter time than ELISA (30 min vs 90 min), and with less steps. The novel liquid-crystal immunoassay has a great potential as a rapid and sensitive detection method for Salmonella.

18. REGULATED DELAYED SHIGELLA FLEXNERI 2A O-ANTIGEN SYNTHESIS IN LIVE RECOMBINANT SALMONELLA ENTERICA SEROVAR TYPHIMURIUM INDUCES COMPARABLE LEVELS OF PROTECTIVE IMMUNE RESPONSES WITH CONSTITUTIVE ANTIGEN SYNTHESIS SYSTEM

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Shigella flexneri (S. flexneri) remains a leading cause of shigellosis in children and continues to be a major public health problem in less-developed countries. We have developed a new attenuated Salmonella vaccine system based on the regulated delayed antigen synthesis (RDAS) and regulated delayed expression of attenuating phenotype (RDEAP) systems for delivering S. flexneri 2a (Sf2a) O-antigen. The new system was developed by integration of the cassettes of araC PBAD lacI and araC PBAD wbaP into the chromosome of the vaccine strain, of which the wbaP gene was

depleted. Plasmid containing genes involved in biosynthesis of the Sf2a O-antigen under the control of the LacI-repressible Ptrc promoter was introduced into the vaccine strain. In the presence of arabinose, the vaccine strain could produce LacI and the Und-P galactose phosphotransferase (WbaP) that is required for synthesis of Salmonella native LPS. Thereby, the vaccine strain could primarily synthesize Salmonella native LPS and repress the production of Sf2a O-antigen. In the absence of arabinose in vivo, the transcription of Sf2a O-antigen genes would initiate and the native Salmonella LPS synthesis would be repressed. After two rounds of oral immunization of mice, the newly developed RDAS strain could induce comparable levels of Sf2a-specific protective immune responses and long-term immunity with the constitutive antigen synthesis system. This system provides another potential option for vaccine development using attenuated Salmonella strains for delivering exogenous LPS-associated O-antigen.

19. DYNAMICS OF INFLUENZA A(H3N2) ANTIBODY LANDSCAPES

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Seasonal influenza virus causes substantial disease burden of human globally due to its fast evolution and escape from human immunity,

despite for the continuous efforts on vaccine development. Antibody landscape quantitatively measures the human immunity to historical influenza strains that are variable both genetically and antigenically. Understanding the dynamics of influenza antibody landscapes and its association with pathogen evolutions would be helpful to predict influenza infection and guide influenza vaccine development. We collected serum from residents in Guangzhou, China in 2010 and 2014, respectively and measured hemagglutination inhibition (HAI) titers against twenty historical strains of influenza A(H3N2) circulated during 1968 to 2014. First, we performed Fourier spectrum analysis to the time series of residuals for each individual, which were derived from a GAM fitted to the time series of HAI titers against historical H3N2 strains adjusted for age at sampling, age at circulation and strains. We plotted the distribution of the peak period identified from the Fourier spectrum analysis across all participants and tested the statistical significance with the distributions from 1,000 permutations of each individual's time series of residuals. In addition, we fitted a generalized addictive model (GAM) to examine the pairwise association between cross-reactivity, observed genetic difference and circulation times across strains. We examined the cross-reactivity using the pairwise association between HAI titers against pre-birth strains and the strains that an individual confronted after adjusted for the age at sampling of the participants. A significantly higher proportion of participants was found to have peak period at ~16 years from the Fourier spectrum analysis. We found the genetic distance of strains have a positive linear association between the circulation times across the strains. The cross-reactivity decreased as the genetic distance/circulation times of the strains increase until the genetic distance was ~10% and the difference between circulation time was ~16 years, after which cross-reactivity appeared to have no association between genetic distance/circulation times of the strains. Our findings seem to suggest a ~16 years period for the antibody landscapes for influenza H3N2 strains, which may be associated with the decreasing cross-reactivity led by the strains evolution.

20. EXPLORING LIPID PROFILES AND THE USE OF MULTIPLEX PCR ASSAYS IN HUMAN SPUTUM SAMPLES

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Respiratory infections are among the top ten leading causes of death worldwide. In the U.S., more than 500 million infections occur annually at a loss of 40 billion dollars and up to 100 million school/work days lost. Because of this substantial health and economic burden, it is imperative that we better understand individual factors that make people more or less susceptible to infection, and then develop ways to improve prognosis and therapeutic interventions. A growing area of research is centered on the role of lipids in respiratory infections, and previous work has demonstrated that lipids (and/or metabolites) can differentiate between disease states, such as community- and non-community-acquired pneumonia cases. In the current study, our objective is to examine the associations between lipid profiles of sputum (obtained via LC/MS lipidomics), respiratory infection status (ascertained using the BioFire FilmArray® Classic Respiratory Panel (RP)), and individual risk factors (collected with retrospective chart review) from patients at Shands Hospital. Preliminary experiments were conducted to optimize the use of lipidomics and the BioFire FilmArray® assay with sputum samples. In partnership with the Southeast Center for Integrated Metabolomics (SECIM), we developed an extraction

protocol and identified more than 500 known lipid species from patient sputum samples, with triglycerides (TG), phosphatidylcholines (PC), ether-PC, and ether-phosphatidylethanolamines (PE) emerging as the most abundant classes. Individually, the lipid profiles of each of four sputum samples shared some similarities in the abundance of lipid classes, but also demonstrated some noticeable differences. Because the BioFire FilmArray® RP has not been used extensively for pathogen detection in sputum samples, we investigated the use of dithiothreitol (DTT) as a mucolytic agent. We observed that the assay is capable of detecting a spike of influenza A successfully with no interference by DTT. These data demonstrate both our feasibility to extract lipids from human sputum samples with excellent coverage of lipid species and substantial detail to visualize differences across samples as well as our excellent detection ability for respiratory pathogens in sputum samples. These optimization experiments have prepared us to begin our current pilot study in which we are examining how different lipid profiles correlate with patient risk factors and causative agents of infection. The hope is that identification of lipid biomarkers for respiratory infections will contribute to improved diagnostics and treatment regimes.

21. NONPARAMETRIC MODELING FOR SPATIO-TEMPORAL DISEASE INCIDENCE RATE DATA

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To monitor the incidence rates of cancers, AIDS, cardiovascular diseases, and other chronic or infectious diseases, some global, national and regional reporting systems have been built to collect population-based data about the disease incidence. Such databases usually report daily, monthly, or yearly disease incidence numbers at the city, county, state or country level, and the disease incidence numbers collected at different places and different times are often correlated: with the ones closer in place or time being more correlated. The correlation reflects the impact of various confounding risk factors, such as weather, demographic factors, life styles, and other cultural and environmental factors. Because such impact is complicated and challenging to describe, the spatio-temporal (ST) correlation in the observed disease incidence data has complicated ST structure as well. Furthermore, the ST correlation is hidden in the observed data, and cannot be observed directly. In the literature, there has been some discussion about ST data modeling. But, the existing methods either impose various restrictive assumptions on the ST correlation that are hard to justify, or ignore partially or entirely the ST correlation. We develop a flexible and effective method for ST disease incidence data modeling, using nonparametric local smoothing methods. This method can properly accommodate the ST data correlation. Theoretical justifications and numerical studies show that it works well in practice.

22. RISK FACTORS FOR RESPIRATORY VIRAL ILLNESS AMONG HEALTHCARE PERSONNEL IN THE UNITED STATES 2012-2015; THE RESPECT STUDY

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Health care personnel (HCP) are exposed to a large number of individuals with respiratory illness while providing patient care. Respiratory precaution and influenza immunization policies recognize the risk that healthcare work presents to HCP and to subsequent patients. However, the etiology of respiratory illness among HCP is poorly characterized and likely changes from season to season.The objective of this analysis was to define risk factors for respiratory infection among HCP that participated in the Respiratory Protection Effectiveness Clinical Trial (ResPECT).HCP were randomized by clusters to either N95 respirators or medical masks each season. Cluster assignment varied by flu season, with each site assigned to both study arms during the multi-year study.The main outcomes of the present study were respiratory infections evaluated included symptomatic laboratory-confirmed respiratory infections, symptomatic and asymptomatic PCR-confirmed respiratory infections , symptomatic and asymptomatic PCR-confirmed respiratory infections not including influenza , and symptomatic and asymptomatic PCR-confirmed influenza. Asymptomatic swabs were collected randomly from participants twice each season. Symptomatic swabs were collected within 24 hours of self-reported respiratory symptoms and again if participants were still symptomatic after seven days. Results are under analysis (to be completed by EPI Research Day). Univariate and multivariate models have been developed to analyze risk factors including demographics, behavior, and work status. Preliminary results show that males had a

reduced risk of respiratory infection relative to females (OR=0.81, CI=0.66-99). As HCP increased in age, the risk of respiratory infection decreased; HCP aged 40-49 (OR=0.81, CI=0.69-0.94), 50-59 (OR=0.68, CI=0.58-0.81), and 60-69 (OR=0.78, CI=0.62-0.98) had a decreased risk of respiratory infection relative to HCP aged 18-29. HCP that identified as Black had a lower risk of respiratory infection (OR=0.66, CI=0.54-0.80). Further analysis is being conducted.

23. STATISTICAL CONSIDERATIONS OF THE TEST NEGATIVE DESIGN

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Increasing discussion on using test negative design to evaluate influenza vaccine effectiveness has been raised recently. The test negative design is a related to the case-control study, while it also shares similar uncertainties with cross-sectional study. The core assumption of the design, that the vaccine has no effect on other diseases with similar symptoms, should be seriously treated. Vaccine effectiveness is estimated from the odds ratio of test positive for vaccinated patients versus unvaccinated patients. The odds ratio estimates the incidence rate ratio without requiring a rare disease assumption. We compare the test negative design with the case-control study. We propose a generic data-generating mechanism following a density sampling strategy. Logistic regression adjusted for correlation among response variables is the main statistical model to estimate odds ratio and its variance. We examine the likelihood and use simulations to compare the Wald test with score test under a range of scenarios. Sample size calculation based on the score test is also considered.

24. APPROACHES TO EVALUATING DRUG EFFICACY AGAINST LEISHMANIA AMAZONENSIS

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The protozoan parasites of the genus Leishmania reside within specialized intracellular compartments known as Leishmania parasitophorous vacuoles (LPVs), within their primary host cell, the macrophage. The LPV is a modified compartment of host endocytic origin, but also exhibits components of the host's secretory pathway, and is a critical component of parasitic infection, as it represents the interface through which Leishmania parasites interact with, and subvert normal host processes. Previous studies have shown that disruption of these interactions with the host secretory pathway results in decreased LPV size and a reduction of parasite replication within host cells. Retro-2 and several of its derivative compounds are drugs that have been shown to cause such disruptions in experimental Leishmania infections by targeting host syntaxin-5, which is a soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) protein involved in vesicular fusion between the ER and Golgi. Two other small molecules of interest are Retro-1 and ABMA. Retro-1 is a drug that also blocks retrograde movement; whereas, ABMA interferes with late endosomal compartments. Collectively, a drug library has been assembled consisting of SAR-derivative compounds of Retro-2, Retro-1, and ABMA to test efficacy against Leishmania infections. Approaches to assessing these compounds' effectiveness include counting infections to determine percentage of infected cells and parasites per infected macrophage, as well as utilizing a strain of Leishmania that expresses luciferase for bioluminescence assays to assess degree of infection. For infection evaluation, RAW 264.7 murine macrophages were infected with wild-type L. amazonensis for 24

hours, then treated with drug derivatives of Retro-2, Retro-1, or ABMA for an additional 24 hours. Cells were then fixed with 2% PFA and immunofluorescence assays were performed utilizing DAPI as a nuclear stain and LAMP-1 as a counterstain in order to visualize the LPV. Percent infection and parasites per infected macrophage were compared to infection controls without any drug treatment. For bioluminescence assays, RAW 264.7 murine macrophages were infected with luciferase-expressing L. amazonensis for 24 hours, then treated with drug derivatives for another 24 hours. Total cell lysates were collected and incubated with luciferin. Luminescence was recorded and compared to infection controls with no drug treatment. Preliminary data suggest that Retro-1 and ABMA and their derivatives may decrease viability of Leishmania infections and warrant further investigation. Compounds of highest interest will be identified and selected for further studies to investigate intracellular drug targets and mechanism of action.

25. BRN1 AS A NOVEL BARCODE FOR CULTURE-INDEPENDENT IDENTIFICATION OF BIPOLARIS SPECIES

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The genus Bipolaris is found worldwide and consists of many plant pathogenic species. Members of this genus cause leaf spots, leaf blights, melting out, root rots, and other diseases. Bipolaris spp. are of particular concern on gramineous crop hosts where they can result in significant economic impacts. Microstegium vimineum is a weedy annual grass that has invaded many temperate forests in the Eastern USA. In recent years, we have studied the emergence of novel Bipolaris spp. within some of these M. vimineum populations as a model system. Bipolaris spp. typically are identified by

morphological features together with phylogenetically informative, multilocus sequences. However, isolation of Bipolaris spp., especially from lesions that also have been colonized by saprophytes, poses a research bottleneck. Further, current multilocus sequencing techniques are not amenable to high-throughput amplicon sequencing directly from plant tissue. We investigated Brn1, a reductase gene involved in melanin biosynthesis, as a potential single-locus barcoding region for the high throughput identification of Bipolaris spp. in diseased plant tissue. Our results suggest high interspecific variation and low intraspecific variation, which supports the potential use of Brn1 as a novel single-locus barcoding region for Bipolaris spp.

26. COMPOSITION OF THE GUT MICROBIOTA TRANSCENDS GENETIC DETERMINANTS OF MALARIA INFECTION SEVERITY AND INFLUENCES PREGNANCY OUTCOME

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Background: Malaria infection in pregnancy is a major cause of maternal and fetal morbidity and mortality worldwide. Mouse models for gestational malaria allow for the exploration of the mechanisms linking maternal malaria infection and poor pregnancy outcomes in a tractable model system. The composition of the gut microbiota has been shown to influence susceptibility to malaria infection in inbred virgin mice. In this study, we explore the ability of the gut microbiota to modulate malaria infection severity in pregnant outbred Swiss Webster mice.

Methods: In Swiss Webster mice, the composition of the gut microbiota was altered by disrupting the native gut microbes through broad-spectrum antibiotic treatment, followed by the administration of a fecal microbiota transplant derived from mice possessing susceptibility- or resistance-associated gut microbes. Female mice were infected with P. chabaudi chabaudi AS in early gestation, and the progression of infection and pregnancy were tracked throughout gestation. To assess the impact of maternal infection on fetal outcomes, dams were sacrificed at term to assess fetal size and viability. Alternatively, pups were delivered by caesarean section and fostered to assess neonatal survival and pre-weaning growth in the absence of maternal morbidity.

Findings: Swiss Webster mice receiving gut microbes associated with resistance to malaria developed low parasite burdens compared to mice receiving gut microbes associated with susceptibility to malaria infection. Parasite burden was negatively correlated with the abundance of five specific OTUs, including Akkermansia muciniphila and OTUs classified as Allobaculum, Lactobacillus, and S24-7 species. The reduced parasite burden observed in resistant mice was associated with reduced maternal morbidity and improved pregnancy outcomes. Pups produced by susceptible dams with high parasite burdens displayed a significant reduction in survival in the first days of life relative to those from resistant dams when placed with foster dams. Thus, high maternal parasite burden, dictated by the gut microbial community, negatively impacts term fetal health and survival in the early postnatal period. Among surviving pups, however, growth prior to weaning was not impacted by maternal infection severity.

Interpretation: The composition of the gut microbiota in Plasmodium chabaudi chabaudi AS-infected pregnant Swiss Webster mice transcends the outbred genetics of the Swiss Webster mouse stock as a determinant of malaria infection severity, subsequently influencing pregnancy outcomes in malaria-exposed progeny.

27. CRYPTOSPORIDIUM: A SMALL PATHOGEN BUT A LARGE PROBLEM

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Cryptosporidiosis is a zoonotic intestinal diarrheal disease caused by the parasite Cryptosporidium spp. Its role is underestimated, especially in developed countries, and knowledge is lacking due to the limited availability and cost of diagnostic methods. The consequences of this parasite in developing countries are significant and related to poverty and poor sanitation. Cryptosporidium was identified in 1912 as a zoonotic agent; however, the awareness of this protozoan in public health was not recognized until a major outbreak in the 1990s. Despite this, little has changed regarding the control, the prevention, and the diagnosis. Today, it is the 3rd leading cause of death in immunosuppressed individuals, it is increasingly becoming resistant to chlorination and ultra-high temperatures, it lacks a specific treatment for infection, it is small enough to penetrate all areas of the environment, and it has a prevalence of up to 88% in developing countries. Cryptosporidium's pervasiveness is apparent, but the potential threat is unnoticed. In developing countries, the lack of production policies and reliability, unsustainable livestock systems, and inadequate sanitation creates a fertile environment for an emerging pathogen. The approach, known as "One Health", is a philosophy that the health of humans, animals, and plants in one environment is interconnected, and this approach is relevant to a wide range of global development goals. By using this approach and examining the complex interactions related to Cryptosporidium, new areas of knowledge could emerge and lead to advances in control and prevention. For example, by examining the relationship of food animal production management, we could evaluate the effect that food animal production has on its

persistency in the environment and in the human and animal populations. The same relationship could be examined between wildlife and domestic life sharing the same environment. Achieving better surveillance systems for control and prevention is not possible until we understand the complexity of the interactions between environmental, social, economic, cultural, political, and geographical factors. Molecular and phylogenetic studies alone do not give the full picture of how Cryptosporidium behaves in the environment. A completely integrated One Health approach is required to encourage collaborations between government, academic institutions, health professionals, and others in order to improve research, establish priorities, and innovate new ideas and solutions for managing this parasite. If Cryptosporidium follows Zika's path, a crisis is on the horizon and preparation is crucial.

28. DEVELOPMENT OF A SALIVA-BASED RAPID DIAGNOSTIC TEST FOR THE DETECTION OF SUBCLINICAL MALARIA PARASITE INFECTIONS

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Malaria is a vector-borne disease caused by parasites of the Plasmodium genus and have evolved complex lifecycles alternating between a vertebrate host and an anopheline mosquito vector. Efforts to eradicate malaria over the past decade has resulted in a substantial decrease in disease-related death, but parasite transmission attributed to low-density subclinical infections remains

a major issue. Microscopy remains the gold standard for the detection of malaria parasites, but is low throughput, and requires trained personnel/specialized equipment. In contrast, rapid diagnostic tests (RDTs) that detect parasite biomarkers found in the blood stream have become widespread in both surveillance campaigns and initial screening at hospitals/clinics due to their low cost and ease of use, and are high throughput when compared to microscopy. The most commonly used RDT in sub-Saharan Africa detects the P. falciparum histidine-rich protein 2 (HRP2), expressed by asexual stage parasites that are responsible for the clinical symptoms characterized by the disease. However, only sexual stage gametocyte are transmissible to the mosquito, and remain undetected by the current HRP2-RDTs. Additionally, low-density subclinical infections and the emergence of parasites lacking pfhrp2/pfhrp3 genes lead to false-negative HRP2-RDT results. These individuals comprise a large proportion of the parasite reservoir that leads to transmission and continuation of the disease. We sought to overcome these challenges by developing a lateral flow immunoassay RDT that is: i) low cost and sensitive, ii) capable of detecting transmissible sexual stage gametocytes, and iii) non-invasive, i.e. saliva based. Using LC-MS based proteomics, we initially identified protein biomarkers unique to gametocyte stages, then selected a single candidate for the development of our RDT. We performed a cross-sectional omics study of saliva/blood from African children with subclinical infections in malaria endemic regions and developed a prototype saliva-based RDT. The results of this study show that our test is capable of detecting submicroscopic parasite carriage that is only surpassed by molecular methods such as qPCR, but is far less costly. The ability to rapidly detect these subclinical carriers using a non-invasive biofluid like saliva will aid not only in clinical settings, but also in surveillance efforts worldwide as we seek to reach our goal of malaria eradication.

29. HOW WOULD THE PITCH CANKER PATHOGEN RESPOND TO A FUTURE CLIMATE? ASSESSING GROWTH, SPORULATION AND VIRULENCE OF FUSARIUM CIRCINATUM AT INCREASED TEMPERATURES

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Pitch canker disease, caused by the fungus Fusarium circinatum, has been responsible for recent outbreaks on pine plantations in the southeast United States, resulting in significant economic losses due to the effect of this disease on growth and timber volume. As climate conditions continue to change, the “Disease Triangle”, which consists of the relationships between host, pathogen, and environment, becomes less predictable and forests may experience a higher risk of disease outbreaks. In anticipation of future environmental conditions, there is a need to develop strategies to obtain trees resistant to the future variants of current pathogens. With this in mind, we tested fifteen F. circinatum isolates from Florida and Georgia, and evaluated their growth response in culture at 25, 28, and 31°C. We also evaluated the sporulation and pathogenicity on a subset of these isolates on loblolly (Pinus taeda) and slash pine (Pinus elliottii) open-pollinated families at the USDA Resistance Screening Center (RSC) in Asheville, NC. The RSC is a public institution that was created to screen seedlings of pine and other tree species for genetically-controlled tolerance to different diseases. It has screened plant material for over 20 industrial, governmental, non-profit, and academic institutions. Our tests showed that although the isolates had slower growth at 31°C, a small number showed no significant growth between 25°C and 28°C,

suggesting that these isolates might be better adapted to warmer climate conditions. Furthermore, some of these new isolates had higher virulence than those that were routinely used at the RSC for screening new pine genotypes from breeding programs. As a result of this study, the RSC is now using these new isolates in their operational screening program. Further evaluations are recommended to include material from the entire geographic range of loblolly pine, which encompasses 14 US states, as well as to test virulence under increased temperatures.

30. PATHOGEN ACCUMULATION ON AN INVASIVE SPECIES: IMPLICATIONS FOR NATIVE-INVASIVE INTERACTIONS

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Invasive species can experience release from pathogens when they are introduced to a new range, which is expected to give them a competitive advantage over native species. However, mounting evidence suggests that invasive species accumulate pathogens over time, some of which are generalists and can infect native species. Changes in host fitness due to disease can lead to multiple outcomes of competition: coexistence, exclusion, or priority effects. Nonetheless, it is unclear how pathogen accumulation affects competition in currently invaded communities. The goal of this research is to characterize the effects of fungal pathogen accumulation on competition between a native and invasive forest understory grass. Microstegium vimineum is an annual grass that has accumulated foliar fungal infections since it invaded the United States more than 100 years ago. Foliar fungal infections on M. vimineum suppress its seed production and can lead to higher biomass of native grasses. We are using data from field experiments, greenhouse experiments, and the literature to parameterize a model that predicts the outcome of competition between M. vimineum and

a native perennial grass, Elymus virginicus, with and without infection present. We constructed communities of M. vimineum and E. virginicus in field sites with infected M. vimineum invasions and applied fungicide or a control. We monitored growth, seed production, and infection of focal plants throughout the growing season to model population dynamics as a function of plant community composition and infection prevalence. In the greenhouse, we examined the potential for senesced M. vimineum tissue (i.e. litter), which accumulates in invaded areas, to affect disease transmission and competition. We found that foliar infection on E. virginicus was more likely to increase due to the presence of live M. vimineum plants than litter accumulation. However, litter suppressed germination rates, particularly for M. vimineum. This case study of pathogen accumulation on an invasive species will contribute to our ability to predict the long-term impacts of invasive species on native communities.

31. FLUOROQUINOLONES IN THE TREATMENT OF MULTIDRUG-RESISTANT TUBERCULOSIS: EXPERIENCE FROM THREE US TB TREATMENT CENTERS

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Background: Fluoroquinolones (FQs) are used to treat multidrug-resistant tuberculosis (MDR-TB). Initially, ciprofloxacin (CIP) and ofloxacin (OFL) were used, then levofloxacin (LVX) and moxifloxacin (MOX). We present accumulated USA experience with FQs in the treatment of TB.

Materials & Methods: A multi-center, retrospective study included three TB centers in the USA: A.G. Holley Hospital (AGH), Texas Center for Infectious Diseases (TCID), and University of Texas Health Science

at Tyler. We included patients admitted between 1984 and 2015, infected with MDR-TB, who received a FQ for at least 28 days. Patients demographics, sputum cultures, susceptibility data, duration of treatment, treatment outcomes, and FQ serum concentrations were collected. Treatment outcome was defined as cured if there was at least 1 negative culture after 6 months of therapy, with no subsequent positive cultures. Failure was defined as positive culture after 6 months of treatment. A time-to-event (TTE) analysis was conducted to compare the time to culture conversion among FQs. The time was defined as the number of weeks from the start of treatment to culture conversion. Population pharmacokinetic (PK) models, established based on both sparse PK data in the current study and rich PK data from other studies, were used to generate the Empirical Bayes Estimates (EBEs) for maximum concentration (Cmax) and area under the concentration-time curve from 0-24 hours (AUC0-24). For the pharmacokinetic/ pharmacodynamic (PK/PD) analysis, we used the epidemiological cut-off values (ECOFF) in liquid medium of 1.0 mg/L for LVX and 0.25 mg/L for MOX as the minimum inhibitory concentration (MIC). Unbound drug fraction was estimated at 70% LVX and 60% MOX. Statistical tests were performed using JMP Pro v13.2 (SAS Institute). PK modelling was done using Monolix v2018R1 (Lixoft).

Results: 106 MDR-TB patients received FQs. The median (range) age was 39.5 years (15.0-92.0) and weight was 59.2 kg (38.2-105.0). Fifty-one patients (48.1%) received CIP or OFL, while 55 received LVX or MOX. In the TTE analysis, LVX/MOX showed faster time to culture conversion in MDR-TB patients compared to CIP/OFL (median 16 vs 40 weeks, log-rank p=0.0577). The median (range) of LVX and MOX serum concentrations were 9.2 mg/L (1.2-19.0) and 3.8 mg/L (0.9-10.4), respectively. EBEs were generated for 25 LVX and 26 MOX patients. The median (range) for LVX Cmax and AUC0-24 were 9.9 mg/L (6.4-16.1) and 118.8 mg.hr/L (76.7-287.6), while for MOX were 4.0 mg/L (2.9-8.3) and 46.1 mg.hr/L (28.7-90.9). The numbers of patients who achieved free Cmax/MIC>10 were 1 for LVX (4%) and 12 (46%) for MOX. For free AUC0-24/MIC>100, the numbers were 8 (32%) for LVX and 19 (73%) for MOX. A total of 8 patients had

evaluable treatment outcome, hence statistical tests were not performed due to the small sample size.

Conclusions: In MDR-TB patients, LVX and MOX showed faster time to culture conversion compared to CIP and OFL. Higher percentage of patients achieved the PK/PD target in MOX compared to LVX, which may indicate that higher doses of LVX are needed.

32. PHARMACOKINETIC-PHARMACODYNAMIC TARGET ATTAINMENT ANALYSIS OF CYCLOSERINE IN TB PATIENTS

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Background: There are limited pharmacokinetic/pharmacodynamic (PK/PD) data for cycloserine (CS) in tuberculosis (TB) patients. We estimated population PK parameters and performed Monte Carlo simulation and target attainment analyses to optimize dosing.

Methods: Our previous model was expanded, including data from healthy subjects and TB patients. The latter came from 6 sites: Georgia, Bangladesh, and four US sites. Monolix (2018R1) was used to build the population PK model. The final PK estimates were used in mlxR package (v3.3.0) in R software to simulate 1000 TB patients (steady state) for each regimen. We used PKPD targets of time above MIC ≥30% and ≥64%, representing bactericidal activity and EC80 (from Deshpande et al. and Dr. Tawanda Gumbo). We assumed 100% unbound drug. A range of MICs was studied (4 to 64 mg/L). Probability of target attainment (PTA) was calculated as the fraction of simulated patients who achieved the PKPD target at each MIC for

each regimen. We selected a PTA of at least 90% for the highest MIC as the PKPD breakpoint.

Results: We included 1069 CS plasma concentrations, from 247 subjects (83% of patients had drug-resistant TB). The average age and weight were 42 years and 61 kg. About three-quarters of the patients were males. We selected a one-compartment model, with first-order absorption and lag phase. The PK parameters were estimated (CV): Tlag 0.326 h (0.43), ka 6.61 h-1 (2.9), V/F 24.9 L (0.17), and CL/F 2.00 (0.36) for healthy subjects and 1.03 L/h for TB patients. Weight and CrCL were found to have a significant effect on V and CL, respectively; and were included in the model. High PTA was achieved with dose increases when we compared 250 mg, 500 mg, and 750 mg given once daily (QD). Dividing the daily dose modestly increased the PTA, reflecting the long half-life of CS, 16.8 h. However, dividing the 750 mg dose to 250 and 500 mg twice daily (BID) had an MIC PKPD breakpoint of 16 mg/L, compared to 8 mg/L in the 750 mg QD regimen (PTA of 92 vs 84%, respectively). Dividing the dose reduced the Cmax significantly; for example, Cmax for 500 mg QD vs 250 mg BID were 33 vs 26 mg/L, respectively. The 250 mg regimens failed to achieve PTA >90% for MIC >16 mg/L. The 500 mg TID regimen achieved the targets for MIC of 32 mg/L. The 500 TID regimen and higher produced Cmax >55 mg/L, which may not be tolerable.

Conclusion: A PK model for CS was established and used for target attainment analysis. Although dividing the dose resulted in a slight increase in the PTA, it resulted in a significant decrease in Cmax, which might reduce the adverse CNS effects.

33. USING MOBILE TECHNOLOGY TO MONITOR TUBERCULOSIS TREATMENT AMONG WOMEN WITH TUBERCULOSIS AND TUBERCULOSIS-HIV CO-INFECTION IN THE GREATER ACCRA REGION, GHANA.

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Tuberculosis has for years been a public health menace despite several attempts to contain the situation. The number of deaths and disabilities resulting from TB has made it a global public health concern. Low case detection and multidrug resistant strains of the disease and the emergence of HIV has further compounded the situation with the synergy they present in combination with TB. The prevalence of TB is directly proportional to that of HIV in that where the prevalence of HIV is high, TB prevalence also increases. TB prevalence is mostly higher among men than women. However, the devastating effect of TB on women and their children make the subject of utmost importance among women. In the Greater Accra Region (GAR) of Ghana, women accounted for more than one third of notified TB cases (36.7%) in 2015. The use of the standard treatment, directly observed treatment (DOT) requires the patient to either frequent the health facility to be monitored by the health workers or have someone around them monitoring their treatment. The dearth of information on context specific interventions to control TB necessitated this study. Access to mobile technology is increasing in Ghana and has been used for several health interventions. However, its use is limited for TB treatment especially among women, who are most affected. Therefore this study, with support from the NIH-Fogarty International Centre, aims to apply the concept of mobile technology to monitor TB treatment adherence among women of reproductive age in the Greater Accra region of Ghana. Quasi-experimental design will be employed to determine the feasibility of this intervention. The study will enroll TB patients with or without HIV who are booked to begin TB treatment or have

been on treatment at the selected DOT units for at most one month. Also, various factors influencing treatment adherence will be explored among the study participants. Ethical approval for this study will be sought from the Ethics Review Committee of Ghana Health Service. Participants would be informed of the objectives of this study and will be required to either provide verbal or written consent to participate in this study. It is envisioned that the findings from this study will add to knowledge on interventions for TB control in the Ghanaian context and Africa at large.

34. A PYRETHROID-RESISTANT STRAIN OF AEDES AEGYPTI DISPLAYS LOWER REPELLENCY AND OLFACTORY SENSITIVITY TO INSECT REPELLENTS

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The mosquito, Aedes aegypti (Diptera: Culicidae), is a vector of dengue fever, zika, chikungunya, and yellow fever. Although the repeated use of chemical control measures has brought about serious resistance problems, insecticides and repellents are still major tools for vector control. For assessing repellent performance, behavioral analysis was measured in a glass tube repellency assay using 15, 30, and 60 min exposures. Three contact repellent standards were used, including N, N-diethyl-3-methylbenzamide (DEET), ethyl 3-[acetyl(butyl)amino] propanoate (IR3535), and 2-undecanone, as well as pyrethrum extract and transfluthrin. Studies were performed in susceptible (Orlando) and a pyrethroid-resistant Puerto Rico strain of Aedes aegypti. Additionally, we investigated the sensitivities to these compounds in electroantennographic studies on adult female Aedes aegypti antennae. Different levels of behavioral resistance were found to all the tested insect repellents

on the Puerto Rico strain of Ae. aegypti when compared with the susceptible Orlando strain. Resistance ratios at the different time points varied from 2.1-2.3 for DEET and 11.6-12.7 for IR3535 in the Puerto Rico strain of Ae. aegypti. With 2-undecanone, cocnentrations of 60-70 mg/cm2 were effective in repelling Orlando females, and while higher concentrations were required with the Puerto Rico strain, levels >100 µg/cm2 were toxic, so accurate resistance ratios could not be determined. Resistance was also observed to pyrethrum extract (7-7.9) and transfluthrin (4.6-5.5) in behavioral responses. Electrophysiological tests found decreased antennal sensitivity (electroantennogram amplitude) to these chemical repellents consistent with their behavioral effects. The reduced sensitivity to these repellents presumably represents a fitness cost arising from the kdr mutation present in Puerto Rico Aedes aegypti. This work highlights the need for understanding collateral effects from the evolution of pesticide resistances in mosquitoes, and the importance of finding alternative strategies to control resistance development. This work was funded by the Deployed War Fighter Research Program via the USDA (58-0208-0-068 and 58-0208-5-001).

35. ARE SYMPTOMATIC DENGUE CASES TRULY PREDOMINATED BY SECOND INFECTIONS?

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Dengue is a mosquito-borne virus with four distinct serotypes. Infection of each serotype is believed to confer life-long immunity to the homologous serotype. Heterotypic protection declines within months and leaves the individual with heightened risk of severe outcomes upon heterotypic infections. Since 90% of symptomatic cases exhibit secondary serological responses, numerous transmission models fitted using case data assumed observed counts to be captured manifestations of second dengue infections. Third and fourth infections were often assumed asymptomatic. Though this assumption is often made, the effects of neglecting contributions of third and fourth infections has not been assessed. Here we formally compared models that could incorporate different probabilities of having symptomatic disease upon primary, secondary, tertiary and quaternary infections using maximum likelihood approaches as well as Bayesian Monte Carlo Markov Chain estimation. We compared the fit of these models to age specific case data from 72 provinces in Thailand over 37 years (1981-2017). We also compared fitted transmission parameters between these models. Results showed that non-second infection dominant scenarios were equivalently likely to generate the observed data. To test the robustness of our model results we assessed the performance of our inference machinery using simulated data for which parameters were known. We found that transmission parameters inferred between the scenarios varied significantly. Recently, the World Health Organization has recommended that countries estimate seroprevalence and transmission intensities in order to make policy surrounding the use of a dengue vaccine. Our results suggest that estimates can depend strongly on model assumptions. Continued uncertainty about the probability of

symptomatic disease upon different types of exposure can contribute to inaccurate measurements.

36. CAUSES OF DEATH IN FLORIDA FARMED WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) DURING 2017 – 2018

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The farming of white-tailed deer (WTD) (Odocoileus virginianus) is an emerging agricultural industry in Florida. Bacterial infections and viral hemorrhagic diseases cause high mortality in fawns and yearling deer, and are a source of significant production losses among Florida deer farmers. Before management can be improved and properly implemented, the causes of death in the farmed herds must first be determined. The University of Florida Cervidae Health Research Initiative (CHeRI) provides a diagnostic service to Florida deer farmers to determine and monitor the proportion of farmed WTD that have died from bacterial infections, hemorrhagic disease-causing viruses, or other causes. From 2017 to 2018, participating Florida ranches provided recently deceased farmed WTD for necropsy or shipped tissues for analysis by the CHeRI diagnostic program. Both necropsy and owner-sampled tissues were tested for hemorrhagic disease viruses using RT-PCR, and as necessary

submitted for microbial culture, histopathology analysis, parasite identification to determine probable cause of death. Of the 128 deceased farmed WTD sampled in 2017, 39% of deaths were associated with bacterial infection, and 44% were attributed to viral hemorrhagic disease, and 17% of deaths due to other or undetermined causes. Of the 152 animals sampled in 2018, 40% of deaths were attributed bacterial infection, 49% were associated with hemorrhagic disease virus, and the remaining 11% of animals sampled died of other or unknown causes. Viral hemorrhagic diseases and bacterial infections are a significant sources of mortality in farmed WTD. These data provide farmed WTD breeders with insight on how to improve management practices, thereby improving herd health.

37. CELL FUSING AGENT VIRUS AND DENGUE VIRUS: THEIR PREVALENCE AND THEIR MUTUAL INTERACTIONS WITH REGARD TO REPLICATION

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Insect-specific viruses (ISVs) are agents that infect insects but have no direct effects on humans. Recent research has indicated that ISVs—like Cell Fusing Agent Virus (CFAV)—may impact the

reproductive success of viruses of human health significance like dengue virus (DENV), and vice-versa, when present in the same organism (e.g. a mosquito). The presence of two viruses in one organism is common, but the nature of their interactions and the potential outcome with regard to public health is often unknown. The mosquito-specific virus CFAV primarily infects mosquitoes of the species Aedes aegypti. The virus was originally discovered in Aedes aegypti cell lines and later found in mosquitoes from the wild. The distribution of this virus in the wild, as well as in cell lines, is of particular interest as its prevalence could affect local mosquito population susceptibilities to viruses like Dengue. Additionally, the presence of CFAV natively (i.e. present without contamination or intentional introduction) in mosquito-derived cell lines may also affect studies using these cells. Furthermore, the location of DENV in the environment is of critical importance, as it could one day establish itself in Florida. In this work, we attempt to study the relationship between DENV serotypes 1-4 and CFAV in terms of their propagation when in each other's presence using RT-qPCR and Sanger sequencing. We also search for the presence of CFAV and DENV serotype 4 (DENV4) in populations of wild mosquitoes from Florida and laboratory cell lines using RT-PCR and Sanger sequencing. So far, we have determined the presence of native CFAV infection in the Aedes aegypti¬-derived cell line Aag2 from our lab. Furthermore, RNA-Seq analysis of Aedes aegypti mosquitoes from Manatee County, Florida revealed the presence of a DENV serotype 4 genome sequence that is similar to Caribbean strains of the virus, implying the possibility of its migration to Florida from nearby countries. Our results have indicated the presence of CFAV and DENV4 in surprising and concerning locations. Further work will involve continued investigation into the prevalence of CFAV, DENV, and their interactions with each other during co-infections.

38. CHANGING PHENOLOGY IN RESIDENTIAL RHIPICEPHALUS SANGUINEUS INFESTATIONS: MODEL DEVELOPMENT

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Residential infestations of the brown dog tick, Rhipicephalus sanguineus, appear to initially be synchronized by stage but later all stages may be apparent simultaneously. Homeowners often report periods when no ticks are visible and the infestation appears to be over, only to have ticks reappear later. Presumably, these are periods when most ticks have fed and are developing and molting in refugia. To improve understanding of this phenology and the visibility of ticks through the infestation, we are developing an individual based model of residential infestations. The model will consider ticks, dogs as hosts, and human ability to see ticks either on-host, host seeking, or in refugia (visibility). Areas of a residence are characterized by environment, visibility, accessibility to hosts, accessibility to acaricide application, and connectivity. Tick movement off-host is limited to connected areas, while on-host movement can occur to any location a host can access. States include (for each stage and sex) resting, questing, feeding and development. Transitions between states and development rates are functions of location, stage/sex, environment and individual variation, including insecticide resistance. Model outcomes include the population size over time, characteristics of phenology such as how frequently stages overlap in time, and the duration of periods when ticks are present but not visible to humans. Results of initial

development in a simplified environment and definition of transition equations will be presented.

39. CHANGING PHENOLOGY IN SUSCEPTIBLE RHIPICEPHALUS SANGUINEUS LATREILLE

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The brown dog tick, Rhipicephalus sanguineus Laterille, has the ability to complete its entire life cycle both indoors and outdoors, which can lead to a severe residential infestation. A residential infestation may start with few engorged females, but reach a high infestation level due to their high reproductive rate. Variation in environmental conditions influences development and survival, affecting the phenology of tick life stages. Phenology and development are critical in population assessment and effective control. To improve understanding of brown dog tick development and how environmental factors and acaricide resistance affect development, we proposed this project to evaluate tick population development under multiple environmental conditions and resistance expression to permethrin and fipronil. Three brown dog tick colonies, including one acaricide-susceptible colony and two Florida derived, field-collected colonies, will be used. A preliminary evaluation was conducted using two temperature (20 and 35°C) and two humidity (33 and 92% relative humidity) conditions to estimate developmental and survival rates in the susceptible strain ticks. Data indicate that immature development time was affected by treatment (ANOVA, $P<0.001$), with larvae and nymphs at low temperature taking longer to develop than those at high temperatures. Molting success of larvae, but not nymphs, was affected by treatments (ANOVA, $P=0.001$), with more larvae at the high humidity level

molting than those in the low humidity treatment. The highest survival time of both nymphs and adults was at low temperature and high humidity (12.35 and >27.57 weeks on average, respectively). The lowest survival time of nymphs and adults was at low temperature and high humidity levels (<1.00 and 10.97 weeks on average, respectively).

40. CHARACTERIZE THE ROLE OF AUTOPHAGY IN DENGUE VIRUS INFECTION IN AEDES AEGYPTI MOSQUITOES

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Dengue virus (DENV) has been a major mosquito-borne disease worldwide and impacts 50 to 100 million people every year and is spread by the main mosquito vector of the genus Aedes, especially Aedes aegypti. Since the current dengue vaccine is still not fully protective, it is only recommended for people who had DENV before, thus interfering with the virus transmission cycle in the mosquito becomes the major control method. The most commonly used control method involves spreading insecticide, but this strategy has resulted in mosquito's adjusting to the lethal situation by developing insecticide resistance. Having new control strategies becomes an important issue to eliminate dengue virus. In humans, autophagy was found to enhance dengue virus replication, but to date there have been limited numbers of publications investigating these pathways in the mosquito. We started investigating several Aedes aegypti populations to identify genes expressed in the autophagy pathway in connection to the virus titer. After DENV infection, several populations showed autophagy- related gene expression change. The vector competence also differed by population. Here we show the autophagy pathway in Aedes aegypti might have a role in mosquito –DENV interactions. We believe these result will give us a more comprehensive view of mosquito immunity and provide a

greater understanding of the involvement of autophagy in dengue virus infections in mosquitoes.

41. CHARACTERIZING HUMAN TARGET CELL INFECTION BY THREE GEOGRAPHICALLY DISTINCT ISOLATES OF MAYARO VIRUS

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Background: Mayaro virus (genus Alphavirus, family Togaviridae) is an emerging arthropod-borne virus transmitted by Haemagogus mosquitoes in sylvatic regions of Central and South America. Like Chikungunya virus, Mayaro virus (MAYV) infection leads to fever, maculopapular rash, and arthralgia. Limited knowledge exists pertaining to regional differences in MAYV in vitro infectivity in human cells. We aim to describe viral kinetics, cytopathic effects, and human target cell susceptibility to three geographically distinct MAYV isolates represented genotypes D and L (Uruma, Peru and Brazil).

Methods: MAYV susceptibility of key human target cells (human dermal fibroblasts, human embryo kidney cells (HEK293), monocytes and skeletal muscle satellite cells) as well as Vero E6 cells was visualized using immunofluorescence confocal microscopy at 0, 24, 48 and 72h post infection (p.i.). Viral kinetics were determined for each cell line from 0h to 72h p.i. at MOI=1, followed by viral plaque assays in Vero E6 cells to determine viral titers. Cytopathic effect was observed and compared across viral isolates and cell lines by staining with crystal violet.

Results: Immunofluorescence and flow cytometry revealed that human dermal fibroblasts, skeletal muscle satellite cells, and Vero E6 cells were all susceptible to each MAYV isolate, though to differing degrees (MAYV-Uruma > MAYV-Peru > MAYV-Brazil). HEK293 became infected at lower rates, and monocytes were nearly refractory to infection. Viral replication kinetics assays revealed that peak viral titers occurred for all three viral isolates around 24h p.i, reaching 1x108 pfu/ml. MAYV-Uruma reached this peak the fastest, followed by MAYV-Peru and then MAYV-Brazil. Crystal violet staining also demonstrated lower viral pathogenesis with greater cell survival and decreased cell apoptosis for MAYV-Uruma, Peru, and Brazil, respectively.

Conclusions: These results indicate that MAYV can infect human dermal fibroblasts, which are abundant at the initial site of exposure. Further, skeletal muscle satellite cells are quite susceptible to MAYV, in keeping with clinical symptoms associated with this virus. Some differences in infectivity are apparent across different MAYV isolates and may contribute to variable virulence and pathogenicity. These findings advance our understanding of MAYV infection of human target cells and provide some initial data with regards to MAYV phenotypic variation according to geography.

42. CORRECTING DETECTION BIAS FOR INFECTIOUS DISEASE SURVEILLANCE

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Introduction: Surveillance data, while useful, are usually subject to detection bias, namely, disproportional detection rates among particular subpopulations. For example, females in fertility age are much more likely to be detected because of the association between Zika infection and microcephaly. Such detection bias leads to bias in the quantification of important epidemiological parameters.

Methods: We propose a Poisson transmission model to correct such detection bias and jointly estimating infection probability, age- and sex- relative susceptibility, environment factors and reporting probability. Markov chain Monte Carlo algorithm is constructed to estimate model parameters and the latent case numbers jointly.

Results: The proposed method is applied to the Zika surveillance data in Colombia from 2015-2016. The study period for this analysis was July 27, 2015 to November 21, 2016. In total, 102482 cases were included in the final analysis. Models accounted for transmission within or between department in Colombia. Assuming the reporting probability was 0.7 for children (aged 0-19), the reporting probability for adults (aged >19) was 0.61 (95% CI: 0.59, 0.62). When the public was aware this such association, this probability for females in fertility age group (aged 20-39) was 1.00 (95% CI: 0.99, 1.00). A male was 35% (95% CI: 34%, 36%) less susceptible than a female. Children (age<20) and older adults (age>40) were 48% (47%, 49%) and 28% (27%, 30%) more susceptible than young adults (aged 20-40),

respectively. Higher temperature, higher precipitation and less population density was associated with higher susceptibility.

Conclusion: Important epidemiological parameters such as infection probability and relative susceptibility could be biased if the detection bias in the surveillance data is not adequately accounted for. Our method offers reliable estimates of these parameters which could be useful in forecasting epidemics or designing control strategies.

43. CULEX QUINQUEFASCIATUS SAY (DIPTERA: CULICIDAE) FROM FLORIDA TRANSMITTED ZIKA VIRUS

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Zika virus is a single stranded RNA mosquito-borne arbovirus that can cycle between mosquitoes and humans, mainly by the bite of the Aedes aegypti mosquito. However controversy exists about the competence of Culex pipiens quinquefasciatus Say (Diptera: Culicidae) for Zika virus and therefore a Gainesville, Florida population of Cx. quinquefasciatus was investigated for its ability to be infected with and transmit ZIKV. RNA from whole adult Cx. quinquefasciatus mosquito bodies collected 16 days after imbibing blood containing ZIKV were screened by qRT-PCR and revealed 9 female mosquito bodies with ZIKV RNA (Infection Rate = 28%). The mean titer of ZIKV in the 9 bodies was 5.85 ± 5.8 log10 ZIKV pfue/ml (average ± SEM). Saliva on honey coated filter paper cards provided

as a sugar source to the same mosquitoes revealed that the eluted RNA had an average titer of 5.6 ± 4.5 log 10 ZIKV pfue/ml per card. A repeat of this experiment validated that individual Cx. quinquefasciatus mosquitoes from this population can be infected with ZIKV and qRT-PCR detected ZIKV RNA in the mosquitoes from two cages (Infection Rate = 55%). Plaque assays of the saliva samples eluted from the filter paper cards attached to the two cages revealed the presence of live virus with an average titer of 5.02 log 10 ZIKV pfu/ml. We report a laboratory colony of Culex quinquefasciatus mosquitoes were experimentally able to salivate Zika virus (ZIKV, Flaviviridae; Flavivirus) at 16 days post infection (dpi). ZIKV RNA was detected in bodies and in saliva deposited on filter paper cards with subsequent studies demonstrating the presence of live ZIKV in saliva.

44. CUTANEOUS FEATURES OF ZIKA VIRUS INFECTION: A CLINICOPATHOLOGICAL OVERVIEW

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Zika virus (ZIKV) is an emerging mosquito-borne flavivirus transmitted mainly by Aedes species of mosquitos. Although the infection is usually mild and self-limiting, it is a public health challenge in tropical and subtropical countries owing to its unprecedented pathogenicity and increased risk for fetal malformations and neurological symptoms. Cutaneous manifestations as for other mosquito-borne viruses remain a

hallmark of the disease. The cardinal cutaneous manifestation of ZIKV is the maculopapular rash and prurtitis. However, in our experience there is marked diversity in the characteristics of the rash and in the severity of the illness, ranging from a conspicuous, diffuse, mildly pruritic, maculopapular rash to cases with nearly universal erythrodermia. In contrast to Chikungunya virus (CHIKV) and Dengue virus (DENV) infections, in which the rash occurs generally after the fourth day of onset of symptoms, the cutaneous manifestations of ZIKV commonly occur within the first 24–48 h after the onset of symptoms. We provide a detailed overview of ZIKV infection, including its varied cutaneous clinical manifestations and diagnostic aspects, as well as detailed insights into its pathogenesis in human skin.

45. DENGUE SINCE ZIKA: CHARACTERIZING POTENTIAL IMPACTS OF ZIKA EMERGENCE ON ENDEMIC DENGUE TRANSMISSION

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In 2015 and 2016, Zika virus (ZIKV) swept through many Latin American countries where dengue virus (DENV) is endemic. Dengue and Zika viruses are of the same family, share a vector and may interact competitively or synergistically through human immune

responses. We examine dengue incidence data from Brazil and Colombia from before, during, and after the Zika epidemic. We find evidence that dengue incidence was atypically low in 2017 in both Brazil and Colombia. We investigate whether Zika incidence at the state or department level is associated with changes in dengue incidence and find mixed results. We use simulations to investigate potential outcomes of incorporating cross-protection or enhancement between dengue and Zika. Our simulations show that regardless of the mechanism, low periods of dengue incidence are followed by a resurgence in dengue cases. It is therefore possible that countries currently experiencing low levels of dengue incidence may experience large dengue seasons in the near future.

46. DETECTION AND ISOLATION OF HEARTLAND VIRUS FROM TICKS COLLECTED OFF A PET CAT IN GAINESVILLE, FLORDA

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Heartland virus (HRTV), order Bunyavirales, family Phenuiviridae, genus Phlebovirus, is a human pathogen that was discovered in the United States in 2009. The virus was subsequently isolated from Amblyomma americanum ticks collected on properties owned by HRTV patients, suggesting the virus was vectored by the ticks that are known to feed on humans. Serology tests performed by others suggest that various vertebrates such as white-tailed deer (WTD) (Odocoileus virginianus) and raccoons (Procyon lotor) in the Eastern and Southern USA, including Florida, get exposed to the virus, but until the work presented here, HRTV had not yet been directly detected in or isolated from Florida ticks. From Oct. 2016 to Feb.

2018, a total of 12 Amblyomma americanum comprised of 6 deplete nymphs, and four female and two male adults, and two adult female Ixodes affinis ticks, were collected off a pet cat in Gainesville, Florida. The cat spent part of the day outdoors and lived in a house bordered by a woodland preserve with WTD, raccoons, and a variety of other vertebrates that may be reservoirs of the virus. RNA purified from the ticks was analyzed by reverse transcription-polymerase chain reaction (RT-PCR) for the genomic RNAs (vRNAs) of Bourbon -, Heartland-, Lone Star-, and Powassan viruses, which are the tick-borne viruses predicted to occur in the Southeastern USA. Two deplete nymph A. americanum ticks were RT-PCR positive for HRTV vRNA, and infectious HRTV virus was isolated from both. The results of this work raise the question whether HRTV is a cause of undiagnosed illness in Florida.

47. DETECTION OF ZIKA VIRUS IN FIELD-CAUGHT AEDES AEGYPTI IN HAITI, JANUARY TO MAY 2017

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Zika virus (ZIKV) remains a pathogen of public health concern even though media coverage of the virus has progressively diminished since the end of year 2016. Our research group collects mosquitoes at specific study sites in Haiti for our ongoing arbovirus/vector studies. The mosquitoes are identified using taxonomic keys, sexed, then pooled (maximum of 25 individuals/pool) according to collection site, and homogenized. Total RNA extracted from pooled mosquitoes is tested by RT-PCR for the presence of ZIKV, and dengue and chikungunya virus genomic RNAs (vRNAs). Here, we present the results of ZIKV RT-PCR analyses of RNA from mosquitoes collected weekly between January and May 2017 at three separate sites in Léogâne and Gressier, Haiti. Three different traps (BG sentinel trap, CDC gravid trap, and CDC light trap) were used at each site. Out of a total of 296 Aedes aegypti mosquito pools collected from Léogâne and Gressier, Haiti, 28 (9.5%) were positive for ZIKV via real-time RT-PCR. Of the 206 all-female A. aegypti pools, 22 (10.7%) were ZIKV positive, and of the 90 all-male pools, 6 (6.6%) were ZIKV positive. We found a similar proportion of ZIKV-positive mosquitoes from the two sampling locales, Léogâne and Gressier, which are coastal towns within 10km proximity. The largest proportion of ZIKV-positive mosquitoes (15.4% and 16.7%) were from January and February. These data show that ZIKV was present in the A. aegypti population in Haiti despite reduced numbers of Zika fever cases in Haitians in 2017 compared to those during the outbreak of 2016. Moreover, the detection of ZIKV in all-male mosquito pools suggests transovarial or venereal transmission was occurring, maintaining the virus in an endemic fashion in Ae. aegypti mosquitoes.

48. DEVELOPING STERILE INSECT TECHNIQUE FOR FLORIDA MOSQUITOES:ANOXIA PRE-TREATMENTS IMPROVE PERFORMANCE OF IRRADIATED MALE AEDES AEGYPTI MOSQUITOES

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The use of sterile insect technique (SIT) is emerging as a promising control method in mosquito management programs. Ionizing radiation can be used very efficiently to sterilize insects by fragmenting their genomic DNA as part of SIT programs. However, radiation exposure can also induce off-target effects from oxidative stress to critical somatic cellular components. Subjecting insects to anoxic conditions prior to and during irradiation has been found to reduce oxidative stress and improve performance of sterilized males of some species. The objective of this study was to determine the extent to which anoxia pre-treatments can induce a hormetic response in male Aedes aegypti mosquitoes prior to irradiation. Male Ae. aegypti pupae were exposed to 1 h of anoxia by being placed in sealed containers and flushed with nitrogen to determine effects of anoxia on longevity and performance when exposed to irradiation at doses of 0, 20, 50, or 100 Gy. Pupal mortality and successful adult emergence were assessed 3 days after irradiation. Survival was assessed daily to compare longevity among treatments. A subset of males was monitored for daily activity using activity monitors. Mating trials with fertile females were performed to assess sterile male mating competitiveness. Anoxia pre-treated males had shorter longevity and performed worse than control males at low irradiation doses but performed as well as or better than control males after exposure to 100 Gy, without impacting sterility. Anoxia may improve performance when high doses of irradiation are necessary to achieve sterility.

49. DIEL TRENDS IN MOVEMENT AND HABITAT SELECTION OF WILD AND RANCHED WHITE-TAILED DEER IN FLORIDA: IMPLICATIONS FOR VECTOR EXPOSURE

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Epizootic hemorrhagic disease virus (EHDV) and blue-tongue virus (BTV) are viral threats to white-tailed deer (Odocoileus virginianus) and heavily impact the Florida deer farming industry. These vector-borne orbiviruses are transmitted by the Culicoides family of biting midges and may result in hemorrhagic disease (HD) among white-tailed deer. Complications due to HD and secondary bacterial infections may ultimately result in death. In subtropical Florida, high ambient temperatures experienced by white-tailed deer during summer may act as an environmental stressor, especially among ranched deer of larger body mass. Environmental stressors (e.g. thermal stress) can reduce the overall fitness of herds, making deer more susceptible to HD if they are unable to physiologically or behaviorally adapt. By adjusting diel and seasonal foraging strategies, Florida white-tailed deer may be selecting habitat that reduces operative temperatures and indirectly increases vector-host interactions. Our previous studies indicated higher HD seroprevalence in ranched white-tailed deer relative to neighboring wild deer. While both groups had similar home range sizes, and inter-annual fidelity, differences in diel activity levels and habitat selection between wild and ranched deer during the HD risk period

may indicate high risk behaviors. For 3 years, GPS collars recorded the location of deer at each hour from May 1st to October 31st. Trends in diel movement and hourly habitat selection were investigated using Manly's selectivity measure. We observed primarily crepuscular activity with ranched deer being more active at dawn and wild deer more active at dusk. Ranched deer selected mesic bottomland habitat with increased frequency and appeared to avoid upland pine during the hottest hours of day. Wild deer showed similar but less pronounced results. We postulate that the high selection of mesic-bottomland habitats by ranched deer during daytime hours may increase host contact with vectoring midges and contribute to their higher HD seroprevalence. We recommend that managers of ranched deer populations in the southeast U.S that have selected for larger body mass and other phenotypes incompatible with high ambient temperatures consider managing their landscapes in a way that provides thermal refuge while simultaneously considering vector exposure. Likewise, managers of wild deer populations should consider the extent of adequate thermal cover provided within home ranges to improve population health.

50. DIFFERENCES IN LANDING BEHAVIOR BETWEEN TWO STRAINS OF AEDES AEGYPTI EXPOSED TO PYRETHROID INSECTICIDE-TREATED FABRIC

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Emerging insecticide resistance is a major issue for vector control, especially in disease endemic areas. Resistance is detrimental to a mosquito control program because it is associated with a higher cost in order to achieve a comparable level of chemical control, has the potential to result in disease resurgence. Pyrethroid resistance has previously been documented in Puerto Rican populations of Aedes aegypti mosquitoes. In this study, behavioral differences in the landing activity for pyrethroid resistant and pyrethroid susceptible strains of Aedes aegypti when exposed to pyrethroid-treated cloth are explored. In order to observe the landing behavior of both pyrethroid-resistant and susceptible laboratory colonies of mosquitoes, these populations were exposed to four different pyrethroid-treated uniform fabrics and an untreated control fabric. The two doses for each pyrethroid treatment corresponded to the approximate ED25 and ED75, based on previous results (Agramonte et al. 2017). Excito-repellent irritancy behaviors were evaluated with individual non-blood-fed, 5-10d females, which were introduced into a glass arena placed over a treated uniform fabric. Repellency was evaluated using several variables, including the time to first take-off (TFF), number of landings (NOL), cumulative flight time (TFT), and maximum time in contact with the surface (MSC) (WHO 2006, Licciardi et al. 2006, Hougard et al. 2003). In addition to the

differences in strain and insecticide treatment, half of the mosquitoes were surgically altered, with antennae bilaterally ablated with fine forceps. Treatments were randomly assigned according to a complete block design and replicated 10 times for each treatment combination. A computer with a close-range digital video camera was used to record each 3 min exposure period. The digital recordings of each treatment exposure were analyzed and corrected using Ethovision XT 11.5 (Noldus). Results from the landing assays showed significant differences in antennal status and strain all response variables in both a three way analysis of variance with contrasts and Dunnett's test in SAS 9.4. Additionally, significant differences were found between treatments for the MSC and TFF response variables.

51. DIFFERENTIAL SUSCEPTIBILITIES AND IMMUNE RESPONSES OF AEDES AEGYPTI TO TWO DENGUE 4 VIRUS STRAINS

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Dengue virus (DENV) causes the largest number of arbovirus infections among humans globally. There are four serotypes: DENV1, -2, -3 and -4. A large proportion of studies that evaluated the susceptibility of Aedes aegypti mosquitoes, the primary vector, to DENV infection of their midgut, carcass and/or salivary glands used DENV2. However, DENV1 to 4 differ genetically, and there are genetic differences within DENV of the same serotype. Thus, studies based on one serotype (DENV2) or one DENV2 strain are biased. We compared mosquito susceptibilities and immune responses within

the midgut and carcass of A. aegypti infected with different strains of the same DENV serotype (DENV4 strains H241 and Haiti). Orlando, Florida strain A. aegypti were chosen for this study and infected with DENV4 lab strain H241 or with field isolate of DENV4 (DENV4/Haiti/0075/2015) that we isolated from a child in Haiti in 2015. When A. aegypti ingest natural viremic blood or DENV-spiked blood, the blood passes into the midgut, wherein midgut epithelial cells are a target for infection. If DENV passes the midgut barrier, it disseminates into the hemolymph and the remainder of the body, commonly around day 7. In our work, mosquitoes were fed either naïve blood (no virus) or virus-spiked blood, then collected on day 7 and day 10 post-blood meal for plaque assay to quantify virus titer. The median virus titers were significantly higher (between 2-4 logs) within DENV4 Haiti-infected mosquitoes than in DENV4 H241 for midgut and carcass samples collected at day 7 and day 10. These results suggest that the DENV4 strains that were tested have different phenotypes. The mRNA levels of transcription factor Rel 2 and its negative regulator, Caspar, of the Immune Deficiency (IMD) innate immune pathway of A. aegypti were measured via quantitative real-time RT-PCR, relative to an internal control gene transcript (RPS7). Rel 2 and Caspar were significantly different within DENV4- Haiti-infected mosquito pools from that of the naïve blood control group but no significant changes were noted between DENV4 H241 and the naïve blood control group. These data further suggest that in addition to higher susceptibility of A. aegypti to DENV4 Haiti, there may be a concurrent negative effect on the immune response, but not for the lab strain. Further research is needed to pinpoint the genetic difference(s) between the two DENV4 strains responsible for the different responses in Orlando A. aegypti.

52. EVALUATING A TRANSMISSION BLOCKING VACCINE USING NOVEL PARTICLE-BASED DELIVERY TO DECREASE MALARIA TRANSMISSION

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Malaria remains a devasting disease throughout the world, and novel strategies are needed to achieve global eradication. Malaria is a vector-borne diseased caused by parasites belonging to the Plasmodium genus and involve a complex life cycle between a vertebrate host and mosquito vector. Current efforts to develop vaccines targeting malaria specific antigens have been largely unsuccessful, due in part to low immunogenicity of the antigen, resulting in short lived immune responses and the need for multiple boosters during a given transmission season. Therefore, an ideal vaccine would provide long lasting, high titer, protection after just a single dose. Transmission of the parasite from host to mosquito vector, and back to another host takes place over several developmental stages and requires the parasite to escape the mosquito midgut. Previously, our lab developed a transmission blocking vaccine (TBV) that generates antibodies against specific epitopes on a highly conserved mosquito midgut surface protein, the Anopheline alanyl aminopeptidase N (AnAPN1). Upon taking a blood meal containing malaria parasites, the mosquito will simultaneously take up AnAPN1 specific antibodies produced following vaccination, and subsequently preventing parasite infection of the mosquito to disrupt the transmission cycle. It was previously shown that >80% reduction in parasite burden could be seen when mosquitoes were fed an infectious blood meal containing antibodies produced in mice, show the potential for AnAPN1 to be used in a TBV. Additionally, this vaccine is effective against both Plasmodium falciparum and P. vivax malaria, the two species responsible for the majority of the

morbidity and mortality worldwide. Having established a potent TBV using AnAPN1 as the antigen, we sought to overcome the challenge of a long-lasting vaccine that provided protection throughout an entire transmission season from a single dose. We are using biodegradable nanoparticles for the antigen delivery, as they have been previously shown to quickly traffic to draining lymph nodes where they elicit a strong immune response. We also engineered larger microparticles containing the antigen that can be injected into an individual, where they slowly break down and release antigen, providing a natural boosting up to several months after the person was inoculated. With the implementation of these micro- and nano-particles, this vaccine will provide a safe, effective, and long-lasting way to limit malaria infections.

53. EVALUATION OF MULTIPLE TRAP TYPES FOR THE CAPTURE OF VECTORS OF EASTERN EQUINE ENCEPHALITIS IN SAINT JOHNS COUNTY, FLORIDA

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Eastern Equine Encephalitis is a highly dangerous mosquito-borne alphavirus endemic to the United States. Vector surveillance and control is the primary tool used to prevent the spread of EEE, so highly efficient and attractive traps are needed to accurately assess mosquito abundance. This study tested the following trap types to determine which captured the most EEE vectors in Saint Johns County: Biogents Sentinel Traps, Centers for Disease Control Light traps, the Sentinel Mosquito Arbovirus Capture Kit, Mosquito Magnet X trap, CDC resting trap, gravid trap with a hay infusion, and a gravid trap with a cat-tail infusion. BG traps caught the highest abundance of EEE vectors compared to all the other trap types analyzed. The EEE vectors trapped in this assay were Aedes atlanticus, Aedes vexans, Culex erraticus, and Culex nigripalpus. Aedes atlanticus was the most prominent of the vectors captured. The two main vectors of EEE, Culiseta melanura and Coquilletidia

perturbans, were never captured during the testing period at the chosen site.

54. EXPERIMENTAL APPROACH TO INVESTIGATING THE GENETICS OF ARBOVIRAL COMPETENCE IN AEDES AEGYPTI POPULATIONS

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Background: A key factor governing the emergence and spread of mosquito-borne viruses is vector competence (VC), a property of mosquito populations defined as their ability to transmit virus to a new host. VC can be estimated as the proportion of mosquitoes that produce virus in saliva (i.e., bite becomes infectious) following oral exposure and the extrinsic incubation period (EIP). During the EIP, virus particles negotiate physiological barriers that must include the midgut and salivary-gland epithelia. We have begun to investigate variation in VC for Zika virus in Ae. aegypti populations from Florida with the long-term goal of identifying genetic polymorphisms in the genome that influence this trait in nature.

Methods: Aedes aegypti eggs were collected from locations in Miami-Dade County in 2016, including Miami Beach (Aeg-MB) and Little River (Aeg-LR), and from Palmetto in Manatee County (Aeg-PT) in 2018. Eggs were hatched in the laboratory and maintained as distinct populations. For the Miami-Dade mosquitoes, adults from the F2 generation were challenged with ZIKV in blood meals along with an established control colony (Aeg-Orlando). Midgut infection

rates were determined by plaque assay 7 days post-feeding to investigate susceptibility to infection. In follow-up studies with Aeg-MB, Aeg-PT, and ZIKV strain PRVABC59, plaque assays were used 14-16 days post-feeding to investigate rates of dissemination and transmission. To examine genetic factors contributing to variation in VC, over 100 isofemale lines have been generated from Aeg-PT and will be used to measure heritability of VC and select lines with divergent phenotypes (i.e., low-VC and high-VC) for genetic analysis.

Results & Conclusions: For Miami-Dade mosquitoes, midgut-infection rates were highly variable among ZIKV strains tested. Comparisons among Aeg-MB, Aeg-LR, and the control show similarities for some ZIKV strains but variation for others, indicating that genetic background of both the mosquito and virus influence VC. Rates of infection and dissemination for the Aeg-MB/PRVABC59 combination were high in replicate experiments (> 90%), while transmission rates were less than 50%. VC experiments for the Aeg-NP/PRVABC59 combination are in progress. In conclusion, these data suggest that (i) a relatively high proportion of Ae. aegypti in Miami-Dade County are non-competent for ZIKV and (ii) that this non-competent phenotype is likely due to a physiological barrier to salivary-gland infection. In the near term, our plan is to use laboratory selection and genomic analyses to pinpoint genetic factors contributing to this physiological barrier that prevents transmission.

55. FEASIBILITY OF AN OVINE MODEL OF MALE ZIKV INFECTION

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Zika virus (ZIKV) is an arbovirus known for sexual transmission in humans, causing asymptomatic persistent male infection. Thus, models of male infection are needed to study the potential effects of ZIKV on male reproductive physiology. The purpose of this study was to develop a male ovine (sheep) model of ZIKV infection. In the first experiment, we sought to serially passage ZIKV in sheep. One intact, male sheep (ram) was inoculated with 1x106 pfu of Asian lineage ZIKV and blood was collected twice daily for one week. After one week, a second ram was transfused with the first ram's blood; this was repeated with a third ram. Rams were sacrificed after two weeks. While the first ram in the transfusion experiment did seroconvert, no viral RNA was detected in tissue samples; the second and third rams transfused did not seroconvert and no viral RNA was detected. In a second experiment, six intact rams were inoculated with a high dose of Asian lineage ZIKV (1x107 pfu) and were serially sacrificed on days two through six and day nine post-infection (PI). On each day of euthanasia, tissue samples were collected for viral culture and real time PCR (RT-PCR). Spleen, liver, testes, and accessory sex glands from rams sacrificed on days two, three, six, and nine PI were RT-PCR-positive for ZIKV, and ZIKV was cultured from the spleen and testes of three rams. Tissue from rams sacrificed on days four and five PI was neither RT-PCR-positive nor

culture-positive for ZIKV. Rams given a high dose of ZIKV seroconverted and exhibited neutralizing titers upon screening assays, indicating successful infection. These two experiments helped to establish the minimum infective dose of ZIKV that leads to viral dissemination to the tissues in rams. RT-PCR, viral culture, and serology results of these experiments indicate that rams are susceptible to ZIKV and that viral dissemination and replication occurs in highly vascular tissue—including those of the male reproductive tract.

56. FIRST ISOLATIONS OF MELAO VIRUS FROM HUMANS: THE HAITI STORY

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The order Bunyavirales contains a large group of single-stranded negative-sense RNA viruses. To date, ~300 different bunyaviruses have been identified Melao virus belongs to family Peribunyaviridae. It was first isolated in Trinidad in 1955 and has only been isolated from Aedes spp. mosquitoes. In 2014, a wave of arbovirus infections occurred in Haiti, following an outbreak of chikungunya disease caused by chikungunya virus. We are determining the causes of febrile illnesses of unknown origin among a cohort of school children in Haiti. From May of 2014 to February of 2015, a cluster of children showed signs of acute febrile illness consistent with an arbovirus infection. Preliminary RT-PCR analyses of plasma from 5 sick children were negative for chikungunya, dengue, and zika virus genomic

RNAs. Upon virus isolation attempts in Vero E6 cells, cytopathic effects consistent with bunyavirus infections were observed for each of the 5 specimens. Sequencing data from cell culture media confirmed identity of Melao virus. To our knowledge, these are the first isolates of Melao virus from humans. Our findings suggest Melao virus is endemic in Haiti.

57. HABITAT ASSOCIATIONS OF LONESTAR TICKS (AMBLYOMMA AMERICANUM) IN FLORIDA

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Distribution maps are an ideal tool to understand the geographic spread of species, but their utility in identifying meaningful environmental associations is often limited. Inferences are further complicated when species are difficult to detect or vary in their detection probability throughout the year. Animals, such as ticks, that are seasonally active are a specific example. The species-specific and life stage dependent patterns of activity are rarely accounted for and may have significant impacts on conclusions associating environment and ticks. Failure to account for this subsequently impacts predicted patterns of disease transmission. Here, we leverage data from repeated site visits within an occupancy framework to account for changes in detection due to the ticks' biology. Our results from adult A. americanum indicate that the average annual detection probability of this species is 16%. Overall, detection is highly variable throughout the season; but during peak activity detection probability rises to nearly 50%. Accounting for this bias we found that whether a site was occupied by A. americanum was strongly correlated with NDVI, annual precipitation and temperature of the driest quarter of the year.

58. HOMOLOGS OF HUMAN DENGUE-RESISTANCE GENES, FKBP1B AND ATCAY, CONFER ANTIVIRAL RESISTANCE IN AEDES AEGYPTI MOSQUITOES

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Dengue virus (DENV) is transmitted by mosquitoes and is a major public health concern. The study of innate mosquito defense mechanisms against DENV have revealed crucial roles for the Toll, Imd, JAK-STAT and RNAi pathways in mediating DENV in the mosquito. Often overlooked in such studies is the role of intrinsic cellular defense mechanisms that we hypothesize to work in concert with the classical immune pathways to affect organismal defense. Our understanding of the molecular interaction of DENV with mosquito host cells is limited and we propose to expand upon the recent results from a genome-scale, small interfering RNA (siRNA)-based study that identified mammalian host proteins associated with resistance to dengue/West Nile virus (DENV/WNV) infection. The study identified 22 human DENV/WNV resistance genes (DVR) and we hypothesized that a subset would be functionally conserved in Aedes aegypti mosquitoes, imparting cellular defense against flaviviruses in this species. We identified 12 homologs of 22 human DVR genes in the Ae. aegypti genome. To evaluate their possible role in cellular resistance/antiviral defense against DENV, we used siRNA silencing targeted against each of the 12 homologs in an Ae. aegypti cell line (Aag2) infected with DENV2 and identified that silencing of

the two candidates, AeFKBP1 and AeATCAY, homologs of human FKBP1B and ATCAY were associated with viral increase. We then used dsRNA to silence each of the two genes in adult mosquitoes to validate the observed antiviral functions in vivo. Depletion of AeFKBP1 or AeATCAY increased viral dissemination through the mosquito at 14 days post-infection. Our results demonstrated that AeFKBP1 and AeATCAY mediate resistance to DENV akin to what has been described for their homologs in humans. AeFKBP1 and AeATCAY provide a rare opportunity to elucidate a DENV-resistance mechanism that may be evolutionarily conserved between humans and Ae. aegypti.

59. IDENTIFIABILITY ANALYSIS OF ZIKA EPIDEMIOLOGICAL MODELS

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The Zika virus (ZIKV) epidemic has caused an ongoing threat to global health security and spurred new investigations of the virus. Use of epidemiological models for arbovirus diseases can be a powerful tool to assist in prevention and control of the emerging disease. We introduce six models of ZIKV, beginning with a general vector-borne model and gradually including different transmission routes of ZIKV. These epidemiological models use various combinations of disease transmission (vector and direct) and infectious classes (asymptomatic and pregnant), with addition to loss of immunity being included. The disease-induced death rate is omitted from the models. We test the structural and practical identifiability of the models to find whether unknown model parameters can uniquely be determined. The models were fit to obtain time-series data of cumulative incidences and pregnant infections from the Florida Department of Health Daily Zika Update Reports. The average relative estimation errors (AREs) were computed from the Monte Carlo simulations to further analyze the identifiability of the models. We show that direct transmission rates are not practically identifiable; however, fixed recovery rates improve identifiability overall. We confirm a reproduction number greater than one at the

start of the Florida epidemic. Basic reproduction number, $R0$, is an epidemiologically important threshold value which gives the number of secondary cases generated by one infected individual in a totally susceptible population in duration of infectiousness. Elasticity of the reproduction numbers suggests that the mosquito-to-human ratio, mosquito life span and biting rate have the greatest potential for reducing the reproduction number of Zika, and therefore, corresponding control measures need to be focused on these control strategies.

60. INSIGHT INTO ENERGETICS OF MALARIA PARASITE – MOSQUITO VECTOR INTERACTIONS

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Malaria remains a devastating vector-borne parasitic disease globally. Malaria is caused by species of the Plasmodium genus, evolving a complex life cycle alternating between Anopheles spp. mosquitoes and vertebrate hosts. In sub-Saharan Africa, which bears more than 90 % of the disease burden, the primary vector is Anopheles gambiae s.l. Although significant gains have been made over the last decade in reducing malaria cases and mortality, the latest WHO statistics show a worrying trend of stagnation and even resurgence in a number of malaria endemic countries. Development of drug resistant parasites, lack of effective vaccines, and widespread insecticide resistance among major malaria vectors underpin these setbacks and present a threat to malaria elimination and eradication efforts. Thus, there is need for new control strategies to combat these challenges. One promising avenue entails exploiting the mechanisms that underlie vector-parasite interactions that influence parasite transmission success that could in turn guide the development of transmission blocking interventions such as vaccines

and therapeutics. The co-evolutionary relationship between malaria parasites and the Anopheles vectors is not well understood, but there is evidence of parasite-induced behavioral and physiological changes to the host vector. In the mosquito vector, the parasite undergoes multiple developmental stages and replication before becoming mature human-infective sporozoite stages. To meet its own metabolic needs and ensure the survival of the host vector through the sporogonic development, we hypothesized that the parasite utilizes the conserved adipokinetic hormone (AKH) signaling in the mosquito to mobilize energy reserves. However, little is known about the AKH signaling pathway in the malaria vectors. To understand, we employed molecular and chemical analyses to elucidate AKH signaling in sugar-fed, sugar-starved and blood-fed female A. gambiae. We will present our results and discuss their implication on malaria transmission potential.

61. INVESTIGATING THE ACTIVITY OF HALOGENATED AROMATIC AMIDES AGAINST ANOPHELINE SPECIES

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Mosquito-borne pathogens affect millions of people worldwide. It is important to identify safe repellents that can provide long and reliable protection from arthropod biting and prevent arthropod-borne diseases. In the present study, the vapor repellent activity and the toxicity of three halogenated aromatic amide derivates (two of which are chemically novel) were evaluated against insecticide-susceptible strains of Anopheles albimanus (El Salvador, 1975) and Anopheles quadrimaculatus (Orlando, 1952). A novel vapor phase

bioassay was used to evaluate the three experimental compounds as potential spatial repellents and their toxic effects on each mosquito species. DEET (N, N-Diethyl-meta-toluamide) and transfluthrin were used as standards against which to compare the new compounds. Adult mosquitoes (4-7 days old) of each species were collected and chilled on ice. Sixteen females were transferred to a glass tube (12.5 cm long, 2.5 cm outer diameter) and covered with netting. Each compound was dissolved in 1mL of acetone and serial dilutions (ranging from 0.01-30 µg/ml) were made. Circular filter papers (5 cm2) were saturated with 50 µL of each concentration and allowed 10 min for evaporation. The filter papers were then placed in clear conical polypropylene caps and fixed to the end of the glass tubes. For repellency bioassays, the tubes were placed vertically on a white foam board with the treated caps on the bottom side and the acetone control on the top side. Mosquitoes on the treated and control sides of the tube midline were counted separately at 15, 30, 60 min. Half maximal effective concentration (EC50) was determined. For toxicity bioassay, the tubes were placed horizontally on a white foam board. Knock down activity at 1 hr and 4 hr, and mortality at 24 hr was recorded. All of the experimental compounds (1: EC50 = 37 µg/cm2, 2: EC50 = 36 µg/cm2, 3: EC50 = 55 µg/cm2) were more effective at spatially repelling Anopheles albimanus than DEET (EC50 = 124 µg/cm2). Only experimental compound 2 (EC50 = 90 µg/cm2) acted as an effective repellent against Anopheles quadrimaculatus in comparison to DEET (EC50=115 µg/cm2). For both species, all experimental compounds demonstrated higher toxicity levels than DEET. However, transfluthrin exhibited the highest spatial repellency and toxicity in both species.

62. INVESTIGATING THE PREVALENCE OF IXODES SCAPULARIS ON REPTILE HOSTS IN FLORIDA

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In Florida, only a few cases of Lyme disease are acquired in state. In the southeastern US, reptiles are important hosts for Ix. scapularis that vector Borrelia burgdorferi. Previous studies have implied that large numbers of available reptile host in Florida could be diluting Borrelia burgdorferi transmission; however, the prevalence of Ix. scapularis on reptiles has not been well investigated in Florida. The objective of this investigation was to determine how Ix. scapularis utilizes reptile hosts in Florida. In order to look at host utilization, I examined reptiles for ticks from the Florida Museum of Natural History Herpetology collection. Four species of lizards were examined for ticks: Plestiodon laticeps (Broadhead skink), Plestiodon fasciatus (common five lined skink), Plestiodon inexpectatus (south eastern five lined skink) and Sceloporus undulates (Eastern fence lizard). I examined 1,948 reptiles, collected from 1902 to 2018, which came from 60 counties. Ticks were present on reptiles from the Pan-handle to the southern tip of Florida. Wakulla County, located in northern Florida had the greatest number of reptiles infested with ticks (85/238). The majority of reptiles infested with ticks were found in the Pan-handle and north-central Florida and 14.5% (282/1,948) of all specimens examined were infested with ticks. Plestiodon latieps had the highest prevalence of ticks 29.7% (114/383). Nymphal and larval ticks were the most common life stages found on reptiles. This investigation found a higher prevalence of infected reptiles than previous studies conducted in southeastern US.

63. INVESTIGATING THE PREVALENCE OF THEIRLERIA SPP. WITHIN AMBLYOMMA AMERICANUM IN FLORIDA

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In Florida, deer farming is a rapidly increasing livestock industry. Theileria cervi commonly infects white tailed deer in Florida where it is endemic. The obligate intracellular protozoan poses substantial risk to naïve deer; thus neonates and imported naïve animals are at risk of infection and high levels of mortality. Symptoms of Theileriosis include anemia, asymptomatic infections, and death. The most common tick species in Florida, is Amblyomma americanum, a known vector for T. cervi. The main purpose of study was to identify the prevalence of Theileria spp. in A. americanum in Florida. Ticks were collected by flagging at 37 sites of Florida from March 2016 to August 2017. A total of 465 A. americanum ticks, both adults and nymphs, were found in 19 sites. DNA was extracted from each tick and tested for Theileria, using a pan-Theileria PCR assay. Gel electrophoresis was used to detect positive/negative samples for Theileria spp. We found that Theileria spp. prevalence was high in both nymphs (97%) and adult (95%) ticks which mirrors the high T. cervi prevelence within wild white-tailed deer in Florida (97.6%). The high prevalence found in ticks suggests a high risk for farmed ruminants within Florida. DNA sequencing is currently being conducted to identify the species/subspecies of Theiliera in Florida.

For future studies, comparing prevalence of Theileria regionally could be beneficial. Due to an increase in ticks and tick-borne pathogens in the United States, additional surveillance should be conducted on other species of ticks and their vectored pathogens.

64. ISOLATION AND IDENTIFICATION OF EPIZOOTIC HEMORRHAGIC DISEASE -, BLUETONGUE -, MULE DEERPOX -AND NOVEL VIRUSES FROM DEAD FARMED FLORIDA WHITE-TAILED DEER (ODOCOILEUS VIRGINIASUS)

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The growth of the cervid farming industry has raised questions regarding the contemporary viral pathogens of white-tailed deer (WTD) throughout Florida. Following an outbreak of hemorrhagic disease in Florida cervids in 2012, the need for rapid and accurate

diagnoses became crucial to the industry. Hemorrhagic disease in Florida WTD has up to now typically been attributed to either one of two orbiviruses: epizootic hemorrhagic disease virus (EHDV) or bluetongue virus (BTV). The University of Florida Cervidae Health Research Institute (CHeRI) actively performs RT-PCR screens of specimens taken from dead farmed deer for the viral genomic RNAs (vRNAs) of known viral pathogens of Florida deer: EHDV, BTV, West Nile - and Eastern Equine Encephalitis viruses. To cast a wider net and uncover additional pathogens, spleen, lung and other tissues are homogenized and inoculated onto cell cultures, including C6/36, Vero E6, BHK-21 and OHH1.K cells. An advantage of isolating viruses in cell cultures is that the amount of vRNA is amplified, simplifying virus genome sequencing. Moreover, novel viruses are detected, as RT-PCR screens are virus-species specific. Using a combination of RT-PCR screens, virus isolation and next generation sequencing methods, we have verified the continued circulation and association with fatal infections of hemorrhagic disease viruses EHDV 1, 2, and 6 and various BTV strains/serotypes, in farmed WTD of Florida. Moreover, we have identified Mule Deerpox -, a novel mammalian orthoreovirus, mobuck -, big cypress -, and three novel orbiviruses as significant pathogens of farmed WTD. These findings inform farming and management practices and candidates for vaccine development important for the protection of farmed WTD and other cervids.

65. ISOLATION OF MAYARO VIRUS FROM A PATIENT THAT DEVELOPED GUTTATE PSORIASIS DURING THE 2016 EPIDEMIC OF ZIKA VIRUS IN VENEZUELA

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An 18 year-old female was examined at the outpatient clinic at the Hospital Internacional Barquisimeto in Cabudare, Venezuela. She had fever, chills, a rash and small joint tenderness. She denied any travel history, pet or tick exposure. Three days prior, with the onset of fever she had developed a pruritic maculopapular rash, which evolved into erythematous fine scaling oval shaped macules and plaques distributed mainly in trunk and proximal aspects of the limbs. She had no bleeding manifestations, conjunctivitis, retro-ocular pain nor myalgias. Full blood count and chemistry were unremarkable except for mild lymphocyte leukocytosis and mildly elevated erythrocyte sedimentation rate (ESR). Peripheral blood smear was negative for hemoparasites. Serology tests for antibodies to dengue viruses (DENV) serotypes 1 - 4, Epstein Barr virus, cytomegalovirus, and parvovirus were negative. RT-PCR tests for the genomic RNAs of Zika virus (ZIKV) and DENV serotypes 1-4 were also negative. Plasma and urine were inoculated onto cell culture and cytopathic effects (CPE) were observed 48 hours post-inoculation. RT-PCR tests of virus genomic RNAs purified from the spent cell-culture media were negative for ZIKV, DENV 1-4 genomic RNAs, as was a pan-flavivirus RT-PCR assay. In contrast, a pan-alphavirus RT-

PCR assay was positive; additional RT-PCR tests and nucleotide sequencing revealed that the virus was Mayaro virus (MAYV). Cases of Alphavirus-associated psoriasiform lesions are either rare and understudied. To our knowledge, this is the first laboratory confirmed case of MAYV-associated psoriasis.

66. LANDSCAPE INFLUENCE ON DISTRIBUTION OF TICKS IN SOUTHERN AFRICA

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Land use change can influence the prevalence and distribution of ticks due to their intimate relationship with both vertebrate hosts from which they acquire blood meals and vegetation which provides suitable off-host microclimates. Given the risks to human and animal health associated with pathogens transmitted by ticks, there is a need to understand the influence of environmental drivers on tick distribution. Here, we assess how landscape features, neighborhood effects, and edges influence tick occupancy and abundance across an agricultural landscape in southern Africa. We found that Rhipicephalus appendiculatus and R. simus increase in abundance when far from homesteads and near to protected savanna, while Haemaphysalis leachi increase in abundance when close to homesteads. The composition of the landscape surrounding savanna patches also differentially influenced the likelihood of finding each tick species; H. leachi was more likely to be found in savanna patches surrounded by subsistence agriculture while Rhipicephalus spp. was more likely to be found in savanna surrounded by sugarcane. The availability of hosts in commercial agriculture, subsistence agriculture, and savanna likely drives the distribution of ticks at the landscape scale. At the local scale we found that Rhipicephalus avoided savanna edges. Understanding the

how anthropogenic landscape changes can influence ticks is useful for land use planning and for assessing public and animal health risks associated with ticks and tick-borne diseases.

67. LEVERAGING ARBOVIRAL SURVEILLANCE DATA TO INFORM PUBLIC HEALTH RESPONSES IN BARBADOS

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The island nation of Barbados (pop. ~285,000), located in the Caribbean, has long contended with public health threats posed by Aedes-transmitted diseases, in particular, dengue fever. More recently, both Chikungunya and Zika joined dengue as challenges to public health. Management of arboviral diseases in Barbados is necessary not only for protecting the health of the resident population, but also ensuring the continued success of the island's thriving tourism industry. The establishment of Aedes mosquitoes in Barbados and throughout most of the Caribbean has rendered islands vulnerable to emerging pathogens and outbreak events. In 2015, Zika spread rapidly throughout the Americas, and its proliferation through the Caribbean followed suit. Barbados reported its first confirmed autochthonous Zika transmission to the Pan American Health Organization (PAHO) in January 2016, a month before the global public health emergency was declared. Following detection of suspected Zika cases on Barbados in 2015, 926 individuals were described as suspected cases, and 147 lab confirmed cases were reported through December 2016. Our work on dengue incidence in Barbados from 2013-2016, in which

georeferenced cases of dengue fever reported to the Ministry of Health (n=1,117) were aggregated to health districts, demonstrated that cases were not randomly distributed in space. Utilizing local Moran's I and spatial scan statistics, we detected areas of significantly high and low disease activity that shifted between years, an indication of spatially and temporally variable disease transmission risk. The ability to detect critical hotspots of transmission activity enables public health agencies to target mosquito control efforts, strengthening the capacity to curtail outbreaks and respond to, and reduce, the threat of emerging mosquito-borne pathogens in the Caribbean. Moving forward, we recommend further incorporation of spatially explicit methods of outbreak detection into existing surveillance frameworks.

68. LYME DISEASE CASES ACQUIRED IN EUROPE REPORTED TO THE FLORIDA DEPARTMENT OF HEALTH, 2009–2018

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Background: The majority of Lyme disease cases reported to the Florida Department of Health (Florida Health) are acquired outside the state. This includes cases acquired abroad. Lyme disease outside North America is caused by different serotypes or genospecies. Differences in testing and lack of awareness of Lyme disease among travelers abroad may lead to under-reporting of internationally acquired cases. Multiple health agencies in Europe have reported increased geographical distribution of tick vectors and a rise in incidence of cases being reported. This study analyzes Lyme disease cases with exposure in Europe.

Methods: Probable and confirmed cases of Lyme disease were classified per the Council of State and Territorial Epidemiologists' national case definition. Cases investigated and reported to Florida Health between January 1, 2009, through December 31, 2018, (as of January 2, 2019) were reviewed and sorted according to import status of acquired outside of the U.S. and origin of exposure in

Europe or a European country. Lyme disease incidences in European countries were obtained from data reported by European Centre for Disease Prevention and Control (ECDC) and the World Health Organization.

Results: Twenty-eight cases of Lyme disease with exposure in Europe were reported, with an average of three cases and range of one to five cases per year. Countries of exposure included Austria, Belgium, Czech Republic (3), Denmark, Finland (2), France (3), Germany (5), Hungary, Netherlands, Poland, Scotland, Sweden (2), Switzerland (2), Ukraine, and unspecified European countries (3). Sixteen cases (57%) reported known tick bites abroad, with an additional six (21%) reporting exposure to tick habitats. The majority of cases reported exposure in central Europe, which the ECDC recognizes as the highest area for Lyme disease infection rates on the continent.

Conclusions: The ECDC currently provides specific Lyme disease educational materials for travelers to Europe. Centers for Disease Control and Prevention has online resources where travelers can search disease information by country of destination, but these resources do not include information on Lyme or other tick-borne diseases by location of travel. Including tick-borne disease risk information in the destination query would be helpful for both travelers and their health care providers. Florida Health is adding information on international tick-borne disease risks to existing resources. A protocol was also created for county epidemiology staff to use when investigating Lyme disease cases with exposure in other countries, as the current Lyme disease case definition only references exposure in high and low incidence U.S. states.

69. MODELING THE AGREEMENT AND COST OF INDOOR RESIDUAL SPRAY IMPLEMENTATION STRATEGIES TO CONTROL MALARIA TRANSMISSION

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Background: Indoor residual spraying (IRS) is an effective method to control malaria-transmitting Anopheles mosquitoes, and often complements insecticide-treated mosquito nets, the predominant malaria vector control intervention. With insufficient funds to cover every household, malaria control programs must balance the malaria risk of a particular human settlement against the financial cost of spraying that settlement. This study creates a framework for modeling the cost of IRS implementation, and applies it to potential risk prioritization strategies in four provinces (Luapula, Muchinga, Eastern, and Northern) in Zambia.

Methods: We used optimal network models, with average fuel economy, commodity price, and service price data for 2015 and 2016 as inputs, to assess the travel cost of routes between operations bases and target households in settlements identified through remote sensing. We compared network travel distances to Euclidean distances, to demonstrate the importance of accounting for road route costs. We then compared the cost of spatial prioritzation strategies assuming sufficient funds to spray 50% of households, using four underlying malarial risk maps: a) predicted Plasmodium falciparum parasite rate in 2-10 year olds (PfPR), or b) predicted probability of the presence of each of three main malaria transmitting anopheline vectors (An. arabiensis, An. funestus, An. gambiae).

Results: The estimated one-way costs of reaching settlements to deliver IRS ranged from $0.01 to $37.87, with 75% of settlements

costing $5.80 or less. Simple Euclidian distance models generated a range of +$23.98 - -$80.70 (over- and under-estimates) in modeled cost per trip, as compared to the network route method. There was little overlap between risk map prioritization strategies, both at a district-by-district level, and across all four provinces. At both scales, < 10% of houses were either completely included or completely excluded by all four strategies. The costs of reaching prioritized settlements were either lower, or not statistically different from non-prioritized settlements, at both scales of strategy.

Conclusion: Differences were observed in the cost of reaching settlements with higher estimated PfPR and Anopheles vector capacity, both in cases of applying blanket IRS and in cases of applying IRS based on risk maps. These findings contradict the idea that reaching areas with higher malaria burden is more expensive than reaching areas with lower malaria burden.

70. MODELING THE GEOGRAPHIC DISTRIBUTIONS OF LONE STAR AND BLACK-LEGGED TICK IN FLORIDA

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The Lone star (Amblyomma americanum) and Black-legged ticks (Ixodes scapularis) are species of great public health importance as they are competent vectors of several notable pathogens. While the regional distributions of these species are well characterized, more localized distribution estimates are sparse. We used records of field collected ticks and an ensemble modeling approach to predict habitat suitability for the Lone star and Black-legged ticks in Florida. Of the seventeen climatic and habitat variables considered, vegetation health (NDVI), annual precipitation, and low mean winter temperatures were the most important determinants of habitat suitability. Agreement between the modeling algorithms used in this study was high and indicated the distribution of suitable habitat for

both species was reduced at lower latitudes. These findings are important for raising awareness of the potential for I. scapularis and A. americanum transmitted pathogens in Florida.

71. MOLECULAR EPIDEMIOLOGY OF LETHAL BRONZING DISEASE OF PALM

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The palms (Arecaceae) are important specialty crops due to their aesthetic roles as ornamentals in landscapes and value as a significant agricultural commodity. One of the greatest threats to the sustainability of palm production is lethal decline that is caused by multiple strains of phytoplasmas. Subgroup A of the16Sr-IV group of phytoplasmas is the causal agent of Lethal Yellowing Disease (LYD) and has been a devastating disease of coconut palms in Florida. Subgroup D is a relatively new strain that is responsible for Lethal Bronzing Disease (syn. Texas Phoenix Palm Decline [TPPD]). This disease affects Phoenix spp., Syagrus romanzoffiana, and Sabal palmetto palms and is increasingly widespread in Florida. Key to the life cycles and management of LYD and LBD are their insect vectors. Previous transmission studies on the LYD phytoplasma were conducted prior to the advent of modern molecular tools and

techniques such as quantitative PCR (qPCR), digital PCR (dPCR), and genomic analyses to confirm strain movement. The objectives of this new collaboration are to describe the diversity of phytoplasmas in Florida and the Caribbean that cause decline of palms, identify the vectors and alternative hosts of LBD, and provide genomic data that can be used to study host-vector-pathogen interactions and to manage the disease. Identifying putative insect vectors is the first step in studying transmission and the role of the vector in disease spread. A population survey of auchenorrhynchan insects in palm canopies was conducted at the Fort Lauderdale Research and Education Center, FL where the disease is actively spreading. Yellow sticky traps were used for insect collection to determine insect species that have feed on the palm trees in the disease area. Insects were tested for phytoplasma by PCR. Four families of auchenorrhynchans were consistently collected during the one-year collection period. Among them, a cixiid planthopper, Haplaxius crudus, was the most abundant and approximately 1.11% of these tested positive for phytoplasma. Based on these results, we suggest H. crudus is a potential vector of the Lethal Bronzing Disease phytoplasma and appears to be the same as for the Lethal Yellowing phytoplasma. The low number of positive vectors is consistent with the challenges to obtaining transmission rates for Lethal Yellowing disease in the 1970-80s.

72. MOSQUITO NEUROTOXICITY OF METHOXYLATED STILBENES AND N-METHYLBENZAMIDES.

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Stilbenes are common phytoestrogen compounds. One of the most famous representatives of the stilbene class is resveratrol, a commonly used supplement naturally found in peanuts and grapes. In addition, the benzamides constitute a diverse synthetic group, of which substituted analogs can be used in human health for instance, as analgesics, antipsychotics, or neuroleptics. In particular, DEET (N, N-diethyl 3-methylbenzamide) is used for mosquito control through repellent effects via insect olfaction. Derivatives of stilbenes and N-methylbenzamides have been screened for mosquito toxicity. One specific conformation in both compound groups seems to yield insecticidal properties: the 2-methoxy group on the phenyl ring of stilbene (S943916) or N-(phenylcyclopentyl) methylbenzamide (2S-65465). Compared to analogs such as 4-methoxystilbene (S880159), and 3,4-dimethoxystilbene (T274844), S943916 had greater toxicity on Anopheles gambiae. At 1 µg/mg topical, S943916 killed 100% mosquitoes, while S880159 and T274844 killed 20% and 0%, respectively. Also, 2S-65465 and its analog 2-hydroxy N-(phenylcyclopentyl) methylbenzamide (PRC1358) killed respectively 85% and 77% of the mosquitoes at the same dose. For S943916, 2S-65465, and PRC1358, LD50 values at 24 hours were found to be 540, 488, and 874 ng/mg, respectively. We also explored further the actions of these compounds on headless larvae, a preparation that reports on effects of test compounds that normally cannot penetrate the insect cuticle. In this assay, their paralytic concentrations were 5.8, 9.4, and 12.8 ppm respectively. Originally, according to 2S-65465 design by Laggner and colleagues (https://www.nature.com/articles/nchembio.732), its putative molecular target was predicted to be a voltage-gated potassium (Kv)

channel, because it phenocopied the known inhibitor psora-4. Through patch-clamp electrophysiological recordings, we evaluated the effects of 2S-65465 on HEK cells expressing mosquito AgKv2.1 channels. The K+ currents were inhibited at an IC50 of 0.24 µM, while PRC1358 = 1.14 µM; S943916 = 22 µM; and S880159 = 336 µM. This is the first time the inhibitory action of 2S-65465 on Kv channel is reported. These results altogether indicate an important role for the 2-methoxy group on the phenyl ring for the inhibition of the voltage-gated potassium channel. The possible mode of action is discussed. This finding may open interesting new leads toward synthetizing new mosquitocide compounds. This work was funded by the FNIH (BLOO11VCTR) and the Deployed WarFighter Research Program via the USDA (58-0208-0-068 and 58-0208-5-001). The authors would like to thank Dr. Baonan Sun for providing the patch clamp data.

73. OPTIMAL INSECTICIDE DISTRIBUTION TO PROTECT NEW CITRUS PLANTINGS FROM HUANGLONGBING

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The citrus industry in Florida has dealt with the impact of Huanglongbing (HLB), a vector-transmitted disease, since its local discovery in 2005. The primary vector of HLB is the Asian citrus psyllid, Diaphorina citri Kuwayama, henceforth referred to as psyllid. Citrus production has fallen severely in Florida and growers need to replant groves in order to maintain production levels so that the citrus industry will not fail. It is important that these new plantings

are protected from infection. Despite promising new psyllid management strategies, traditional pesticides are the primary means of controlling the psyllid population. We investigate the problem of how to optimally distribute pesticides in a plantation if the goal is to minimize infection in certain regions. Using a spatially explicit simulation model for HLB transmission (a modification of [1]), we consider the situation of two blocks of citrus adjacent to one another, one full of mature, partially infected trees and the other an uninfected, recently replanted block. We assume that psyllids migrate directly into the mature block, but not into the replanted block. Contrary to intuition (and what is commonly practiced), our simulation predicts that the bulk of the insecticide should be expended on the mature block since the primary danger to the replanted block is the development of a small but resilient psyllid population in the mature block which leads to constant ingress into the replanted block. We also consider how this changes if experimental control measures such as RNAi are introduced into the system. [1] Lee JA, Halbert SE, Dawson WO, Robertson CJ, Keesling JE, Singer BH. Asymptomatic spread of huanglongbing and implications for disease control. Proc Natl Acad Sci U S A. 2015 Jun 16; 112(24): 7605–7610.

74. OPTIMIZING THE CDC BOTTLE BIOASSAY: MOSQUITO NUTRITIONAL STATUS SIGNIFICANTLY AFFECTS TOXICOLOGICAL ENDPOINTS

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With insecticide-resistant mosquito populations becoming an ever-growing concern, convenient screening methods that can identify insecticide resistance in mosquito populations are needed. The current CDC Bottle Bioassay serves as a relatively cheap and effective way to screen field-caught mosquitoes against a wide variety of insecticidal active ingredients and commercial formulations with the goal of characterizing insecticide-susceptibility. While a very useful and practical bioassay for insecticide-resistance screening, there are numerous issues that affect the reproducibility of the findings between experimenters and different agencies. Standardization of this assay is needed to ensure consistency of results throughout the scientific community. We explored whether sucrose-water (10% w/v) feeding status had an impact on the response of mosquitoes to currently utilized insecticides. Sugar water starved mosquitoes were more sensitive to permethrin at 24-hr (LT50 = 68.5 min) and 48-hr (LT50 = 60.5 min) compared to sugar water fed mosquitoes (LT50 = 97.6 min). This effect was also noted for malathion, as mosquitoes starved for 24 hr (LT50 = 40.3 min) and 48 hr (LT50 = 31.8 min) were more susceptible compared to the fully fed mosquitoes (LT50 = 47.5 min). Similar findings were also observed in a permethrin-resistant strain challenged with permethrin. To test if this effect was due simply by the change in mosquito weight over time, we measured the weight of mosquitoes in each treatment group (i.e. starved vs. fed). Our findings indicate that changes in weight do not fully account for the differences observed in this experiment. These findings will advise amendments to the CDC Bottle Bioassay protocol

to increase its reproducibility among different experimenters. We would like to thank the CDC for their funding support of this project.

75. PHENOTYPIC AND GENOTYPIC RESISTANCE TO COMMONLY USED INSECTICIDES IN AEDES AEGYPTI AMONG FOUR CITIES IN SOUTHERN ECUADOR

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The development of insecticide resistance (IR) can undermine efforts to control vector species of public health importance. Aedes aegypti is the primary vector of resurging diseases in the Americas such as yellow fever and dengue, and more recently emerging chikingunya and zika viruses, which have caused unprecedented epidemics in the region. While a yellow fever vaccine exists, vector control is the sole public health intervention option for dengue, chikungunya and zika. IR monitoring has not been conducted regularly in the dengue hyperendemic region of southern Ecuador. In this study, IR was measured across four cities in El Oro Province, Ecuador using phenotypic assays and genetic screening for alleles associated with resistance to pyrethroid insecticides. Bottle bioassays showed phenotypic evidence of significant inter-seasonal variation in resistance to deltamethrin and alpha-cypermethrin, and some evidence of differences between cities for deltamethrin. Despite low sample sizes, there was a significant difference in phenotypic response to the organophosphate, Malathion, between two of the cities during the second sampling season. Genotyping showed

moderate to high frequencies of the F1534C resistance allele and moderate frequencies of the V1016I resistance allele in all four cites. Frequency of resistance genotypes varied significantly between cities in the first sampling season (February-April), and not in the later seasons, suggesting a possible selective response to vector control activity. Interestingly, despite statistically significant evidence suggesting that the mutant resistance alleles conferred phenotypic resistance, there was not precise correspondence between phenotypic and genotypic evidence for resistance. We found that 17.6% (F1534C) and 45.6% (V1016I) of genotypically resistant mosquitoes were phenotypically susceptible in the bottle bioassays. This study indicates there is spatiotemporal variability in IR in southern Ecuador, and serves as an initial examination of the status of and relationship between genotypic and phenotypic markers of resistance in this region.

76. RAPID DIAGNOSTIC TEST FOR ZIKA VIRUS IN DRIED BLOOD SPOTS WITH LOW DEMANDS ON INSTRUMENTATION

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Diagnosis of Zika virus (ZIKV) infection typically involves the collection of blood, saliva or urine from the patient, followed by reverse-transcription polymerase chain reaction (RT-PCR) to detect ZIKV genomic RNA (vRNA). The preparation of dried blood spots (DBS), wherein a drop of the patient's blood is blotted onto filter paper, is one promising low-cost method for specimen storage and subsequent nucleic acid extraction. Moreover, it has been reported

that whole blood is better than serum and plasma for the detection of ZIKV vRNA, as the virus binds to red blood cells. Recent advances in the design of diagnostic molecular tests include reverse-transcription loop-mediated isothermal amplification (RT-LAMP), a nucleic acid-based test that can be performed using minimal instrumentation (e.g. a water bath). Results can be obtained in as little as 8 minutes, compared to the 1.5 hours required for RT-PCR. We first determined the sensitivity and specificity of RT-LAMP to purified ZIKV vRNA. Next, RNA purification method, filter paper type, storage temperature (room temperature, -20oC, -80oC), and duration of storage (one to 30 days) for DBS were evaluated. We then tested our methods using clinical specimens obtained during the 2016 ZIKV epidemic in Venezuela. Our results indicate that ZIKV vRNA is detectable by real-time RT-PCR and by RT-LAMP in DBS that have been stored for up to two years post specimen collection. RT-LAMP reaction times as short as 8 min could be used to detect ZIKV in some circumstances. Storage temperature has a minimal effect on the detectability of ZIKV vRNA within 30 days of preparation of DBS. We conclude that DBS and RT-LAMP are useful and practical, as their use reduces costs and minimizes the time required for the detection of ZIKV vRNA.

77. SELECTIVE PRESSURE OF AMERICAN MOSQUITO VECTORS ON P. FALCIPARUM GENES

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The malaria parasite Plasmodium falciparum was introduced into the Americas through the transatlantic slave trade by European colonial powers who imported >12 million slaves from Africa to work in mines and plantations. Since malaria is holoendemic in West Africa, nearly all of the slaves imported during the 300-years of the transatlantic slave trade were infected with P. falciparum, thus resulting in millions of parasite lineages potentially being introduced. In contrast, the phylogenetic tree calculated using whole genome sequence data from Haitian and South American isolates specifies a monophyletic origin of the parasites in relation to Africa. This discordance is postulated to be due to the extreme bottleneck the parasite population underwent as result of the importation. While the intermediate host (Homo sapiens) stayed the same, the parasite had to adapt to new definitive host species, where the anopheline mosquito host vectors in Africa and those in the Americas had diverged over ~ 100 million years. We hypothesize that the divergent molecular, immunological, biochemical and ecological environment of the American vectors presented an intense selective pressure resulting in a severe population bottleneck, where an extremely reduced number of strains were able to survive on the new continent. Published in vivo studies assessing alternative alleles of P47 (PF3D7_1346800, 6-cysteine protein) describe four key non-synonymous single nucleotide polymorphisms (SNPs) which are necessary for successful infection of A. albimanus, the malaria vector present on Hispaniola. Since it is unlikely that these mutations are the only ones involved in adaptation to new vectors, we assessed mutations with differential frequency between Haitian and African

strains. Supporting our hypothesis was the discovery that non-synonymous SNPs were 7 fold more frequent among stage-specific genes expressed in the sexual and mosquito stages of the lifecycle relative to mappable genes expressed in the blood stages (excluding multigenic hypervariable surface antigen gene families). Two of the genes potentially under selection by the vector were PSOP26 (PF3D7_1244500) and CTRP (PF3D7_0315200) with mutliple non synonymous mutations which were conserved in all nine lineages from Haiti and present at low frequency in African isolates. These alleles are candidates for further in vivo experiments to determine their influence on the successful completion of the mosquito phase of the lifecycle in A. albimanus. Gene products identified via mutations selected in order to adapt to a new vector species perform critical functions in the life cycle, thus potentially leading to new strategies for malaria control or elimination.

78. STRUCTURE-ACTIVITY RELATIONSHIP ANALYSIS OF POTENTIAL NEW INSECTICIDES AND REPELLENTS

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Commercial insecticides and repellents are some of the most effective tools for decreasing the propagation of mosquito-vectored infectious diseases. The emergence of target site mediated resistance threatens the success of this tactic and necessitates research into the next generation of resistance-breaking chemical control agents. In our efforts to discover potential candidates to this

end, we have successfully identified multiple novel lead compounds exhibiting excellent repellency and/or toxicity while simultaneously displaying negative cross-resistance. Herein, we report the biological results of our ongoing investigation into the lead optimization performed and the structure-activity relationship (SAR) analysis that has rationally guided the design and synthesis of our lead analogs. Topical applications to anesthetized mosquitos were used to screen insecticidal activity (75 compounds), and a horizontal spatial glass tube assay, developed within our group, was used to quantify repellency and vapor-phase toxicity (116 compounds). The benchmarks used for comparison are N,N-diethyl-meta-toluamide (DEET), one of the most commonly used insect repellents, and propoxur, a carbamate insecticide. Following the synthesis and evaluation of a series of novel derivatives based on our lead compounds, we were able to identify several key features contributing to the observed toxicity and repellency. To date, we have identified a vapor-phase active derivative that is 13 times more potent than DEET, and while a proper mosquito LC50 was not obtained due to DEET concentration limitations, many of our most repellent derivatives are at least 15 to 1000 times more toxic than DEET. Additionally, a preliminary mouse oral toxicity study indicates that our most potent repellent exhibits an LD50 greater than 2 g/kg, well above the minimum level set by the Innovative Vector Control Consortium (50 mg/kg). Our investigation into topically applied insecticides, likewise, has provided us with very promising data. While our derivatives have not yet reached the efficacy of propoxur against wild-type mosquitos, we are within twofold as toxic, and against resistant strains our derivatives are 20 to 67 times more potent. Utilizing SAR analysis, we have successfully synthesized a variety of lead analogs for the bio-control of mosquitos. We are pleased to report that not only are our most potent vapor-phase acting compounds significantly more repellent and toxic than DEET, but our topical insecticides are within twofold activity of propoxur against wild-type mosquitos and do not exhibit cross-resistance. Future work will focus on further structural modification in attempts to increase the efficacy of our derivatives.

79. SYNERGISTIC EFFECTS OF THE PYRETHROID PERMETHRIN WITH POTASSIUM CHANNEL BLOCKERS ON ANOPHELES GAMBIAE

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Chemical insecticides remain a major component of vector control, and resistance to some chemicals, especially pyrethroids, challenges our efforts to control vector borne diseases. The purpose of this research was to explore the possibility of co-applying permethrin (an agonist of voltage-sensitive sodium channels) with the potassium channel blockers 2S-65465 (2S) and 4-aminopyridine (4-AP). We hypothesized that they would potentiate the neurological effect of this pyrethroid and reduce the amount of permethrin needed for effective control of Anopheles gambiae. Toxicity should increase because the ability of pyrethroids to cause persistent sodium currents will be augmented by blockage of outward potassium current flow, which normally repolarizes the membrane potential during a nerve membrane action potential. Topical treatments were performed on the An. gambiae G3 (susceptible) and Akdr (pyrethroid-resistant) strains. On G3, permethrin had a 24 hr LD50 value of 0.18 (0.16-0.22) ng/mg of mosquito weight; the LD50 values of the compounds 4-AP and 2S were 0.11 (0.08-0.15) μg/mg and 0.5 (0.4-0.6) μg/mg, respectively. Co-application of compound 2S at 125 ng/mosquito (a dose causing 10% mortality, the LD10) with increasing amounts of permethrin showed an Abbott corrected LD50 value for permethrin of 0.021 (0.015-0.029) ng/mg, which was around 9-fold lower than permethrin alone. For comparison, 4-AP and the mono-oxygenase inhibitor piperonyl butoxide (PBO) had synergist ratios of ca. 2 on the G3 strain. Interestingly, the synergism ratio of 2S with permethrin was reduced from 9 to 3 on the Akdr strain. Another significant aspect of pyrethroid toxicity is rapid knockdown, where mosquitoes are unable to stand or fly normally long before death ensues. Topical co-application of 2S and

permethrin increased knockdown effects, with 8-fold lower 1 hr KD50 (half knockdown dose) compared to single permethrin treatments. Intracellular recordings from electrically stimulated Periplaneta americana central giant axons revealed that 2S and 4-AP (> 10 µM) caused wider action potentials followed by multiple spikes after one stimulation, while effects of permethrin (10 µM) were large depolarizing afterpotentials. When 2S or 4-AP were co-applied with permethrin (3 µM) at concentrations as low as 0.3 µM and 3 µM, respectively, large depolarizing afterpotentials were observed with no signs of potassium blockage effects present. These data suggest that co-application of either potassium channel blocker with permethrin can synergistically increase the mortality of an An. gambiae susceptible strain, and interactions of 2S or 4-AP with permethrin in the nervous system can augment sodium channel agonism effects.

80. TAILOR-MADE LYOPHILIZED PRIMERS&PROBE REAGENTS FOR REAL-TIME MOLECULAR DIAGNOSIS OF EMERGING VIRUSES: APPLICATION FOR EXPLORING THE CAUSES OF FEBRILE ILLNESS IN CHILDREN IN HAITI

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Real-time PCR and RT-PCR are the gold standard tests for direct diagnosis and detection of microorganisms in medical and veterinary studies but also in studies aiming at screening the environment for existing or new microorganisms. These tests are easy to develop and implement and can be developed in weeks for detection of an emerging pathogen. However, in practice, implementation at the field level remains much more complicated as showed by the poor results observed in recent External Quality Assessment studies of European expert laboratories concerning the detection of arboviruses such as yellow fever, West Nile, Usutu, Toscana, Tick-borne encephalitis viruses, among other. To be better prepared for re-emerging pathogens, we set-up a platform for preparing quality controlled and lyophilized primers and probes (QC-Lyoph-P&P) that target a large variety of emerging viruses and their differential diagnosis. QC-Lyoph-P&P are (i) can be shipped at room temperature and stored for years without degradation, (ii) amenable to different formats from 2 to 96 reactions preventing contamination and successive freeze-thaw cycles, (iii) can be used with a large variety of enzyme kits routinely used in the laboratory, (iv) can be provided with the ad-hoc synthetic positive control, (v) are adapted to any diagnostic platform, (vi) have been pre-validated through limit-of-detection (LOD) determination, (vii) can be deployed within days in the local laboratories where emergence has occurred, and (viii) will be accessible on the European Virus Archive on-line catalog (https://www.european-virus-archive.com/). At this time, QC-Lyoph-P&P have been prepared for 110 viral or bacterial targets, among which there is a large number of arboviruses and zoonotic viruses. Preparation process and validation steps will be presented with examples. The knowledge about the causes of febrile illness affecting children in Haiti is inexistent. Here we took advantage of samples collected during a NIH-supported program aiming at investigating Zika virus circulation to address other causes of infectious diseases. Samples from this cohort have been tested for Zika virus (7 positives / 198), Mayaro virus (no positive / 137),

PanFlavivirus (1 positive / 47 samples, negative for Zika virus). The screening will be continued on all samples and other viruses will be tested such as hepatitis E virus, cytomegalovirus, adenovirus, spondweni virus, Oropouche virus. In this specific case, it appears that detection of Zika virus can be deployed locally in Haiti using QC-Lyoph-P&P for timely detection and prospective monitoring.

81. WHY SHOULD WE DO PCR? ASSESSMENT OF CLINICIAN ACUTE ARBOVIRUSES TEST ORDERING PRACTICES

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Background: Zika (ZIK) and dengue (DEN) viruses are flaviviruses with similar geographic distributions and clinical presentations and can be cross-reactive on antibody testing. Polymerase chain reaction (PCR) testing can detect viral ribonucleic acid (RNA) in acute infections and is available commercially. Additionally, secondary DEN infections may result in a low immunoglobulin (Ig)M antibody titer and early IgG elevation, making PCR the best way to confirm an acute DEN infection. Understanding provider test ordering practices can help inform clinician outreach and improve public health surveillance.

Methods: Florida Department of Health epidemiologists work with commercial laboratories to forward select flavivirus positive samples to the state public health laboratories (SPHL) for confirmatory testing. Obtaining acute symptom onset date, required symptoms, recent travel history, and sample collection dates are pivotal to the laboratory testing algorithm. SPHL relies on state and county epidemiologists to acquire this information before assigning testing if samples arrive without appropriate paperwork. Epidemiologists confirm and evaluate other epidemiologic information from

providers to request additional arboviral testing from SPHL and to obtain convalescent samples as needed. SPHL and commercial laboratory results from confirmed and probable DEN and ZIK cases with symptom onset between January 1, 2016, and December 18, 2018, were reviewed to assess gaps in testing requested by providers.

Results: Of the 70 PCR-positive DEN cases at SPHL, only one had provider-ordered PCR testing. Sixty-seven of these had DEN serology ordered commercially; thirteen had only significantly elevated IgG, which does not meet national DEN case definition criteria. Two additional cases had only ZIK or chikungunya commercial testing ordered. Among the 76 PCR-positive ZIK cases at SPHL, 51 had provider-ordered PCR and nine had only ZIK serology ordered. Commercial DEN serology was ordered for 62 of the ZIK cases.

Conclusion: Thirteen DEN and sixteen ZIK cases were identified that likely would not have been reported due to initial testing selection. Arboviral disease reporting helps drive mosquito control activity in Florida. In addition, molecular testing provides more disease-specific testing, which is particularly relevant for ZIK-linked birth defects. PCR serotyping of acute DEN cases also provides important epidemiologic information. The Centers for Disease Control and Prevention recommends both DEN and ZIK PCR when either is suspected as an acute illness; relying exclusively on IgM testing is not recommended. Health care providers should be encouraged to order PCR testing for suspected acute flavivirus patients and consider all clinically similar arboviruses endemic to the exposure location.

82. WOLBACHIA INFECTION DETERMINATION IN WILD AEDES AEGYPTI POPULATIONS IN PALMETTO, FLORIDA

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Aedes aegypti and Aedes albopictus are the primary vectors of arboviral diseases such as zika, dengue, yellow fever, and chikungunya. Aedes albopictus and Aedes aegypti pose a greater threat to global health due to their increasing distribution and vectorial competence. Novel methods of controlling these species offer a viable alternative to traditional pesticide use. Wolbachia is an intracellular endosymbiont found in wild A. albopictus populations but rarely in wild A. aegypti populations. In laboratory-based studies, various strains of Wolbachia have been found to increase A. aegypti immunity to viral pathogens and decrease viral transmission. In Florida, Wolbachia-positive A. aegypti mosquitoes were documented in Jacksonville in 2014 and St. Augustine in 2016. Wolbachia-positive A. aegypti were collected from Palmetto in 2016 and 2017. However, the source of the Wolbachia infection in Florida wild A. aegypti is unknown. The aim of this study was to compare the Wolbachia infection rates in four populations of wild A. aegypti mosquitoes to determine if these mosquitoes were becoming infected with Wolbachia through interactions with A. albopictus. Infection rates were compared between two populations of A. aegypti mosquitoes that occur with A. albopictus against two

populations of A. aegypti mosquitoes that occur independently of A. albopictus. From April to August of 2018, a total of 60 oviposition traps were placed in 4 zones in Manatee County, Florida (Anna Maria Island, Cortez Fishing Village, West Palmetto, and North Palmetto) to collect A. aegypti and A. albopictus eggs. Larvae were hatched from the eggs and reared to adulthood. Adults were identified by sex, species, zone, and location collected. So far, DNA extraction has been performed on 865 out of the total 5650 A. aegypti collected and 347 have been tested for Wolbachia. No Wolbachia-positive A. aegypti have been identified. The processing and testing of samples will continue over the next year.

83. A NOVEL COMPOUND INHIBITING PSEUDOMONAS AERUGINOSA AMPG GREATLY REDUCES THE TRANSPORT OF SIGNALING MOLECULES FOR INDUCIBLE AMPC B-LACTAMASE EXPRESSION AND RESISTANCE TO B-LACTAM ANTIBIOTICS

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Pseudomonas aeruginosa was designated as a high priority for developing new antimicrobial agents by the World Health Organization due to its extraordinary capacity to develop resistance to all available antibiotics. One of the widespread mechanisms of resistance is the overexpression of inducible chromosomal AmpC β-lactamase. AmpG transports the signaling molecule GLcNAc-1,6-anhydromurNAc-peptide into the cytoplasm to induce AmpC β-lactamase. We showed that deletion of the ampG gene leads to a very low level of AmpC β-lactamase expression and high sensitivity to β-lactams, suggesting that AmpG is a potential target for

controlling the resistance to β-lactam antibiotics by AmpC. Through high-throughput screening of more than 645K compounds in P. aeruginosa PAO1 we previously identified compounds that decreased ampC-lux expression and increased sensitivity to ampicillin. We report here the further characterization of one of the lead compounds, UF-S-4. To examine AmpG-mediated transport we used spheroplasts and measured the uptake of a fluorescent probe (GLcNAc-1,6-anhydromurNAc-fluorophore conjugate). Carbonyl cyanide m-chlorophenylhydrazone (CCCP), a toxic proton motive force inhibitor that inhibits AmpG activity, was used as a positive control. UF-S-4 inhibited AmpG transport of the probe, similarly to CCCP. The β-lactamase activity of cefoxitin-induced PAO1 was inhibited about 5-fold by UF-S-4 as compared to 9-fold by CCCP. Unlike CCCP, compound UF-S-4 showed no cytotoxic activity to HeLa cells. We conclude that compound UF-S-4 significantly reduces AmpC β-lactamase activity in P. aeruginosa PAO1 cells by blocking the entry of the inducing molecules for AmpC β-lactamase into the cytoplasm. More compounds are being tested for their potential activities against P. aeruginosa PAO1.

84. APPLICATION OF CRISPR INTERFERENCEIN BURKHOLDERIA PSEUDOMALLEI

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There are numerous genetic tools available for studying bacterial gene function. Only few of them can be used with B. pseudomallei due to the strict regulations associated with its classification as Tier 1 select agent in the United States and most traditional tools are cumbersome and time consuming. B. pseudomallei is the causative agent of melioidosis, an infectious disease with high mortality and relapse rates that is endemic in Southeast Asia and Northern Australia and other parts of the world. Studies of gene and protein function are required to understand bacterial pathogenesis and drug resistance mechanisms, which may lead to improved treatment of the disease. The discovery of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9, an RNA-guided DNA endonuclease that causes a double-strand break (DSB) at a targeted genomic location, has revolutionized the ability to perform targeted genome editing in many organisms. CRISPR interference (CRISPRi) system, an inducible gene repression using an inactive Cas9 protein (dCas9) and single guide RNAs (sgRNAs) was demonstrated as a less time consuming, powerful genetic tool enable depletion of specific protein especially in GC rich microorganism like Pseudomonas aeruginosa1. We tested this system in B. pseudomallei by targeting motA, encoding a component of the flagellar motor complex involved in bacterial swimming and penA, encoding a Class A □-lactamase involved in □-actam antibiotic resistance. When compared to wild-type B. pseudomallei Bp82 and Bp82 with chromosomally-integrated dCas9 in the absence of sgRNA, depletion of motA gene expression by the complete CRISPRi system resulted in

complete inhibition of bacterial swimming and significantly decreased resistance to carbenicillin (16 fold) and ampicillin (4 fold). The results show that CRISPRi functions very well in B. pseudomallei and we anticipate application of this system for depletion of specific genes, either individually or multiple genes simultaneously. References 1. Tan SZ, Reisch CR, Prather KLJ. 2018. A robust CRISPR interference gene repression system in Pseudomonas. J Bacteriol 200:e00575-17. H

85. ASSESSMENT OF THE PCR-BASED ASSAYS FOR IDENTIFICATION AND DIFFERENTIATION OF BRUCELLA SPECIES

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Brucellosis is one of the most significant zoonoses affecting a vast range of animals, and leading to major economic losses. Humans can be infected by direct contact with a sick animal or its raw products. Human brucellosis is rarely fatal, but it can cause severe and often irreversible damage to almost all systems. Due to the lack of effective means for vaccination and treatment of human brucellosis, the timely identification of infection from Brucella spp., the bacterial species causing disease, among domestic and wild animals is most important. The traditional methods of Brucella spp. identification are usually characterized by low specificity and sensitivity, and put the laboratory personnel at risk of infection. More reliable and effective approaches for Brucella diagnosis is the application of polymerase chain reaction (PCR) assays. Nowadays there is a wide variety of PCR-related techniques, such as conventional PCR, qualitative real-time PCR, high-resolution melt analysis, multiple locus VNTR analysis (MLVA) and so on. Some of these approaches allow for genus-specific identification, while more advanced assays provide discrimination at the level of species, biovars and even individual

populations. This is important, as the animal vaccination strategy depends on the pathogen species, as well as the species of its host. The goal of this work was to evaluate the advantages and disadvantages of various PCR-based assays. Most of them were designed to detect, in whole or in part, the six classical (core) Brucella species: B. abortus, B. melitensis, B. suis, B. canis, B. ovis, and B. neotomae. Five assays were able to identify marine mammal Brucella species (B. ceti and B. pinnipedialis). The species B. inopinata and B. microti could only be detected by the assay of Kang et al. (2011), while no tests were reported as far for detection of the recently isolated species B. papionis and B. vulpis. As a result, it was found that the most accurate and simple technique for identification and differentiation of a wide range of Brucella species was Bruce-ladder assay described by Garcia-Yoldi et al. (2006) and modified by Kang et al. (2011). The last one was able to identify 13 individual genotypes belonging to 10 of 12 known Brucella species, including three most popular vaccine strains (B. abortus S19, B. abortus RB51, and B. melitensis Rev1). For intraspecific differentiation of strains, the methods of MLVA or DNA sequencing may be more relevant, but they are significantly more expensive and complicated.

86. BURKHOLDERIA UBONENSIS MEROPENEM RESISTANCE: A STORY OF A NEAR NEIGHBOR OF BURKHOLDERIA PSEUDOMALLEI

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Burkholderia ubonensis (Bu) is a non-pathogenic bacterium co-isolated from the environment with B. pseudomallei (Bp), the causative agent of melioidosis. Concerns arise as Bp might increase its already significant drug resistance by acquisition of DNA from drug resistant near neighbor species such as Bu. In contrast to Bp, meropenem resistance (MEMr) is common in Bu but the mechanisms are unknown. To dissect the mechanisms of MEMr in Bu, we used genetic and molecular tools to examine Bu Bp8955, a highly MEMr (MIC ≥ 32 μg/ml) soil isolate from Puerto Rico. A random transposon mutagenesis approach was used to identify mutants with increased MEM susceptibility (MEMs) (MIC <8 μg/ml). Such mutants were obtained at a frequency of 0.28%. Transposon insertion sites were determined by sequencing chromosome-transposon junction sequences. Two highly MEMs mutants (MIC = 1.5 μg/ml) were further studied. They contained transposon insertions in slt and nagZ, encoding a soluble lytic transglycosylase and β-N-acetylglucosaminidase, respectively. In other bacteria, Slt and NagZ are required for β-lactamase induction in response to β-lactam challenge. Unlike Bp, Bu encodes two Class A β-lactamases, PenA and PenB, but sequence and functional analysis indicate that only PenB is catalytically active. In B. multivorans PenB was shown to be a carbapenemase. B. ubonensis also encodes an Class C β-lactamase, AmpC, an inducible cephalosporinase in B. cenocepacia. Deletion of penA, penB and ampC and MIC analyses confirmed that

only PenB possesses activity against MEM, imipenem (IMP) and ceftazidime (CAZ) but not AmpC and PenA. RT-qPCR showed that when Bp8955 cells were challenged with MEM and IMP, penB and ampC mRNA levels were highly induced but not those of penA. When expressed in the E. coli periplasm, PenB and AmpC but not PenA supported bacterial growth in the presence of ampicillin. Lastly, a penB'-lacZ transcriptional fusion under control of the cognate PenR transcriptional regulator exhibited the same expression pattern in MEM and IMP, but not CAZ challenged Bu and B. thailandensis, a close relative of Bp, cells. Taken together, the results suggest that: 1) unlike Bp, Bu PenA is inactive and PenB is the main MEMr determinant induced in β-lactam challenged cells; and 2) B. pseudomallei complex bacteria process the functions necessary for PenR-dependent PenB expression.

87. CASE REPORT: FIRST SUCCESSFUL TREATMENT AND MANAGEMENT OF CANINE MELIOIDOSIS

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This case study presents a guideline for treatments and management of canine melioidosis, a fatal disease caused by Burkholderia pseudomallei. A 10-year-old male dog was trapped with barbed wires causing severe wound infection around its neck and back. The wounds were treated with amoxycillin/clavulanic acid 30 mg/kg

orally twice daily at a local primary care clinic and became worse after seven days of treatment. The dog was then referred to our animal hospital at Prince of Songkhla University in Hatyai, Thailand for proper diagnosis and appropriate treatments. At the admission, the dog was presented with mild fever, severe anemia, thrombocytopenia, increased liver enzymes and blood parasites. Bacterial culture from infected wound swabs revealed Proteus mirabilis, Pseudomonas aeruginosa, and Escherichia coli, while hemoculture grew Burkholderia pseudomallei. The presence of abscess-like mass at splenic tail was confirmed by ultrasonography and x-ray imaging. The dog was treated based on a modification of human protocol by giving 20 mg/kg intravenous meropenem, twice daily to prevent death from sepsis for 14 days, then followed by a short term of oral doxycycline 10 mg/kg daily for blood parasite treatment and 20 weeks of sulfamethoxazole trimethoprim (co-trimoxazole) 25 mg/kg twice daily for eradication of the bacteria. The multi-locus sequence typing was used to genotype the B. pseudomallei isolate from blood. Sequence type (ST) 366 was identified. Epidemiological analysis has demonstrated that this ST is a local genotype of B. pseudomallei that is widely spread in environment and caused human disease in southern Thailand. Canine melioidosis is an unusual infection in dogs even in Thailand where melioidosis is highly endemic. The bacterial agent can persist in host immune cells causing fulminant or recurrent disease. Most of the publications recommended to euthanize the infected animal due to the environmental contamination concern. There was no guideline for canine melioidosis treatment. This successful case management was solely based upon proper diagnosis and appropriate treatments. This case report may be used as a guide for melioidosis case management in other companion animals.

88. CHARACTERIZING AND OPTIMIZING LIGNIN'S ANTIBACTERIAL ACTIVITY AGAINST STAPHYLOCOCCUS AUREUS

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As an aromatic plant cell wall polymer, lignin provides mechanical strength, contributes to forming a water-impermeable coating of vascular tissue, and serves as a physical barrier against pests and pathogens. Each year, lignocellulosic biofuel production generates millions of tons of lignin for which there are currently few valuable applications; as such, deriving value-added applications of lignin are predicted to offset lignocellulosic biofuel production costs, which remain significantly higher than fossil fuel production costs. Increasing interest in using lignin-based materials for biomedical applications necessitates characterizing the effects of lignin on mammalian and microbial cells. However, to accomplish this task, lignin must be soluble in aqueous media. Lignin has demonstrated antibacterial activity in existing literature, however, lignin's hydrophobicity has prevented comprehensive characterization of its antibacterial activity without using model compounds or sodium lignosulfonate, a chemically-modified water-soluble lignin derivative. We have discovered that 1 M 3-morpholinopropane-1-sulfonic acid (MOPS, pH 7.2 ± 0.05) effectively solvates at least 100 mg lignin/mL within 30-60 minutes at room temperature. These lignin solutions can then be diluted in water or aqueous media and filter-sterilized, thereby providing opportunities for novel experiments using lignin in aqueous systems. Moreover, the pKa value of MOPS (7.2) is close to optimum biological pH (7.3 ± 0.1), thus making a MOPS solution where pH=pKa an ideal lignin solvent for biological experiments. To

demonstrate this, we compared viability of the opportunistic, pervasive pathogen Staphylococcus aureus grown in the presence and absence of soluble lignin. At a concentration of 5 mg/ml, MOPS-solubilized lignin alone was able to modestly inhibit S. aureus growth in tryptic soy broth (TSB) by 92%, and when combined with sub-inhibitory concentrations of the antibiotic tunicamycin, elicited >99.9% growth inhibition. Cell death and damage in the presence of MOPS-solubilized lignin was also found be 5-fold increased, as assessed by staining with propidium iodide (PI). We also added MOPS and MOPS-solvated lignin to tryptic soy agar plates and performed parallel antibiotic disk diffusion assays with β-lactam antibiotics against S. aureus. Lignin restores β-lactam susceptibility to a β-lactam-resistant S. aureus strain and induces β-lactam hypersusceptibility in a β-lactam-susceptible strain. These results suggest that MOPS-solvated lignin may potentially be a low-cost adjuvant to antibiotic therapy and provide opportunities for developing value-added applications of lignin, ideally to cultivate an economic environment favorable for lignocellulosic biofuel production while combating the rise of antibiotic-resistant pathogens.

89. CHLAMYDIA TRACHOMATIS RESIDING IN INCLUSIONS EXHIBIT CELL DIVISIONS IN HOST CELL-FREE CONDITION

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The limited metabolic capabilities due to the reduced genomic size of the obligate intracellular chlamydial pathogens make them dependent on a wide range of host metabolic precursors, intermediates, and cofactors. The growth and nutrient requirements are so complex that the axenic cultivation of pathogenic chlamydia has not been successful causing severe restrictions in studying cell division, metabolism and physiology. Here, we take a step forward in achieving the growth of Chlamydia trachomatis in axenic cultivation. We reasoned that chlamydial inclusions acquire necessary host factors to allow cell divisions to continue even in axenic medium. We utilized C. trachomatis L2 transformed with a plasmid expressing green fluorescent protein and harvested inclusions containing reticulate bodies. The inclusions were isolated and purified by using anti-IncA antibody and magnetic beads conjugated with secondary antibody. The isolated and intact inclusions were imbedded into an agarose matrix and supplied with Chlamydia intracellular phosphate-1 medium (CIP-1) developed by Omsland et al. When examined by fluorescence microscopy, we observed a 97% increase in inclusion size over three days of incubation. Individual reticulate bodies were observed undergoing cell divisions. This observation indicated that the intact inclusions are capable of supporting the cell divisions of C. trachomatis in a host cell-free condition.

90. CLONALITY AND CLOSED PANGENOME OF XANTHOMONAS CYNARAE PV. GARDNERI

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Xanthomonas cynarae pv. gardneri (formerly, X. gardneri, Xg) is a globally-distributed pathogen causing bacterial spot of tomato and pepper. For a very long time, only the reference strain isolated from Yugoslavia in 1957 was known. In the early 1990s, two strains were isolated from Costa Rica. In the late 1990s and post-2000, strains were recovered from several other countries around the world. Previously, a clonal global population was found using multi-locus sequence analysis of six genes. In this study, we examined genomic variation in this species using strains isolated from eight countries: Brazil, Canada, Costa Rica, Ethiopia, Reunion, Uruguay, USA and South Africa. In all, the dataset consists of 68 genomes. We carried out pangenome analyses using the Roary pipeline which identified 2899 core genes and 3566 accessory genes. Aligned core genes was used to infer a maximum likelihood phylogeny using the iQTree pipeline. Type three secreted effector, which are usually targets of resistance breeding were identified by BLAST using a database of Xanthomonas effectors. We used ClonalFrameML to infer evidence of recombination within the core genome. Our results point to a closed pangenome and limited genetic variation in the core genome, while accessory genes provided additional population structure. The profile of effectors was largely uniform among strains, with allelic

variation being the most pronounced. Some strains from Brazil, Ethiopia and the USA lacked the XopAQ effector. ClonalFrameML inference of recombination suggests a low rate of import of genetic materials into this species, with few strains exhibiting unique recombination events. Overall, we found a closed pangenome and largely clonal population. This is very different from what is observed in the other major species causing bacterial spot disease of tomato, X. perforans. This information therefore suggests that a more durable global management strategy, especially resistance breeding targeting effectors, can be developed for this species compared to X euvesicatoria and X. perforans.

91. CONNECTING ENVIRONMENTAL CUES, BACILLUS ANTHRACIS PHYSIOLOGY, AND LOCALIZED INFECTIOUS ZONE FORMATION

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In the soil environment, the bacterium Bacillus anthracis exists as a dormant spore, waiting for the necessary signals to permeate through the exosporium layer and initiate germination. Vegetative organisms are sensitive to most disinfectants and heat but when exposed to certain signals they can sporulate quickly. The spore surface, or exosporium, is coated with glycoprotein that is involved in spore binding to environmental surfaces, generates spore hydrophobicity and affects spore germination. Spores contact a host through ingestion, inhalation, or cutaneous inoculation then germinate to the vegetative form and elaborate the A2B-type anthrax toxin made up of protective antigen (PAG), lethal factor (LF),

and edema factor (EF) which combine and ultimately cause host death (typically grazing mammals). Outbreaks still occur globally, including areas where vaccination reaches livestock but not wildlife. Outbreaks are episodic with pronounced seasonality. In the mid-latitudes, such as the United States and Australia, outbreaks have long been associated with early wet springs followed by hot, dry summers. It has been shown that animal behavior and herd population dynamics impact outbreak severity. Besides animal behavior and landscape level ecological factors, what drives the high infectiousness of primary localized infectious zone (LIZ) formation are unknown. Our work investigates the role soil microenvironmental characteristics have on B. anthracis physiological flux in the soil, an often-overlooked aspect. Bioluminescent reporter strains were engineered and utilized to understand the effect of simulated environmental conditions on B. anthracis vegetative growth, toxin elaboration, and sporulation induction. The B. anthracis PAG (pagA) and small sporulation protein B (sspB) promoters were cloned to express a Gram-positive optimized luxABCDE as reporters of vegetative growth and toxin elaboration, respectively. Expression of bioluminescence from the promoters was verified in aerated and static growth. High soil pH and calcium levels have long been anecdotally and observationally linked to high local anthrax prevalence. We measured B. anthracis growth and luminescent dependence, as measures of toxin elaboration (PpagA-lux) and sporulation (PsspB-lux), in relation to pH and calcium levels. B. anthracis grew better at alkaline pH while high soluble calcium levels inhibited growth. Toxin expression was highest at alkaline pH that was shifted higher in the presence of calcium. Sporulation experienced high positive association with increased calcium levels and somewhat alkaline pH. The findings lay the groundwork for more complex soil models involving plants and other microorganisms. This work sheds light on how B. anthracis physiological behavior in the soil may contribute to LIZ formation adding to the evolving knowledge of the drivers of anthrax outbreaks.

92. CORE GENOME AND EFFECTOR PROFILE ANALYSIS OF XANTHOMONAS PERFORANS SUGGEST GLOBAL STRAIN MOVEMENT

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Xanthomonas perforans (Xp) is a rapidly evolving pathogen that causes bacterial spot disease of tomato. We have observed population change due, in part, to multiple recombination events that introduced novel genes and allele types in to the Xp gene pool, including type III secreted effectors (T3Es). T3Es are involved in modulating the interaction with its host, and effector content is usually associated with host range and virulence. Xp exhibits genetic variation in effector genes at local and global scales. Our hypothesis is that the evolutionary history of the strains is reflected in their effector content because effector genes are evolving together with the core genome. The objectives of our study are to i) describe the phylogenetic relationships among a global sample of Xp strains, ii) determine their effector content, iii) compare effector profiles and core genome phylogenetic associations. This will help us understand the geographic distribution of strains as influenced by worldwide human trade and movement, and potential impacts to the host-pathogen interaction mediated by T3Es. We conducted a

phylogenetic analysis of 887 core genes from whole genome sequences of 269 Xp strains representing a worldwide population. Effector profiles were obtained through a homology analysis with tBLASTn. We queried 125 effectors that were previously reported in genomes of Xp and other Xanthomonas species. Phylogenetic analysis shows the majority of the strains can be grouped geographically with a high likelihood of multiple events of strain introduction in different countries. Geographical grouping was also reflected in effector profiles, as strains isolated from same geographic region had similar effector profiles. Notably, the effector XopJ4, considered a core effector of tomato pathogenic Xp strains, was missing in a specific group of strains from USA, Nigeria, and Thailand. Likewise, novel effectors AvrXa3, AvrXacE2 and XopAQ were found in strains from USA, Nigeria and Thailand. TAL effectors were also observed in several strains isolated from Ethiopia, Brazil, and Nigeria. Our findings suggest that Xp populations are geographically structured but also share variation likely due to the global movement of strains. We speculate that rapid and independent recombination events have further increased the gene pool, as observed in the effector profiles, leading to emergence of novel lineages of Xp strains.

93. DETECTION OF ANTIBIOTICS-RESISTANT BACTERIA IN HOUSEFLIES IN FLORIDA

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In the agricultural industry, the use of antibiotics is common practice in order to promote growth, improve efficiency, and treat diseases of animals. However, the antibiotics used are often the same ones used to treat human pathogens. The rise in antibiotic resistance is becoming a global issue due to their overuse. An often-overlooked link from antibiotic treated livestock to human populations is the common house fly. Musca domestica are the most abundant insect associated with livestock, poultry and other animals. They are also notorious as the mechanistic vector for multiple pathogens in humans and other mammals. In this project, we ask the question of whether the house flies bear antibiotic-resistant bacteria (ARB) in the field and can potentially promote their dissemination as carriers. We sampled houseflies from a livestock farm owned by the University of Florida College of Veterinary Medicine. The prevalence and abundance of antibiotic resistant bacteria were assayed by plating homogenate of individual house fly whole bodies and guts on selective antibiotics media. Identifies of the ARB were revealed by Sanger sequencing of the full 16S rRNA gene. Our results showed that house flies indeed harbor a surprising amount of ARB such as Enterococcus faecium. Additionally, we sampled house flies monthly through the course of one year and found that houseflies harbor ARB from various genera such as Bacillus, Kytococcus, and Oceanobacillus. This is concerning as the number of infections caused by enterococci and other enteric bacteria have increased in recent years. The results suggest houseflies pose a serious threat to

human health as they are a direct link between livestock and urban centers.

94. EDWARDSIELLA PISCICIDA: A VACCINE DELIVERY PLATFORM FOR MULTIPLE FISH PATHOGENS

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Construction of (i) RAEV strains with balanced-lethal Asd+ vectors encoding Ich i-antigen, (ii) E. piscicida strains with regulated delayed attenuation and (iii) RAEVs to display a regulated delayed lysis phenotypes. Insertion of defined deletion mutations with and without insertions was accomplished by conjugational transfer of suicide vectors to E. piscicida J118 using the suicide vector donor strain □7213. The codon-optimized sequences of I-antigens were inserted into the E. piscicida Asd+ plasmid pG8R8022, which were electroporated into E. piscicida χ16022. Synthesis of I-antigen was confirmed by western blotting. We developed a balanced-lethal vector-host system, without using antibiotic-resistance markers, using E. piscicida ΔasdA mutants with requirement for diaminopimilic acid (DAP) and a plasmid with the wild-type asdA gene to specify synthesis of recombinant i-antigen from the fish pathogen I. multifiliis. Regulated delayed attenuation was achieved by replacing the fur and crp promoters with the tightly regulated araC PBAD cassette so that fur and crp expression is dependent on arabinose provided during growth. Following colonization of lymphoid tissues, Fur and Crp protein synthesis ceases such that attenuation is gradually manifest in vivo to preclude induction of diseases symptoms. The regulated delayed lysis system relies on araC PBAD regulated expression of the asdA and murA genes required for DAP and muramic acid synthesis that are essential for the cell wall peptidoglycan layer. The regulated programmed cell lysis was achieved by using χ16017 and complementing the two mutations (asdA and murA) by a plasmid vector pYA4763 with asdA and murA genes controled by araC PBAD. In the presence of

arabinose, the plasmid encoded asdA and murA and the chromosomally encoded murA are transcribed from their respective PBAD promoters, allowing for bacterial growth. In vivo, no synthesis of AsdA and MurA leads to cell lysis. RAEV strains displaying regulated delayed lysis and regulated delayed attenuation will be highly immunogenic for bath vaccination of fish.

95. GENETIC DIVERSITY OF CLINICAL AND ENVIRONMENTAL BURKHOLDERIA PSEUDOMALLEI ISOLATES FROM SOUTHERN THAILAND

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Burkholderia pseudomallei, a soil dwelling bacterium, is the causative agent of melioidosis, a potentially fatal disease endemic to most parts of Thailand. Prevalence of melioidosis in southern Thailand and the influence of rainfall has not been well defined. The environmental diversity of B. pseudomallei in southern Thailand has not been characterized since the 1960s. As such, the risk of melioidosis and the current genetic diversity of B. pseudomallei in this region is poorly understood. We employed the multidisciplinary "One Health" approach to investigate relationships between clinical and environmental isolates as well as the impact of weather patterns on melioidosis incidence and genotype frequency. In the 1960s, Dr. Richard Finkelstein, a US researcher from the South East Asian Treaty Organization (SEATO) isolated B. pseudomallei from soil and water samples collected across southern Thailand. This historic collection of environmental isolates was compared to isolates from recent melioidosis patients collected from 3 major hospitals in Songkhla Province during 2014 - 2018. These culture-confirmed clinical

isolates were analyzed with species-specific real time PCR assays and further genotyped by multi-locus sequence typing (MLST). The eBURST algorithm was used to visualize the evolutionary relationships between these clinical samples and those found in Finkelstein's collection. A quantitative timeline of melioidosis cases in southern Thailand was compared to regional rainfalls. Genetic analysis using MLST identified 57 different sequence types (STs) among the clinical samples. Of these STs, 40.35% were common to Finkelstein's environmental isolates, but the most frequent genotypes in each collection differed. While ST371 was the most frequent strain in Finkelstein's collection, it was only found once in the clinical isolates. Likewise, ST288 was most frequent in the clinical isolates, but only found once in Finkelstein's collection. These findings suggest that B. pseudomallei populations in southern Thailand are so much diverse, and only subsets of known environmental strains were found in human cases nowadays. Genetic analysis with real time PCR determined that flagella and LPS genotypes of these clinical isolates were consistent with those characteristics of Southeast Asia. Interestingly, 6 patients were confirmed to be infected by multiple STs, suggesting a high genetic diversity of B. pseudomallei in the natural sources of infection. Our analysis found that the incidence of melioidosis in southern Thailand rose in November – December during rainy season. We suspect these infections are associated with the weather patterns unique to the Malay Peninsula. Our current findings suggest that continued implementation of the "One Health" approach to B. pseudomallei epidemiology in southern Thailand would be integral for assessing the threat of B. pseudomallei in Thailand and Southeast Asia.

96. GENOMIC COMPARISON REVEALS NATURAL OCCURRENCE OF CLINICALLY RELEVANT MULTI-DRUG RESISTANT EXTENDED-SPECTRUM B-LACTAMASE PRODUCING ESCHERICHIA COLI

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The effectiveness of antibiotics has been challenged by increasing frequency of antimicrobial resistance (AMR), which has emerged as a major threat to global health. Despite its negative impact on the development of AMR, there are few effective strategies for reducing AMR in food-producing animals. Using whole genome sequencing and comparative genomics of 36 multi-drug resistant (MDR) Escherichia coli, isolated from beef cattle with no previous exposure to antibiotics, we provide evidence that occurrence of MDR E. coli arises in the environment as well as selection by antibiotics. Extended-spectrum β-lactamase producing E. coli with enhanced virulence capacities for toxin production and adherence have evolved while implicating important ramifications among animal and human health. Gene exchanges by conjugative plasmids and insertion elements have driven widespread antibiotic resistance in

clinically relevant pathogens. Phylogenetic relatedness of E. coli strains from various geographic locations and hosts, such as animals, environments, and humans suggests that transmission of MDR E. coli occurs intercontinentally without host barrier.

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

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Vibrio cholerae is the causative agent of the disease cholera. This bacterium is ubiquitous in aquatic environments and toxigenic V. cholerae O1 serves as a source for recurrent cholera epidemics around the globe. In January 2010, a massive earthquake struck Haiti, causing severe damage to the public health infrastructure. The following October, cholera appeared in Haiti for the first time in over

150 years. Previous studies proved that the original cases of cholera are consistent with a clonal, single-source introduction of V. cholerae O1 from Nepalese U.N. peacekeeping troops sent to Haiti after the earthquake. After the initial epidemic waves, cholera may now be endemic in Haiti, showing outbreak patterns associated with the rainy season. This single-source introduction of V. cholerae presents a unique opportunity to study the evolution and selective pressures acting on this microorganism. Since the start of the cholera outbreaks in 2010, the dominate serotype of V. cholerae circulating in Haiti was the Ogawa serotype. In 2015, the Inaba serotype became the dominant serotype in Haiti, surpassing the number of cases caused from original Ogawa serotype. The driver producing the switch from the Ogawa to Inaba serotype is a nucleotide substitution in the wbeT gene. Though the switch from Ogawa to the Inaba serotype is a common phenomenon in the V. cholerae genome, if the Ogawa serotype is dominant in the population and an outbreak of the Inaba serotype occurs, this is typically caused by a separate introduction into the population. By performing phylodynamic analysis with genome-wide single nucleotide polymorphisms (SNP), we are able to investigate the ongoing cholera epidemic in Haiti and the underlying evolutionary processes and selective pressures at a remarkable resolution. By using genome-wide SNPs to perform our analysis, we are able to assess potential evolutionary changes that occur in the V. cholerae genome to generate this switch in serotype. Our results propose that the V. cholerae O1 strains circulating in Haiti have evolved from their initial clonal, single-source introduction of the Ogawa serotype to the new, unintroduced Inaba serotype. Outbreaks of different serotypes are usually indicative of a separate introduction, but our results promote that the V. cholerae O1 Inaba serotype circulating after 2015 in Haiti is the outcome of the initial Ogawa serotype evolving to the Inaba serotype. This suggests that Haiti is potentially becoming a source for V. cholerae in the Caribbean and could lead to cholera outbreaks throughout the region.

98. HOST-TARGETED APPROACH AGAINST MDR GRAM-NEGATIVE BACTERIA

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Targeting bacteria with conventional antibiotics eventually leads to the emergence of antibiotic resistance. In addition, development of novel antibiotics has almost completely ceased. With increasing antibiotic resistance and limited treatment options, bacterial infections are once again becoming untreatable, leaving a disastrous socio-economic footprint on humans. The high-priority bacteria known as the ESKAPE pathogens: Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species, are the leading cause of death from nosocomial infections, claiming 23,000 lives in the United States each year. It is estimated that by 2050 this number will increase tenfold. The ESKAPE pathogens are considered to be extracellular and are cleared by the host phagocytic immune cells. Therefore, these pathogens do end up in the intracellular environment of various phagocytic cells, including macrophages, dendritic cells, and neutrophils. While the immune system is able to clear these pathogens, its ability to do so is reduced in immunocompromised individuals. Our current understanding of the mechanisms involved in the detection and removal of these bacteria in vivo is limited. With the increase of antibiotic resistance and limited availability of effective antibiotics, the development of new antimicrobial approaches is crucial to maintain human and animal health and prevent further spread of antibiotic resistance. As such, we have been adapting a host-targeted approach by engineering immune cells to: (1) enhance bacterial uptake, (2) inhibit

bacterial growth inside of the host, and (3) enhance the ability of the host cell to kill bacteria. We have demonstrated a proof-of-concept of this approach using various infection models.

99. IDENTIFICATION AND BIOCHEMICAL CHARACTERIZATION OF A DUAL FUNCTIONALITY ENZYME INVOLVED IN PENTA-PEPTIDE SYNTHESIS OF PEPTIDOGLYCAN IN CHLAMYDIA TRACHOMATIS

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Peptidoglycan is a sugar based polymer that is unique to bacteria and the enzymes that synthesize it have been attractive targets for antibiotic development. Chlamydia trachomatis, which causes infectious blindness and sexually transmitted bacterial infections worldwide, possesses a thin layer of peptidoglycan that is cross linked by penta-peptides. While most peptidoglycan synthesizing enzymes are present in the members of Chlamydiae, several enzymes remain undiscovered. One of the crucial enzymes, glutamate racemase (MurI), is involved in synthesizing D-glutamate and has not been annotated in the genome. Thus, our knowledge of how C. trachomatis synthesizes the penta-peptide of the peptidoglycan is incomplete. This remains an obstacle to designing inhibitors to prevent crosslinking in peptidoglycan leading to inhibition of Chlamydia infections. We have determined that a chlamydial enzyme, diaminopimelate epimerase (DapFCt) complements an Escherichia coli murI mutant as well as a murI dapF E. coli double mutant. These results support an in vivo dual functionality of DapFCt as both a glutamate racemase and a Dap

epimerase. We confirmed efficient glutamate racemization activity in an in vitro coupled assay using highly purified DapFCt. Unlike, the majority of glutamate racemases in other organisms, DapFCt required pyridoxal 5'-phosphate (PLP) as a cofactor. Since DapFCt exhibited the dual function of catalyzing two different substrates, we tested these substrates in a competition assay. Racemization of D-glutamate to L-glutamate by DapFCt in the presence of equimolar concentrations of LL-DAP decreased by 50%, suggesting competition for the same catalytic site. This is the first report that demonstrates that DapFCt epimerase also functions as a glutamate racemase (MurI) suggesting its broad substrate specificity. These results suggest that disturbing the concentration balance between competing substrates can lead to inhibition of synthesis of one of the critical components of Chlamydia peptidoglycan penta-peptide. Furthermore, a unique inhibitor of DapFCt would simultaneously shut down epimerization and racemization reactions leading to the absence of cross linking in Chlamydia peptidoglycan and arrest of bacterial growth.

100. IDENTIFICATION AND GENOMIC SURVEY OF GENE TRANSFER AGENTS IN PLANT PATHOGENIC BACTERIA

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Gene Transfer Agents (GTAs) are phage-like mediators of gene transfer between bacterial cells and were first discovered in the bacterium Rhodobacter capsulatus in the 1970s. Typically, bacteria with GTAs show high recombination compared with strains of the same species without GTAs. Since their first discovery, gene transfer agents have been reported in several alphaproteobacteria. In a previous study, we identified evidence of large-scale genomic recombination in a Nigerian lineage of Xanthomonas perforans compared to other worldwide strains. Comparison of the mobilome identified a unique GTA cluster which was absent in other X. perforans strains. The GTA cluster bears the hallmark of a defective prophage locus which could potentially contribute to the high recombination events previously observed. We further surveyed the genomes of major plant pathogenic bacterial species for the presence of a GTA locus. Recombination analyses were carried out on genomes of strains with GTAs and compared to strains of the same species without GTA. We identified GTA clusters in other plant pathogenic bacteria, including several Xanthomonas species with similar GTA clusters to the Nigerian X. perforans strains. Additionally, we inferred more recombination events in strains with GTA than strains without GTA. Our findings show that plant pathogenic gammaproteobacteria, among other plant pathogens, and contain GTA clusters and provide additional insights into mechanisms of evolution in these pathogens.

101. IDENTIFICATION OF A NOVEL ACTINOMYCES SP. IN GRANULOMATOUS CLOACAL AND HEMIPENAL INFLAMMATION IN CAPTIVE MALE BALL PYTHONS (PYTHON REGIUS)

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Ball pythons (Python regius) are one of the most commonly kept and bred reptiles in captivity. In a large ball python breeding colony, a unique syndrome characterized by granulomatous inflammation of the cloaca and phalli (hemipenes) was observed in 139 of 481 (29%) breeding males, but absent in virgin males (n=201). In contrast, only 1 out of 1446 breeding females and no virgin females (n=293) exhibited similar cloacal lesions. On postmortem examination (n=13, 12 males, 1 female), there were numerous pericloacal, hemipenal, and pericoelomic granulomas; fewer animals exhibited intracoelomic and/or intrahepatic granulomas. Histopathologic examination revealed chronic granulomatous inflammation of the affected tissues associated with mixed morphology bacteria. Aerobic culture of a hepatic granuloma of one snake resulted in the isolation of a non-filamentous, fastidious Gram positive bacilli; amplification and sequencing of the 16S gene and subsequent phylogenetic analysis of the isolate identified the bacterium as a novel species of Actinomyces. A specific, heminested PCR targeting a 16S gene of the bacterium confirmed the presence of the agent in granulomas of all 13 snakes, as well as cloacal swabs taken at the time of necropsy in 11/13 snakes. To determine if the bacterium was present in unaffected snakes, cloacal swabs (n=74) of breeding and virgin males and females of the affected colony and two unrelated, grossly unaffected breeding colonies were screened by PCR. The Actinomyces sp. was identified in swabs from all three colonies, and prevalence rates in all colonies in breeding snakes were similar, with

86% of males (n=22) and 57% of breeder females (n=23) testing PCR positive. However, in virgin snakes, while 18% (4/22) females were PCR positive, no virgin males (n=20) were positive for the bacterium by PCR. This study characterizes a novel disease syndrome of breeding male ball pythons associated with a novel Actinomyces. While the bacterium can be detected in both breeding and virgin females, it was only very rarely associated with clinical disease. Together this data suggests a role for the bacterium in the disease syndrome observed in captive male ball pythons and the potential for sexual transmission of the pathogen.

102. IRON DEPRIVATION INHIBITS REPLICATION OF CHLAMYDIA TRACHOMATIS BY PREVENTING ISOPRENOID SYNTHESIS

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The essential trace metal, iron, is required for normal development of the obligate intracellular, sexually transmitted pathogen, Chlamydia trachomatis. C. trachomatis development alternates between the extracellular, infectious elementary body, and the intracellular, replicative form called the reticulate body. In Chlamydia, several conditions including iron deprivation halt chlamydial cell division and cause the development of a morphologically enlarged, but viable form known as an aberrant body (AB). Together, these phenotypes constitute the chlamydial developmental state known as “persistence”. The methylerythritol phosphate (MEP) pathway of isoprenoid synthesis (IPS) is absent in

higher eukaryotes and can be specifically inhibited in parasites and bacteria using the antibiotic fosmidomycin (FSM). FSM targets the MEP pathway by inhibiting the essential enzyme, 1-deoxy-D-xylulose 5-phosphate reductoisomerase (Dxr). Bioinformatic analysis indicates that C. trachomatis encodes Dxr, but its function and the requirement for IPS in chlamydial development is unknown. We utilized FSM as a tool to test the hypothesis that normal chlamydial development requires isoprenoid synthesis via the MEP pathway, expecting MEP pathway inhibition to be lethal in C. trachomatis. Overexpression of chlamydial Dxr (DxrCT) in Escherichia coli under FSM exposure and in a conditionally lethal Dxr mutant demonstrates that DxrCT functions similarly to E. coli Dxr. When Chlamydia-infected cultures were exposed to FSM, production of infectious progeny was significantly reduced. However, titer recovery assays, electron microscopy, and peptidoglycan labeling revealed that FSM inhibition of IPS is not lethal to C. trachomatis, but instead induces persistence and prevents normal peptidoglycan ring formation. These results indicate that the inhibition of isoprenoid synthesis prevents availability of key isoprenoid, bactoprenol, which is essential for peptidoglycan synthesis and, ultimately, cell division. Furthermore, the MEP pathway requires the activity of two iron-sulfur cluster-containing enzymes downstream of Dxr. We propose that iron deprivation, like FSM exposure, inhibits isoprenoid synthesis, resulting in persistence.

103. MELIOIDOSIS IN INTENSIVE PIG FARMING SYSTEMS IN SOUTHERN THAILAND

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Melioidosis, a severe tropical disease, is endemic in Southeast Asia and northern Australia. It is caused by Burkholderia pseudomallei, a facultative intracellular Gram-negative bacterium that occurs naturally in water and soils. Melioidosis has been reported in various animal species including pets, livestock and wildlife. Melioidosis in pig is frequently asymptomatic, often with lesions detected during routine abattoir inspection. In 2018, there were 5 incidences of melioidosis in finishing pigs in Nakhon Si Thammarat, a southern province of Thailand. Lungs, liver, and spleen with multiple abscesses of the infected pigs were found in the slaughter house. Cultures of pig tissues, soil and water supply samples in the pig farms grew B. pseudomallei. The preliminary study has also identified that 2 out of 38 healthy sows were seropositive by indirect hemagglutination (IHA) test for melioidosis (a cutoff value of

≥1:320). In present study, we aim to investigate the apparent prevalence of melioidosis in finishing pigs in southern Thailand providing the estimated risk of melioidosis in pig farms. Serum samples and visceral organs including lungs, livers and spleens were collected from 131 pigs (25 weeks old, various breeds) in the slaughter house. Gross pathology found moderate to severe pneumonia with normal spleens and livers in 15 pigs, 3 of which (20%) were cultured positive for B. pseudomallei. All bacterial isolates were species confirmed by PCR using TTS1 assay, and further classified into YLF genomic group that is common in Southeast Asia. Multi-locus sequence typing (MLST) has shown that all three cases were infected by sequence type (ST) 51, which is frequently found in water reservoir in southern Thailand. There were no seropositive melioidosis in finishing pigs by IHA (titers were range between 1:20-1:160). Histology of H&E stained lung tissue sections revealed patchy interstitial pneumonia. All inflamed tissues of melioidosis from previous and current studies were stained by immunohistochemistry technique. The positive staining has shown the presence of B. pseudomallei in the cytoplasm of phagocytes. Conversely, not all samples from proven cases of melioidosis were stained positive. B. pseudomallei is pathogenic to humans and animals. Pigs show increased resistance to B. pseudomallei infection but are unable to eradicate the infection which is mimic to chronic infection in humans. Using only IHA as a single tool for melioidosis diagnosis may not be useful because it lacks a standard antigen preparation. Our study showed the estimated risk of melioidosis and the zoonotic potential of B. pseudomallei in intensive pig farming in endemic area.

104. OCCURRENCE AND GENOMIC CHARACTERISTICS OF CEFOTAXIME RESISTANT BACTERIA IN WILDLIFE

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Wildlife are important but poorly-studied carriers and sources of antimicrobial resistance bacteria (ARB). Their behaviors can accelerate the dissemination of antibiotic resistance by interacting with livestock, humans, or the environment. However, the transmission of ARBs among livestock, wildlife, and environments is largely unknown. To understand ARB's transmission mechanisms, we characterized ARBs isolated from different niches. We isolated cefotaxime resistant bacteria and extended-spectrum β-lactamase (ESBL)-producing bacteria from cattle (n=48), feral swine (n=52), coyote (n=3), soil (n=6), and water (n=5). The prevalence of ESBL-producing bacteria in cattle, swine, coyote, soil, and water samples was 0.0%, 3.8%, 66.6%, 33.3%, and 20%, respectively. By whole genome sequencing and phylogenetic analysis, we found ESBL-producing E. coli from different sources were unlikely transmitted among each other as isolated ESBL-producing E. coli from different animals contained distinct genomes, with high number of single nucleotide polymorphisms (SNPs). In addition, plasmid types and ESBL genotypes from feral swine were different to coyote isolates, suggesting antimicrobial resistance against extended-spectrum β-lactams were not mediated by common plasmid. Potentially pathogenic Pseudomonas spp., which have diverse virulence factors including toxins and bacterial effector secretion systems, were identified from a coyote. Furthermore, ESBL-producing E. coli isolated from a feral swine and a coyote had same sequencing type 155 and 398 with human clinical strains, suggesting clinically relevant

pathogens are prevalent in wildlife and they may transmit to livestock and humans.

105. PROPERTIES AND FUNCTIONS OF NLPD1 AND NLPD2 IN BURKHOLDERIA PSEUDOMALLEI

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Burkholderia pseudomallei (Bp) is the causative agent of melioidosis, an infectious disease endemic to tropical and sub-tropical regions of the world. Challenges in effective therapy include the scarcity of antibiotics available for therapy due to the bacterium's intrinsic drug resistance and the lack of information available about the nature of the mechanisms that govern Bp's antibiotic resistance. Our lab has documented acquired resistance to ceftazidime, a widely used β-lactam antibiotic, caused by constitutive expression of PenA β-lactamase due to a conserved promoter-up mutation. The gene penA is co-transcribed with the upstream nlpD1 gene. The transcriptional terminator for the gene nlpD1 serves as a penA attenuator and generation of a new promoter immediately upstream of the terminator/attenuator by a conserved G to A transition leads to anti-termination and thus constitutive PenA expression and extended β-lactam resistance. Here we present a summary of progress made by us on identification and characterization of NlpD1 and NlpD2, a second NlpD encoded by Bp. Like penA, nlpD1 and nlpD2 contain a lipobox with a conserved cysteine and an alanine at the +2 position, indicating that they are lipoproteins that localize to the outer membrane. We confirmed outer membrane localization for NlpD1. Similar to E. coli NlpD, the NlpD1 and NlpD2 proteins contain a lysine motif (LysM) that is common in cell envelope-associated proteins and involved in peptidoglycan-binding activity and a degenerate LytM (dLytM) domain that is required for the protein's cell wall

hydrolytic amidase activating activity. Together, this indicates that NlpD1 and NlpD2 are potential activators of periplasmic amidases involved in cleavage of the septal peptidoglycan during daughter cell separation and may facilitate PenA localization to the outer membrane and potentially outer membrane vesicles.

106. RECENT STATE-WIDE SURVEY REVEALS CONTINUED SHIFTS IN XANTHOMONAS POPULATIONS IN FLORIDA COMMERCIAL TOMATO FIELDS

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Bacterial spot of tomato, caused by Xanthomonas perforans, is the most economically important bacterial disease for the Florida tomato industry. Under favorable environmental conditions, this pathogen can reduce yield through defoliation and the infection of fruit. Currently, control measures available to growers are ineffective. Prior studies documented dramatic shifts in the Xanthomonas population associated with Florida tomato production over the past 30 years despite the lack of a clear selective pressure. These population shifts can adversely impact the efficacy of control measures and resistance breeding efforts in tomato. Our goal is to

monitor the Xanthomonas population in Florida and examine the effect of variety, grower, transplant facility, and growing region on genetic variation within and among fields. We collected 585 Xanthomonas isolates from 70 commercial tomato fields across Florida tomato production areas during the fall 2017 growing season. We characterized each strain to identify its species and phylogenetic group, pathogen race, resistance to streptomycin and copper, production of antibiotics, and presence/absence of effectors that impact host range. Initial results indicate that Florida Xanthomonas populations are indeed continuing to shift, including a loss of effectors that limit host range and an increase in the frequency of streptomycin resistant strains. We will compare experimental results of strains with tomato field data (i.e. grower, variety, transplant facility, seed producer, and growing region) to determine whether there are associations between genetic variation and tomato production chain variables.

107. SINGLE NUCLEOTIDE POLYMORPHISM (SNP) ASSAY FOR THE IDENTIFICATION OF BACILLUS ANTHRACIS LINEAGES THAT LACK ANTHROSE, A SPORE SURFACE-ASSOCIATED OLIGOSACCHARIDE.

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The exosporium of Bacillus anthracis endospores exhibits a tetrasaccharide composed of three rhamnose residues and an unusual sugar termed anthrose. Anthrose has been proposed as potential target for immunotherapy and for specific detection of B. anthracis. Although originally thought to be ubiquitous across B. anthracis lineages, previous work has identified a western African lineage isolated from cattle that does not produce anthrose and could represent a vaccine escape mutant. These strains carry the genes required for expression of the anthrose operon but premature

stop codons resulting from an 8-bp insertion in BAS3320 (amino-transferase) and a SNP in BAS3321 (glycosyltransferase) prevent anthrose production. Various other SNPs have been identified throughout the operon and could be the basis for detection of anthrose-deficient strains. Here, we evaluate two such SNPs exhibiting nonsynonymous substitutions when compared to the anthrose-producing Sterne strain. A rhAmp genotyping assay (IDT) was used to interrogate SNPs at positions 892 and 1352 of the BAS3321 gene of the anthrose operon. The rhAmp technology uses blocked primers to prevent extension and minimize non-specific amplification. Extension is conditioned upon cleavage and removal of the blocking group by RNase H2, which itself requires binding of primer to its perfect complement. The two SNP assays were initially tested in triplicate with 5 ng of DNA from both anthrose-positive and negative as recommended by manufacturer. As expected, B. anthracis Sterne produced strong amplification with primers specific for the anthrose-positive alleles, whereas a Nigerian strain produced strong amplification with the primers specific for the anthrose-negative alleles. Ten-fold serial dilutions of DNA from both B. anthracis Sterne and the Nigerian strain were then used to establish the limit of detection of both SNP assays. Both assays were able to consistently detect DNA as low as 100 fg, with infrequent amplification at 10 fg of DNA. Additionally, the QuantStudio 7 software correctly genotyped strains using the SNP 892 assay with at least 100 fg of DNA, whereas the 1352 SNP was only able to discriminate genotypes at or above 1 pg of DNA. This work illustrates the development of two anthrose SNP assays useful in discriminating western African strains from other lineages and in the detection of local B. anthracis strains in western African countries. Additionally, these SNPs may provide a further tool to differentiate Bacillus cereus biovar anthracis strains, also reported in western Africa, from regional B. anthracis, and which display anthrose 892 and 1352 Sterne-like alleles.

108. SPATIAL, SOCIAL, AND MOLECULAR DETERMINANTS OF COMMUNITY-ASSOCIATED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS INFECTIONS AND COLONIZATIONS: AN EMERGENCY DEPARTMENT SERIAL CROSS-SECTIONAL STUDY

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Background: National incidence of community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) is increasing, with three-quarters of CA-MRSA emergency department (ED) cases present as skin or soft tissue infection (SSTI). To understand community-oriented transmission pathways, we investigated spatial, social, and molecular patterns of CA-MRSA SSTI and nasal colonization in patients presenting to the ED.

Methods: From August 2015 – January 2017, pediatric and adult patients presenting to UF Health Shands Emergency Department (Gainesville, FL) with an acute SSTI were prospectively enrolled in a serial cross-sectional study. Nasal and SSTI site specimens were collected and cultured for spa-typing. Geographic, social, and medical epidemiological data was collected via self-reported survey. Patient self-reported home residence was geocoded and spatially joined to corresponding census tracts using ArcGIS. Multivariate analyses was used to test associations of geographic, medical, and social determinants with microbiological and molecular results.

Results: 182 subjects were included, representing 24 counties and 106 unique census tracts. 31 (17.0%) subjects reported living in rural census tracts. 53 (29.1%) subjects had MRSA positive SSTI and 25 (13.7%) had MRSA positive nasal cultures. After adjusting for

demographics, subjects with nasal colonization of MRSA were 34.9 (95CI: 9.5 – 128.7, p=0.0001) times more likely to have positive MRSA SSTI compared to subjects with negative nasal colonization. Subjects with nasal colonization of MSSA were 4.2 (95CI: 1.4 – 13.0, p=0.013) times more likely to have positive MSSA SSTI. MRSA SSTI was associated with recent exposure to livestock (p=0.006) while nasal colonization of MRSA was associated with current alcohol consumption (p=0.027) and current history of smoking (p=0.009). Subjects residing in rural census tracts were 2.4 (95CI: 1.1 – 5.2, p=0.049) times more likely to have MRSA positive wound cultures. Among 46 (86.8%) MRSA SSTI isolates selected for spa-typing, 10 unique spa-types were identified where 75% (n=35) of all isolates were spa-type t008. MRSA SSTI subjects with non-t008 strains were 23.3 (95CI: 2.2 – 246.2, p=0.005) times more likely to report recent livestock exposure compared to MRSA SSTI subjects with t008 strains.

Conclusions: The geographic distribution of CA-MRSA is non-uniform and has a higher prevalence in more rural areas and in those with livestock exposure, highlighting a possible zoonotic association in CA-MRSA transmission. Associations with nasal colonization and social determinants provide insight to potential therapies to reduce CA-MRSA infections. Future research will assess the interactions of neighborhood-level risk factors on CA-MRSA infections as well as temporal transmission patterns across rural-urban-hospital settings using genome-wide SNP analysis.

109. THE IMPACT OF CHANGES TO INFLUENZA VACCINE RECOMMENDATIONS ON SCHOOL-LOCATED INFLUENZA VACCINATION PROGRAMS

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Background: Influenza vaccination is the best way to protect individuals and communities from yearly seasonal influenza outbreaks. School-located Influenza vaccination (SLIV) programs increase vaccination in school-aged children and promote community immunity. Healthy children over 2 years may receive inactivated (IIV) or live attenuated (LAIV) influenza vaccine. For the 2016-17 and 2017-18 seasons LAIV was not recommended due to low effectiveness against the influenza A H1N1 strain. For the 2018-19 season the CDC reapproved LAIV for use citing no preference for either vaccine but the American Academy of Pediatrics (AAP) stated a preference for IIV.

Methods: Data were collected from 2 comprehensive SLIV programs 1) Alachua County, Florida and 2) Maryland school system. Data prior to the 2016-18 seasons using only LAIV were compared to the 2 seasons using only IIV and to the current 2018-19 season again using LAIV.

Results: In Alachua County, 13,909 doses of LAIV were given in 2014-15 and 14,531 in 2015-16 with immunization rates reaching 49% in elementary, 44% middle and 31% in high schools in 2015-16. During the 2 seasons using only IIV, 8,083 doses were given in 2016-17 (44.4% decline from 2015-16) and 8,009 in 2017-18 (45% decline from 2015-16). Immunization rates fell to 24% in elementary, 32% middle and 23% in high schools in 2016-17 and increased slightly to 31% elementary, 33% middle but fell to 21% for high schools in 2017-18. The number of schools participating fell from a high of 70 in 2015-16 to 63 in 2016-17 and 61 in 2017-2018. For the 2018-19 season with LAIV used in Alachua County 8,819 doses were given: a

9.2% increase over the prior year. In Maryland, 96,012 doses of LAIV were given in 2014-15 and 58,672 in 2015-16. The drop in 2015-16 was related to a delay in arrival of LAIV. In 2016-17 28,344 doses of IIV were given (70.5% decrease from 2014-15) and in 2017-18 44,270 doses (54% decline from 2014-2015). In 2014-15 680 schools participated, in 2015-2016 570 participated, in 2016-17 only 331 participated and in 2017-18 439. Data are pending for the 2018-19 season.

Conclusion: Changes in influenza vaccine recommendations can have a significant negative impact on SLIV programs and differing recommendations between the CDC and AAP, may further impact the programs. Lower immunization rates in schools could lead to more influenza infections in both schools and communities.

110. A CITRUS TRISTEZA VIRUS-ENCODED AUXILIARY PROTEIN ENHANCES ACTIVITY OF TWO VIRAL SUPPRESSORS OF HOST RNA SILENCING

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Citrus tristeza virus (CTV) is known for its long RNA genome and for the three virus-encoded silencing suppressors (VSRs): p20, p23, and CP. A VSR is a protein used by the virus to counteract the host RNA silencing (RNA interference, RNAi), one of the main anti-viral defense mechanisms in plants. Recently, understanding the mechanisms of the three VSRs of CTV has become important in management of the CTV-induced diseases. In this work, an auxiliary protein of CTV, p33, was shown to interact with all three VSRs. The effect of p33 on the RNA silencing suppression by these VSRs was examined by using transgenic Nicotiana benthamiana plants (line 16C) constitutively expressing the green fluorescent protein (GFP). The constructs directing ectopic expression of the GFP, p20, p23, CP, and p33

proteins in different combinations were co-agroinfiltrated in 16C plants. Evaluation of GFP expression using observations of GFP fluorescence as well as Western and Northern blot analyses demonstrated that: 1) p20 and p23, but not CP, are able to suppress the RNA silencing at the local level, 2) all three VSRs are not efficient in suppressing the long-distance RNAi, and 3) while p33 alone does not show VSR's activity, its co-expression with other viral VSRs significantly enhances the ability of p20 and p23, but not that of CP, to suppress the long-distance spread of the RNA silencing signal. To our knowledge this is the first case that describes the effect of a non-VSR protein on the activity of other viral proteins possessing the VSR activity.

111. A NOVEL MAMMALIAN ORTHOREOVIRUS TYPE 2 ISOLATED FROM A DEAD WHITE-TAILED DEER IN FLORIDA

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The family Reoviridae is a diverse group of viruses with segmented double-stranded RNA genomes enclosed within a multi-layered icosahedral capsid. Mammalian orthoreovirus (MRV) is the type species of the genus Orthoreovirus and it causes a range of respiratory or enteric diseases of importance in human and veterinary medicine. In 2017, a farmed white-tailed deer (Odocoileus

virginianus) fawn became ill displaying lethargy, dehydration, and profuse foul smelling diarrhea. A necropsy was performed after the three-week-old fawn succumbed and samples were submitted to the University of Florida's Cervidae Health Research Initiative for diagnostic evaluation. Aliquots of homogenized spleen were inoculated onto Vero E6 (Cercopithecus aethiops [African green monkey] kidney, ATCC CRL 1586) cells grown as monolayers at 37°C. The Vero culture displayed cytopathic effects (CPE) and was submitted for transmission electron microscopic evaluation. Numerous icosahedral viral particles (approximately 75nm in diameter) were observed within the cytoplasm of Vero cells. RNA was extracted from Vero cells displaying CPE using a QIAamp viral RNA minikit (Qiagen). A cDNA library was prepared using a NEBNext Ultra RNA library prep kit and sequenced using a V3 chemistry 600-cycle kit on a MiSeq platform (Illumina). De novo assembly of the paired-end reads in SPAdes followed by BLASTX searches of the assembled contigs against a proprietary viral database in CLC Genomic Workbench recovered all 10 segments that displayed highest nucleotide identities to MRV type 2 (MRV-2). A Maximum Likelihood (ML) analysis based on the outer capsid protein sigma-1 supported the Florida white-tailed fawn isolate as a strain of MRV-2 branching as the sister group to a MRV-2 strain isolated from a moribund lion in Japan. To our knowledge, this is the first occurrence of MRV-2 infection in a white-tailed deer. Continued surveillance efforts are needed to determine the threat of this MRV-2 strain may pose to health of farmed white-tailed deer populations.

112. A ROLE OF A NONCODING SUBGENOMIC RNA OF CITRUS TRISTEZA VIRUS IN THE MODULATION OF HOST IMMUNITY

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Upon host infection, viruses produce different RNA species. Not to mention mRNAs for translation of their proteins, many viruses also generate noncoding RNAs (ncRNAs) of various sizes. Compared to the functions of viral small ncRNAs such as small interfering RNAs and microRNAs, the roles of viral long ncRNAs (lncRNAs; RNAs over 150 nts in length) remain to be investigated more. Yet, various functions of a number of viral lncRNAs have been identified, including regulation of host and viral gene expression, virion production, and counteraction of the host antiviral response. During infection, Citrus tristeza virus (CTV) produces a noncoding 5'-terminal subgenomic RNA (sgRNA) of ~750 nucleotides in size referred to as low-molecular-weight tristeza 1 (LMT1). Although LMT1 RNA accumulates in a large amount in the virus-infected cells, it has been considered as a by-product of the complex CTV replication machinery as no specific function of LMT1 has been identified. The LMT1 sgRNA does not have an open reading frame and appears to be a lncRNA of CTV. In this study, we investigated the role of LMT1 in the virus infection cycle using a CTV variant (CTV-LMT1d) that was modified not to produce LMT1 without affecting the generation of other sgRNAs nor the formation of normal virions. However, lack of the LMT1 production did affect the virus amplification: CTV-LMT1d did not accumulate as well as the wild-type virus in the initially inoculated cells of Nicotiana benthamiana.

Also, CTV-LMT1d demonstrated a significant decrease in the invasiveness and systemic spread in N. benthamiana. Moreover, we found a notable difference in the salicylic acid (SA)-mediated host immune responses to the wild type and the CTV-LMT1d viruses, which suggested that LMT1 plays a role in counter-acting host defenses. This was further confirmed by the in planta expression of the LMT1 RNA by itself and observation of the induced changes in the SA-mediated host immune response. Finally, CTV-LMT1d was shown unable to infect citrus plants, indicating that the LMT1 RNA is indispensable for the CTV infection of its natural host.

113. AFRICAN SWINE FEVER IN EUROPE - A TIME BOMB FOR THE EU?

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The authors present an overview of the current outbreaks of ASF in Europe and focus their attention on the implications of massive outbreaks in the European Union. African swine fever (ASF) is threatening the heart of Europe. It has recently emerged in 39 boars in the Southeast of the country, not far from the border with France and Luxembourg. African swine fever is a devastating hemorrhagic fever of pigs with mortality rates approaching 100 percent. It causes major economic losses, threatens food security, and limits pig production in affected countries. ASF is caused by a large DNA virus, African swine fever virus (ASFV). There is no vaccine against ASFV and this limits the options for disease control. ASF has been confined mainly to sub-Saharan Africa, where it is maintained in a sylvatic

cycle among domestic pigs. Wildlife hosts include wild hogs and arthropod vectors. The huge costs associated with the management of a potential massive African Swine Fever outbreak in the EU are not only those directly associated with the death (and necessary slaughtering) of infected pigs, but also the indirect costs related to freezes in exports, production, and indemnities, along with the depopulation of swine. Along with commercial challenges, there are a series of other serious and potentially explosive problems that should not be underestimated: the political issues associated with the slaughtering of clinically healthy, but potentially infected animals; as well as the practical difficulties surrounding the disposal of carcasses; the management of animal waste; and the sanitization of swine. The inevitable disruption which will be linked to the occurrence of major ASF outbreaks in Europe could result in tensions between Member States themselves and between member States and the Commission for reasons including but not limited to trade, export, animal welfare, disease control policies and ethics of animal farming. Animal and plant disease with potential devastating implications continue to threaten health and agriculture worldwide. However, their impact may influence relationships between countries and their trading partners and stakeholders and this is an aspect that should not be underestimated.

114. CHARACTERIZATION OF A NOVEL CIRCOVIRUS FROM A STRANDED LONGMAN'S BEAKED WHALE (INDOPACETUS PACIFICUS)

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Tissues from a juvenile Longman's beaked whale that stranded in Hawaii in 2010 were screened for viruses using a Next-Generation Sequencing (NGS) approach. From the NGS data, the full genome (1,849 bp) of a novel beaked whale circovirus (BWCV) was determined. Two open reading frames (ORF) were annotated, including ORF1 that encodes the capsid gene, ORF2 that encodes the replication-associated gene, and a 9 bp long conserved nonamer on the apex of the open loop found in all circoviruses. Independent phylogenetic analyses based on amino acid sequence alignments of the two CV proteins supported the BWCV as a member of the genus Circovirus, branching as the sister species to the recently discovered canine circovirus. A sequence identity matrix generated from complete genome alignments revealed the BWCV displays between 50.9-56.7% nucleotide identity to other circoviruses, which is lower than the 80% threshold proposed for species demarcation. Considering the genetic and phylogenetic analyses, we propose the formal species designation of beaked whale circovirus to be considered for approval by the International Committee on Taxonomy of Viruses. An endpoint PCR assay targeting the BWCV genome confirmed the presence of the BWCV DNA in every tissue from which DNA was extracted, including spleen, muscle, left ventricle, left adrenal gland, liver, lung, cerebrum, cerebellum, and

lymph node. An in situ hybridization (ISH) assay utilizing RNAscope® technology and targeting the BWCV genome was optimized then used to test for the presence of BWCV nucleic acid in the following tissue sections: diaphragm, liver, lymph nodes (unspecified as to anatomic location), lung, pericardium, oral mucosa and tongue, adrenal gland, testis, aorta, intestine, stomach, spleen, heart, and skeletal muscle. Labeling by the BWCV probe was observed in all tissues, with the exception of two which could not be interpreted (skeletal muscle and spleen). In areas of lymph nodes, there was labeling within individual lymphocytes and monocytes. A consistent feature between tissues was labeling of cells of blood vessels. The presence of BWCV could represent an opportunistic infection without clinical significance, or it could have contributed to that finding in this stranded juvenile Longman's beaked whale. Future research is needed to determine the host range, prevalence, and pathogenicity of the BWCV in cetaceans of Hawaiian waters and elsewhere.

115. CHARACTERIZATION OF A NOVEL ORTHOREOVIRUS ISOLATED FROM DEAD STRANDED HARBOR SEALS IN WASHINGTON STATE, USA

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From October 2007 to July 2008, 24 harbor seals (Phoca vitulina) stranded dead along the shores of Puget Sound, Washington State, USA. The carcasses were necropsied and selected samples were processed for histopathology and ancillary diagnostic testing including virus isolation. Isolates displaying identical cytopathic effects (CPE) in Vero.DogSLAMtag cells were recovered from brain, lymph node, lung, spleen, heart, skin, kidney, and abdominal cavity fluid of four male weaned pups and an adult male seal (5/24). Following inoculation and incubation, infected cells from the five cases became granular and developed a refractile appearance, cells began to round up, detach from the plastic substrate and coalesced into clumps, which progressed over a few days until the entire cell sheet was involved. CPE developed as early as 18 days post infection (DPI) with some flasks developing CPE as late as 33 DPI. There was no evidence of syncytium formation in the progression of the CPE. Vero.DogSLAMtag cells showing CPE were processed for transmission electron microscopy and the ultrastructural examination revealed arrays of non-enveloped, spherical virus particles (approximately 70 nm in diameter) within the cytoplasm of affected cells, consistent with members of the family Reoviridae. RNA was extracted from the same mesenteric lymph node isolate using a QIAamp Viral RNA Mini Kit and a cDNA library was generated using a NEBNext Ultra RNA Library Prep Kit for sequencing on an Illumina MiSeq sequencer. The complete coding sequences of the segmented (n=10) double-stranded RNA genome of a novel reovirus was determined. Genetic and phylogenetic analyses revealed the isolate represents a new strain of the species Mammalian orthoreovirus within the genus Orthoreovirus (hereafter referred to mammalian orthreovirus 5; MRV-5). MVR-5 represents the first mammalian orthoreovirus characterized from dead stranded harbor seals. A study conducted in 1989 reported the presence of reovirus-like virus particles were seen in a single sample from an emaciated harbor seal pup in Washington State, USA; however, the virus was not isolated. In 2005, a novel fusogenic Avian orthoreovirus (SSRV) was isolated in Vero.DogSLAMtag cells from multiple tissues of an aborted Steller sea lion (Eumetopias jubatus) fetus in British

Columbia, Canada. In the present study, gross and microscopic lesions observed in harbor seals from which MRV-5 was isolated were inconsistent, and thus, the role MRV-5 in disease (if any) could not be determined. Although orthoreoviruses are known to induce significant disease in humans and animals, their role in disease of pinnipeds is not well established. Further research is needed to determine the prevalence, pathogenicity, and host range of orthoreoviruses in pinnipeds.

116. DETECTION OF SHEDDING IN ZEBRAFISH (DANIO RERIO) FOLLOWING EXPERIMENTAL CHALLENGE WITH SPRING VIREMIA OF CARP VIRUS

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For a virus to successfully circulate within a host population, sustained transmission from infected individuals to susceptible individuals must be achieved. Specifically, virions must be shed and transmitted to susceptible hosts to continue the chain of transmission. Spring viremia of carp virus (SVCV) is a rhabdovirus that causes significant disease in both wild and farmed carp (Cyprinidae). In this study, we developed a SVCV-Zebrafish model of infection and a sensitive reverse transcription quantitative PCR (RT-qPCR) assay to test for SVCV in water. We inoculated individual zebrafish by spiking their aquaria with SVCV. Each fish was followed for 14-days post-infection, and daily behavioral and morphological observations were taken. Water was sampled from each aquarium containing a zebrafish challenged with SVCV. Each sample was then tested using RT-qPCR to determine the quantity of virus that each treated fish was shedding. This allows us to describe the timing and amount of virus shed post infection, elucidating the time to maximal shedding and the highest chance of SVCV transmission. The developed SVCV-Zebrafish model will permit future study of the

infection dynamics of this environmentally acquired lethal viral disease.

117. DYNAMIC MODELING OF HIV TRANSMISSION AMONG PWID TO ESTIMATE COMMUNITY INTERVENTIONS; RESULTS FROM A CLUSTER RANDOMIZED TRIAL ACROSS MULTIPLE SITES IN INDIA.

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People who inject drugs (PWID) are at high risk of HIV acquisition and may play a central role in ongoing transmission in some populations. Estimating the impact of suites of interventions in these populations is difficult due to stigmatization of individuals by the society, overlap of uptake of interventions and positive and negative interactions between interventions. We used the data from a clinical trial randomized in multiple locations in India conducted in collaboration by the Johns Hopkins Bloomberg School of Public Health and Y.R. Gaitonde Centre for AIDS Research and Education to build a dynamic compartment model. The machinery within the model included stratification based on the drug injection practices, HIV status and compliance with the antiretroviral regimen. Separate dynamic model was fitted for each study location in a single algorithm with some parameters shared between the location and some location-specific parameters that characterized the trial interventions. The model accounted for the initial spatial distribution of prevalence of HIV in the different trial settings as well as the change in prevalence observed within the intervention and control sites. From the trial data a baseline evaluation and two year of follow-up have been recorded from twelve randomized locations in India (six in each). We estimated the differences between the two trial arms; traditional counseling and treatments services which were segregated among multiple addresses (control) and services provided via the integrated care centers and, therefore, aggregated in one location (treatment). Using these data, the relative importance of transmission interventions (needle exchange program,

opioid replacement therapy, counseling) and treatment (antiretroviral therapy) was evaluated. Differences between intervention and control sites were observed suggesting the need for future studies and more detailed investigation due to the importance and time-sensitivity of the topic.

118. ECOLOGICAL CONSEQUENCES OF VIRAL CO-INFECTION IN DROSOPHILA MELANOGASTER

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In nature, organisms infected by multiple pathogen species or different parasite strains are the rule rather than the exception. The co-infecting parasites can interact with each other directly or indirectly via the immune system or host resources establishing interactions that span from synergistic to antagonistic potentially altering the dynamics of infections and the evolution of virulence and long-term, the host-parasite coevolution. Deep sequencing studies have revealed that insects host a vast diversity of viral species that coexist and that can be considered as “persistent” viral infections. But how do these multiple viral species interact between them and what are the consequences of these interactions in the host fitness? In this study we used Drosophila melanogaster and two of its natural viruses (DCV and DXV) as model system to investigate the effects of viral co-infection on host survival and the changes on viral dynamics inside the host. In order to mimic a natural infection, we fed adult female flies with DCV or DXV or both viruses and placed them individually in food vials. We found fruit flies die at a low rate (~ 10%) in all treatments within the first 6 days post-infection and that both single infections and co-infection, have similar effects on host survival. In addition, we detected low levels of virus in alive flies compared to the dead ones and that these levels do not change through time in all treatments. Interestingly we found that DCV

levels are higher in single infected than in co-infected flies. In contrast, DXV loads are higher in co-infected flies compared to single infected ones. Overall our results suggest that when fed, DCV and DXV establish a chronic infection either when they infect on their own or when they infect simultaneously and that both viruses interfere with each other's replication when co-infected.

119. EXPLORING HIV-RELATED STIGMA IN THE SOUTHERN UNITED STATES: A QUALITATIVE STUDY

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Background: The Southern United States (U.S.) has the highest transmission rates of HIV with a rate of 16.1 per 100,000 people, as compared to 10.6 (Northeast), 9.4 (West), and 7.4 in the Midwest, respectively. While the U.S. is seeing an overall reduction in HIV cases, Florida and other Southern states continue to see an increase. Studies suggest that HIV-related stigma may significantly affect ability to achieve viral suppression, medication adherence, psychological and social support, mental health, and a decreased quality of life. Florida is a complex state in terms of geography and population, thus our understanding of HIV-related stigma is not well understood across populations living with HIV in the state. The objectives of this study are to: (1) examine the perceptions of HIV-related stigma in the Southern U.S. and to (2) identify approaches for reducing HIV-related stigma.

Methods: To understand the unique perceptions of HIV-related stigma in the Southern U.S. and to identify potential solutions to reduce stigma, we developed an anonymous, open-ended survey to distribute to community members, community partners, and stakeholders. Consistent with thematic analysis, data were coded and grouped into categories guided by the Social Ecological Model (SEM).

Results: Sixty-six responses were collected from a diverse group including healthcare providers (25.8%), researchers (13.6%), persons living with HIV (34.8%), HIV program staff (10.6%), and others (42.4%). Most respondents were from Florida (71.2%), identified as white (45.5%), female (53.0%), and provided examples from a rural or suburban setting (54.5%). %). Participants described HIV-related stigma and associated examples as individual (fear, lack of knowledge, being perceived negatively), interpersonal (being treated differently by family or friends, refusal of sharing food or utensils), community (social norms, community enacted stigma, discrimination), institutional (healthcare-related issues), and structural (criminalization, healthcare barriers). Similarly, participants described HIV-related stigma reduction examples and recommendations as individual (increasing knowledge), interpersonal (communication between friends and family), community (changing social norms, peer-led groups and campaigns, media), institutional (health services, providers), and structural (education curriculums, updating policy, using first-person language).

Conclusions: HIV-related stigma negatively impacts all dimensions of the HIV care continuum; thus we need approaches that are informed by the communities in which stigma exists. Stigma interventions may be needed to address all of the SEM levels. This study used a convenience sample and may not reflect the full diversity of the population in the Southern U.S. We will be obtaining additional surveys and conducting additional analyses in the future.

120. FINDING NEMO'S PICORNAVIRUS

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Over the last decade, a number of aquaculture facilities have suffered significant mortality events in their clownfish (Amphiprion ocellaris and A. percula). Clinical signs of disease include darkened body coloration, increased gilling, reduced body condition, and abnormal positioning in the water column. Diseased clownfish were processed for parasitologic, bacteriologic, histopathologic, and virologic diagnostic testing. No significant parasite burdens were detected, and while bacteria were isolated from some of the fish, they appeared to be more consistent with a secondary infection. Histopathological examination revealed prominent single cell necrosis and mild inflammation of the mucosal epithelium within the branchial cavity, pharynx, esophagus, and/or stomach. Homogenates from pooled external and internal tissues were inoculated onto striped snakehead (SSN-1) cells, resulting in complete lysis in the

initial infection and upon subsequent passages. Transmission electron microscopy of infected SSN-1 cells revealed small (~25 nm), naked, icosahedral particles within the cytoplasm, occasionally arranged in paracrystalline arrays, consistent with the ultrastructure of a picornavirus. The virus was concentrated by ultracentrifugation prior to RNA extraction, cDNA library generation, and sequencing using an Illumina MiSeq sequencer. Sequencing recovered the full genome of a novel picornavirus most closely related to those recently described from other fish hosts including common carp (Cyprinus carpio), eel (Anguilla anguilla), bluegill (Lepomis macrochirus), and fathead minnow (Pimephales promelas). Future challenge studies are planned to elucidate the clinical significance of this picornavirus in clownfish. Disease progression will be assessed by regularly sampling fish over the study period to assess gross and microscopic lesions (histopathology and in situ hybridization) as well as viral load (virus isolation and RT-qPCR) within external and internal tissues.

121. FIRST REPORT OF A MEGALOCYTIVIRUS INFECTION IN AQUACULTURED RAINBOW SHARKS (EPALZEORHYNCHOS FRENATUS)

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Iridoviruses are a family of double-stranded DNA viruses that infect invertebrates, fish, amphibians, and reptiles. Within the subfamily Alphairidovirinae, members of the genus Megalocytivirus are known to infect >125 species of marine and freshwater ornamental and food fish species around the world. Megalocytiviruses (MCVs) are subdivided into three genotypes: infectious spleen and kidney necrosis virus (ISKNV), red seabream iridovirus, and turbot reddish body iridovirus. MCVs of the genotype ISKNV are notable pathogens negatively impacting the global production of ornamental fishes. In September 2018, chronic low-level mortality was observed in farm-raised rainbow sharks Epalzeorhynchos frenatus. Moribund broodstock were first observed with non-specific clinical signs including lethargy, increased gilling, and abnormal position in the water column. Subsequently, young-of-the-year rainbow sharks exhibited similar clinical signs and mortality rates. Initial diagnostics were performed at the Tropical Aquaculture Laboratory in Ruskin, FL. Wet mounts and bacteriology failed to identity significant parasitic or bacterial burdens; however, histopathological examination revealed microscopic lesions consistent with a MCV infection including the presence of cytomegalic cells (basophilic, intracytoplasmic inclusions) in internal tissues. Additional samples

were submitted to the Wildlife and Aquatic Veterinary Disease Laboratory in Gainesville, FL for virus isolation (VI) and a MCV-specific quantitative PCR (qPCR). Internal tissue homogenates from three fish were inoculated onto the grunt fin cell line and cytopathic effects (e.g. refractility, cytomegaly) were observed three days post-infection in one sample. Extracted DNA from matching tissue homogenates were positive for MCV by qPCR, with the highest viral load detected in the same homogenate that was positive by VI. This DNA sample was used to generate a DNA library for sequencing on an Illumina MiSeq sequencer. The resulting sequence data recovered the full MCV genome of an ISKNV. Similarly, a Maximum Likelihood analysis based on the nucleotide alignment of 18 MCV full genomes supported the rainbow shark MCV as a member of the ISKNV genotype. This study represents the first complete characterization of an ISKNV infection in a cyprinid fish, the rainbow shark. The detection of ISKNV in this highly valued ornamental fish species underscores the need for improved biosecurity measures and mitigation strategies to protect the international ornamental fish industry from MCVs.

122. GENOMIC CHARACTERIZATION OF NOVEL STRAINS OF BIG CYPRESS ORBIVIRUS AND MOBUCK VIRUS ISOLATED FROM DEAD WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) IN FLORIDA

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The genus Orbivirus within the family Reoviridae includes arboviruses with non-enveloped nucleocapsids, composed of outer and inner proteineacous layers that surrounds a segmented, dsRNA genome. Orbiviruses are primarily animal pathogens that are transmitted by ticks, mosquitoes, gnats, and midges. In September 2017, a farmed white-tailed deer (WTD-OV624) from Liberty County, Florida and a white-tailed deer (WTD-OV612) from Gadsden County, Florida were found dead and a necropsy was performed on the fresh carcasses. Specimens were submitted to the University of Florida Cervidae Health Research Initiative for diagnostic evaluation. Spleen specimens from each case were homogenized separately and inoculated onto Aedes albopictus clone C6/36 monolayers resulting in cytopathic effects in both cases. The viral genomic RNA was extracted separately from virions in spent cell culture media and served as templates for the construction of cDNA libraries using a NEBNext® Ultra™ RNA library prep kit. The resulting cDNA libraries were sequenced on an Illumina MiSeq sequencer. The low quality paired-ends reads were filtered and quality trimmed in CLC Genomics Workbench using default parameters. Following removal

of host sequences using Kraken, a de novo assembly of the paired-end reads was performed in SPAdes. The BLASTX analyses of the assembled contigs identified the complete coding sequences (10 segments) for two orbiviruses, Big Cypress orbivirus (BCPOV) and mobuck virus (MBV). A Maximum Likelihood (ML) phylogenetic analysis generated using IQ-TREE, based on the alignment of the T2 protein amino acid sequences from 30 orbiviruses, supported BCPOV strain WTD-OV624 as the closest relative to BCPOV strain BCNP-2-151 previously isolated from Psorophora columbiae mosquitoes in southern Florida in 2014. The ML tree supported MBV strain WTD-OV612 as the closest relative to mobuck virus strain 12-2 that was isolated from a dead white-tailed deer in Missouri in 2012. The inner (T2) and outer capsid (VP2) proteins of BCPOV strain WTD-OV624 showed 99% and 35% amino acid identity, respectively as compared to BCPOV strain BCNP-2-151. Thus, BCPOV strain WTD-OV624 represents a novel BCPOV strain and likely a new serotype. To our knowledge, this is the first report of the occurrence of Big Cypress virus in a vertebrate host. The isolation of BCPOV strain WTD-OV624 from a dead white-tailed deer suggests it may represent a previously unknown mosquito-borne cervid pathogen. The isolation of MBV strain WTD-OV612 expands the known geographic range of this orbivirus into Florida. Further study is needed to determine the vertebrate host range of BCPOV and MBV including their potential roles in disease among farmed and wild white-tailed deer populations.

123. GENOMIC CHARACTERIZATION OF PERCID HERPESVIRUS 1 ASSOCIATED WITH EPIDERMAL HYPERPLASIA IN WALLEYE (SANDER VITREUS)

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Percid herpesvirus 1 (PeHV1), known informally as walleye herpesvirus, was first reported in walleye (Sander vitreus) in 1971 during a spawning event in the Bad Carrot River, Canada and subsequently, in the Northern United States. Infected adults displayed cutaneous whitish plaques during the spring spawning season. Genetic data confirming PeHV1 as a member of the family Alloherpesviridae (i.e. fish and amphibian herpesviruses) is lacking. In this study, a Canadian PeHV1 isolate was propagated on the walleye ovary (WO) cell line and infected WO cells were examined by transmission electron microscopy. As expected for a herpesvirus, non-enveloped virus particles with hexagonal-shaped nucleocapsids approximately 100 nm in diameter were observed in the nucleus and larger enveloped nucleocapsids were observed within the cytoplasm of infected WO cells. DNA was extracted from infected WO cell culture supernatant and used to build a DNA library for sequencing on an Illumina MiSeq sequencer. The 3,447,273 paired-end reads were assembled de novo in SPAdes resulting in two herpesviral contigs that were joined manually by PCR and Sanger sequencing. The complete PeHV1 genome was determined to be 144,645 bp encoding 104 putative proteins including those conserved in all fish herpesviruses. Maximum Likelihood phylogenetic analysis based on the concatenated partial DNA-dependent DNA polymerase (pol) and second exon of the terminase (term) gene sequences (249 amino acid characters including gaps) revealed PeHV1 forms a novel branch

between the alloherpesvirus genera Ictalurivirus and Salmonivirus. The genetic analysis of the partial PeHV1 pol (151 amino acid characters including gaps) and term (98 amino acid characters including gaps) sequences ranged from 34.6-72% and 35.9-77.2% identities to other alloherpesviruses, respectively. Our study provides the first sequence data supporting PeHV1 as a novel species in the family Alloherpesviridae. Primers targeting the PeHV1 term gene were used to screen seven hyperplastic skin samples collected from adult walleye captured in Oneida Lake and held at the Oneida Lake Hatchery in Constantia, NY. Two samples were confirmed positive using the PeHV1 specific PCR assay. This is the first sequence-based confirmation of PeHV1 in the U.S. Challenge studies are planned to confirm PeHV1 is the causative agent of the observed cutaneous disease in adult walleye.

124. HIV DYNAMIC MODELLING FOR IDENTIFICATION OF TRANSMISSION EPICENTERS: HIV-DYNAMITE

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Background: There is a critical need to develop new analytic methods to combat the HIV epidemic. Current methods of defining transmission clusters (i.e., HIV-TRACE and PhyloPart) are based on static analysis and do not adequately capture dynamic variations within subpopulations or are suboptimal for large sequence datasets. Here we present a novel theoretical and technical framework, called HIV-DYNAMITE, able to dynamically model HIV transmission clusters in near-real time.

Methods: Phylogenetic comparative analyses are performed to assess the association between phylogenetic clades and patient demographics, mode of transmission, and comorbidities. In order to dynamically track HIV transmission clusters in our dated tree we will slice the tree at regular time intervals, e.g. every 10th percentile. We identify transmission clusters using PhyloPart under the following parameter distributions to be tested and optimized: 0-5% genetic diversity and 90-100% branch support. Next we link each cluster at different time slices based on their ancestral lineages. Cluster metrics calculated across slices include growth, shrinkage, stability, death, singletons and founder events, which facilitate the estimation of transmission cluster virulence.

Results: When time is considered, clustering is different based on scaling trees – that is, the evolutionary pattern is dependent on the inclusion of time. Time provided a dynamic component to transmission clustering. Clusters sharing a path are represented by different shades of the same color; coloring begins at the most recent common ancestor. Unlike other phylogenetic analysis software, HIV-DYNAMITE can simultaneously identify transmission clusters and infer growth trends of these clusters within epidemics. These trends may be identified either visually or using metrics indicating transmission cluster expansion, stagnation or diminution.

Conclusions: HIV-DYNAMITE provides crucial information for detecting HIV transmission clusters and at-risk groups in near-real time. HIV-DYNAMITE allow for identification and prediction of transmission trends and spread providing the foundation upon which precision public health interventions can be designed and implemented.

125. PARTIAL VALIDATION OF A TAQMAN REAL-TIME QUANTITATIVE PCR FOR THE DETECTION OF TILAPIA LAKE VIRUS

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Tilapia lake virus (TiLV), officially known as Tilapia tilapinevirus, is a significant threat to tilapia production in Asia, Africa, and South America. Clinical signs associated with TiLV infections include lethargy, anorexia, and swimming at the surface away from schooling tankmates. Infected fish display gross lesions including gill pallor, exophthalmia, body discoloration (darkening), scale protrusion and loss, and ascites. The most common microscopic lesions associated with TiLV infections include a syncytial hepatitis and an encephalitis. To date, the presence of TiLV in North America is unknown since there is no coordinated surveillance program due to the lack of a validated diagnostic assay. However, US producers exporting tilapia internationally are increasingly being asked by importing regulatory agencies to ensure that tilapia are TiLV-free. To fulfill this industry requirement, we have developed a TaqMan-based real-time quantitative PCR assay targeting a conserved region within segment 9 of the TiLV genome. A series of experiments using a serially diluted standard revealed high assay analytical sensitivity and efficiency. The assay also shows high specificity since it did not amplify other closely related fish viruses of the order Articulavirales, namely, salmon isavirus and an uncharacterized orthomyxovirus isolated from koi. The TiLV RT-qPCR assay reported here will be vital to safeguarding continued exports from US tilapia producers and has

the potential to form the basis of a nation-wide surveillance program aimed at protecting the young and emerging US tilapia industry from this damaging pathogen.

126. PHYLOGENOMIC CHARACTERIZATION OF ACIPENSERID HERPESVIRUS 1 IN LAKE STURGEON (ACIPENSER FULVESCENS)

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Acipenserid herpesvirus 1 (AciHV1) was first isolated from moribund farmed juvenile white sturgeon (ws; Acipenser transmontanus) in California and later in Europe on an Italian farm rearing ws. Fish infected with this virus (AciHV1-ws) presented with focal white cutaneous plaques that upon histopathological examination revealed keratinocyte swelling and hyperplasia. In spring 2017, two wild, adult lake sturgeon (ls; A. fulvescens) captured from the Wolf River, WI, presented with cutaneous lesions similar to those previously reported in farmed ws in California and Europe. Biopsies were obtained for histopathologic evaluation and molecular diagnostic testing. Microscopic examination of the cutaneous lesions in these two ls revealed hyperplasia and hydropic change of keratinocytes consistent with previous cases of AciHV1-ws disease. A degenerate PCR targeting the DNA-dependent DNA polymerase (pol) of large DNA viruses generated the expected 500 bp amplicons from both skin samples. Sanger sequencing of the purified PCR products followed by BLAST analyses using the National Center for Biotechnology Information non-redundant nucleotide and protein databases confirmed the presence of an alloherpesvirus closely related to AciHV1-ws in both ls samples (AciHV1-ls). A DNA library was prepared from the DNA extracted from biopsied skin lesions and sequenced using a v3 chemistry 600 cycle kit on an Illumina MiSeq sequencer. The de novo assembly of 6,477,748 paired-end reads using the SPAdes genome assembler recovered a large alloherpesvirus contig that was extended and joined to other contigs manually by PCR and Sanger sequencing, resulting in the complete AciHV1-ls genome sequence (201,788 bp). Maximum Likelihood

phylogenetic analysis based on the concatenated amino acid (aa) alignments of the partial pol and exon two of the terminase (term) genes revealed that AciHV1-ls branches as the sister group to AciHV1-ws. The AciHV1-ls and AciHV1-ws partial pol and term aa sequences displayed 93.1 and 100% identity to each other, respectively. This study provides the first complete AciHV1 genome sequence and expands the host range of this virus to include lake sturgeon.

127. PHYLOGENOMIC CHARACTERIZATION OF CARP EDEMA VIRUS

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Double-stranded DNA viruses (dsDNA) infect a wide range of homeothermic and poikilothermic vertebrates. However, only the dsDNA families Alloherpesviridae and Iridoviridae are well studied among poikilothermic vertebrates (e.g. fish, amphibians, and reptiles). Herein, we report the phylogenomic characterization of a fish poxvirus, carp edema virus (CEV) that infects Common Carp (Cyprinus carpio) varieties including koi. CEV is a globally emerging virus that has negatively impacted facilities rearing Common Carp for food, sport, and recreation. In this study, we built a DNA library from CEV infected gill tissue DNA derived from an outbreak that occurred in wild Common Carp in New Jersey in 2017. Sequencing of the library was performed on an Illumina MiSeq and the resulting data trimmed and assembled using multiple assembly softwares. The nearly full genome (463,613 bp) was recovered including the inverted terminal repeats. Ultrastructural examination revealed

abundant large spheroid particles, consistent with previous CEV studies, observed within the cytoplasm of gill epithelial cells. The mature CEV virion appears to possess a single lateral body, similar to previous reports of fish poxviruses in Atlantic Salmon (Salmo salar) and Ayu (Plecoglossus altivelis). Maximum Likelihood phylogenetic analysis based on the concatenated amino acid sequences of seven conserved poxvirus proteins revealed that CEV is the sister taxon to the salmon gill poxvirus and together they form the most basal branch of the Chordopoxvirinae. The genetic distinctness of the fish poxviruses argues that they represent a new genus within the Chordopovirinae that we suggest could be named Piscipoxvirus. However, completion of the CEV genome annotation is needed to determine whether the fish poxviruses share a suite of derived genomic features that support the creation of the proposed genus.

128. SAMPLE PREPARATION AND RNA AMPLIFICATION FOR ZIKA VIRUS DETECTION AT THE POINT OF CARE

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Introduction: The recent outbreaks of Zika virus (ZIKV) infection represent a public health challenge. Rapid, cost-effective, and reliable diagnostic tools for ZIKV detection at the point-of-care (POC) are highly desirable, especially for resource-limited nations. For the diagnosis of Zika disease, the US Centers for Disease Control and Prevention (CDC) recommends a nucleic acid test (NAT) and immunoassay tests. However, they are not performed at POC. To address the need, we have developed an integrated device to achieve sample-to-answer ZIKV detection using RT-LAMP and colorimetric detection. RT-LAMP was chosen due to its high

sensitivity and specificity, short incubation time, and simplified thermal management.

Methods: We developed a valve-enabled lysis, paper-based RNA enrichment, and RNA amplification device (VLEAD). The device features (1) innovative ball-based valves enabling the storage and sequential delivery of reagents for virus lysis and (2) a paper-based unit for RNA enrichment and purification. VLEAD contains three parts, a 3-D printed buffer unit, a mixing unit, and detection unit. The buffer unit is slid into the mixing unit through a sliding mechanism, while the detection unit is inserted by a protrusion at the bottom of the mixing unit. The paper unit is placed in a commercially available coffee mug that provides a constant temperature for amplification in 25 min. For detection by naked eye, we used SYBR green dye and a blue LED flashlight to get an explicit fluorescence signal when virus RNA is present.

Results: The detection limit is 0.5 PFU per device for human urine and saliva samples, and 0.1 PFU for water samples. The device allows a much larger sample volume (140 μL) than traditional microfluidic paper-based analytical devices, which increased the sensitivity of the test. Using VLEAD, we have also tested a de-identified clinical urine sample collected from one Zika patient in Venezuela, 2016, and our result is in agreement with the previous test result by virus isolation and RT-PCR at that time.

Conclusion: We have developed an innovative and highly sensitive POC testing platform, VLEAD, for ZIKV detection within 50 min in human urine and saliva samples.

129. SOCIODEMOGRAPHIC AND SPATIAL-TEMPORAL DETERMINANTS OF HIV DRUG RESISTANCE IN FLORIDA, 2012-2017

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Introduction: Persons living with HIV (PLWH) with resistance to antiretroviral (ARV) medications are vulnerable to numerous adverse health outcomes and can contribute to transmission of HIV drug resistance (HIVDR) if not virally suppressed. In North America, HIVDR prevalence ranges from 11.5%-23.4%, depending on ARV class. Florida is among the highest HIV-incident states in the country with only 62% of PLWH virally suppressed. The degree to which transmitted and acquired HIVDR contributes to disease burden in Florida is largely unknown, however.

Objective: We explored sociodemographic and spatial-temporal associations with HIVDR, in collaboration with the Florida Department of Health (FDOH). This study capitalized on the extensive FDOH electronic database and involves analysis of the largest collection of HIV sequence data in Florida to date.

Methods: HIV-1 nucleotide sequences collected through routine HIV surveillance were selected using consensus B and HXB2 reference nucleotide numbering. HIVDR mutations were categorized according to ARV class: non-nucleoside reverse transcriptase inhibitors

(NNRTI), nucleoside reverse transcriptase inhibitors (NRTI), protease inhibitors (PI), and integrase strand transfer inhibitors (INSTI). Multi-drug resistance (MDR) was defined as resistance to at least two drug classes. Transmitted-drug resistance (TDR) was estimated separately using the World Health Organization's list of surveillance mutations. Sequences were linked to de-identified individual-level patient data available from the FDOH's enhanced HIV/AIDS Reporting System, in addition to county-level health indicators obtained via County Health Rankings. Multivariable (stepwise-selected) logistic regression models were fitted to associate individual and ecological factors with HIVDR.

Results: Prevalence of HIVDR was 19.0% for NRTI, 30.2% for NNRTI, 6.5% for PI, 19.4% for TDR, 13.4% for MDR, and 10.7% for INSTI over the study period. Multivariable analyses revealed older individuals and perinatally-exposed individuals had significantly higher odds of resistance to all drug classes whilst intravenous drug users and Caucasians tended to have lower odds. We observed decreasing odds of TDR and NNRTI resistance as a function of test year, but increasing odds for PI and INSTI resistance. Ecological analyses indicated higher HIVDR rates were associated with lower socioeconomic status, unemployment, and poor mental health whereas lower HIVDR rates were associated with crime rates and percent rural population.

Conclusions: This is one of the most comprehensive studies of HIVDR in Florida. Our findings indicate the prevalence of HIVDR is higher than current North American estimates with considerable variation in HIVDR prevalence between ecological factors and within certain risk groups.

130. THE P33 PROTEIN OF CITRUS TRISTEZA VIRUS CONTRIBUTES TO INDUCING A HYPERSENSITIVE RESPONSE-LIKE RESPONSE IN NICOTIANA BENTHAMIANA

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The hypersensitive response (HR) triggered by the pathogen avirulence factor – host resistance protein interactions is a ubiquitous defense response in plant hosts. The HR-associated necrotic lesions could limit a pathogen to initially infected tissues and impart resistance to uninfected ones. Here, we observed that Nicotiana benthamiana could develop local HR-like necrosis upon Agrobacterium-mediated infiltration of Citrus tristeza virus (CTV) that restricts virus systemic movement. The development of local necrosis correlated with the accumulation of CTV in a dose-dependent manner: virus inoculation using CTV-expressing Agrobacterium cultures at high optical density resulted in severe necrosis at the infiltration sites and lack of systemic infection, while inoculations using low-optical density cultures permitted virus escape to the upper non-inoculated leaves. Furthermore, in the latter case, virus-infected systemic leaves of N. benthamiana showed a similar HR-like response, which correlated with the elevated expression of defense related genes and the accumulation of reactive oxygen species (ROS). As the next step, we discovered that three CTV proteins contribute to such plant response, among which are the p20 and p23 proteins known as viral suppressors of RNA silencing and, unexpectedly, the p33 protein, which is thought to function in virus movement. Overexpression of p33 in N. benthamiana induced ROS accumulation and the development of HR-like necrosis. Furthermore, deletion of the p33 open reading frame in a CTV mutant resulted in a significant decrease in ROS and lack of strong necrosis upon its infiltration in N. benthamiana,

compared to the wild type virus inoculation. These results suggest that p33 contributes to triggering the HR-like response in N. benthamiana and could be one of the CTV avirulence factors.

131. VIRUS – HONEY BEE MOLECULAR INTERACTIONS AND POTENTIAL USE OF VIRUS-BLOCKING PEPTIDES FOR THE PROTECTION OF HONEY BEE HEALTH

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Virus – honey bee molecular interactions and potential use of virus-blocking peptides for the protection of honey bee health Ya Guo and Bryony C. Bonning Department of Entomology and Nematology, University of Florida Colonies of the western honey bee, Apis mellifera have been severely impacted in recent years by a wide range of stressors. The causes of bee declines are multifactorial, but Varroa mites and associated viruses are among the most serious threats to honey bee health. The Varroa mite serves as both mechanical and biological vector of honey bee viruses. The picture that has emerged over the course of a decade of research is that virus load plays an important part in weakening of colonies prior to their demise. The iflavirus, Deformed wing virus (DWV) and the dicistrovirus, Israeli acute paralysis virus (IAPV) are of particular concern in relation to colony losses. Following ingestion, DWV and IAPV are hypothesized to enter midgut epithelial cells by receptor-mediated endocytosis, but the specific molecular mechanisms are unknown. We used honey bee gut membrane enriched preparations (brush border membrane vesicles) in 2-dimensional ligand blots with DWV as ligand to identify candidate receptor proteins. In addition, by feeding adult honey bees on a phage display library, we identified a 7-amino acid, bee midgut binding peptide (BBP2.1). This peptide shares 75% and 85% identity with a region of the DWV and IAPV capsid proteins, respectively. This region of the capsid protein is likely to be instrumental in virus interaction with the honey bee gut receptor. We hypothesize that this peptide can be exploited to interfere with the binding and subsequent entry of these viruses into

the honey bee gut. Increased understanding of the molecular mechanisms involved in honey bee-virus interaction may allow for practical application of such virus-blocking peptides toward suppression of virus entry, and reduction in both virus load and virus-associated mortality.

132. A MODEL UNIVERSITY-SCHOOL PARTNERSHIP TO EXPLORE EMERGING PATHOGEN CONTENT AND CAREERS IN PRECOLLEGE CLASSROOMS

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Now entering its third year, CATALySES (Collaborating to Advance the Teaching and Learning of Science Educators and Students): Emerging Pathogens is a professional development program for secondary STEM teachers developed and implemented by the UF Center for Precollegiate Education and Training (CPET) in collaboration with the Emerging Pathogens Institute. CATALySES is funded by an NIH SEPA grant; teachers participating in the program do not incur any costs. The content of this two-week residential program is focused on infectious diseases and translational research, and is delivered through a combination of hands-on laboratory experiences, workshops, lectures, tours, and discussion groups. The objectives of CATALySES include improving teachers' design expertise, lesson planning, and science identity, leading to improvement of their students' content knowledge, attitudes, and science identity. These students will be better prepared to explore the continuum of paths to the science and health-related workforce. During the two-week summer institute, teachers work with UF science and education researchers to develop lessons and laboratory exercises, translating their CATALySES experiences into classroom action. Each teacher creates a "Research Action Proposal" in which they choose an intervention to implement in their classroom. They decide how they will collect, analyze, and interpret data from their

intervention. These action proposals include specific connections to content from the CATALySES institute. Continued support from the CPET CATALySES research team encourages the teachers' personal enrichment and professional advancement in biotechnology education. CPET provides a variety of resources to CATALySES teachers including a stipend to each teacher attending the summer program, free use of an equipment locker program, and an option to participate in CPET'S Special Explorations for Teachers and Students (SETS) program at no cost to them.

133. A NOVEL APPROACH: THE USE OF MALACHITE GREEN TO TREAT REFRACTORY PYTHIUM INSIDIOSUM AND LAGENIDIUM GIGANTEUM IN 5 DOGS AND 1 CAT

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Pythium insidiosum and Lagenidium giganteum are important pathogens of dogs and cats, and less commonly of humans. These organisms belong to the oomycete class of eukaryotic microbes and are found in warm stagnant waters throughout the Gulf States. Affected animals are often young, otherwise healthy, and present with non-healing cutaneous wounds or gastrointestinal disease. Prognosis is grave and treatment is often unsuccessful. Surgical removal is the treatment of choice; however, lesion size and location frequently make surgical removal infeasible. Increasing recognition of pythiosis and lagenidiosis in veterinary medicine has led to the need for new treatment interventions. Malachite green is an arylmethane dye that is used in aquaculture for its anti-oomycotic and anti-fungal properties. Its use in the commercial fish industry is controversial due to possible carcinogenic, cytotoxic and teratogenic effects; however, no chemical compound has proven as effective against oomycetes in our laboratory studies. The purpose of this report is to describe the clinical application, efficacy and safety of

malachite green administration in dogs and cats when used as an adjunctive therapeutic agent in refractory pythiosis and lagenidiosis. Five dogs and one cat with severe, non-surgical, confirmed oomycosis were treated with malachite green (topically, intralesionally, subcutaneously, orally and/or intravenously) at varying dosages and for varying lengths of time. Adverse effects included vomiting in one dog, hyporexia in the cat and irritation after subcutaneous injection in one dog. Median survival time after initial treatment with malachite green was 94 days. Static disease, defined as no change in lesion size(s), was achieved for 49 weeks in one dog. Initial lesion regression was documented in the cat after intralesional injection of malachite green. Four out of the six dogs were euthanized due to disease progression and one dog and the cat died naturally during the course of their disease. Necropsies were performed on five out of the six animals. Although all animals eventually succumbed to their disease, side effects associated with malachite green administration were minimal and promising results were obtained in two out of the six animals as compared to historical cases. In conclusion, this report is the first of its kind and provides a basis for future prospective research on malachite green and its potential use in the treatment of veterinary oomycosis.

134. ACADEMIC RESEARCH CONSULTING AND SERVICES: EXPERT SUPPORT FOR YOUR RESEARCH DATA NEEDS

Joe Wu - University of Florida; **Joe Joe** - University of Florida; **Tara Cataldo** - University of Florida; **Perry Collins** - University of Florida; **Sara Gonzalez** - University of Florida; **Daniel Maxwell** - University of Florida; **Plato Smith** - University of Florida; **Michelle Leonard** - University of Florida; **Suzanne Stapleton** - University of Florida; **Michele Tennant** - University of Florida

Academic Research Consulting and Services (ARCS) offers a wide range of research support services to the University of Florida community. Hosted by the George A. Smathers Libraries, ARCS has been developed to provide expert services throughout the research process – from data collection through publication and beyond. ARCS can offer consultations on issues regarding best practices for maintaining research integrity. The team also has expertise in several aspects of data science, including:

- Statistical analysis (SAS, SPSS)
- Writing of computer code in languages such as R and Python for analysis of data
- Bioinformatics and genomic data analysis using Galaxy, HiPerGator, and R
- Geospatial analysis (ArcGIS, Erdas Imagine)
- Data management and archiving
- Data and 3D visualizations (3D printing) Post-projects, ARCS can advise on methodologies for
- Evaluating research metrics and impact
- Publishing and preserving your research protocols and results in accordance with funding agency public access mandates and data management requirements

ARCS members are available to bring this expertise to grant submissions at the pre-proposal as well as post-award stages. This poster outlines ARCS services, introduces ARCS consultants, and offers directions to learn more about ARCS and access its services.

135. AEDES AEGYPTI LEUCINE-RICH REPEAT PROTEINS IN RESPONSE TO ARBOVIRUSES

Liming Zhao - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Barry Alto** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Dongyoung Shin** - College of Agricultural and Life Sciences, University of Florida

Aedes aegypti (L.) is the primary vector of chikungunya, dengue, yellow fever and Zika viruses. The leucine-rich repeats (LRR)-containing domain is evolutionarily conserved in many proteins associated with innate immunity in invertebrates and vertebrates, as well as plants. We focused on the AaeLRIM1 and AaeAPL1 gene expressions in response to Zika virus (ZIKV) and Chikungunya virus (CHIKV) infection using a time course study, as well as the developmental expressions in the eggs, larvae, pupae, and adults. RNA-seq analysis data provided 60 leucine-rich repeat related transcriptions in Ae. aegypti in response to Zika virus (Accession number: GSE118858, https://www.ncbi.nlm.nih.gov/gds/?term=GSE118858). RNA-seq analysis data showed that AaeLRIM1 (AAEL012086-RA) and AaeAPL1 (AAEL009520-RA) were significantly upregulated 2.5 and 3-fold during infection by ZIKV 7-days post infection (dpi) of an Ae. aegypti Key West strain compared to an Orlando strain. The qPCR data showed that LRR-containing proteins AaeLRIM1, AaeAPL1 and five paralogues were expressed 100-fold lower than other nuclear genes, such as defensin, during all developmental stages examined. Together, these data provide insights into transcription profiles of LRR proteins of Ae. aegypti during its development and in response to infection with emergent arboviruses.

136. CHANGES IN INFECTIOUS DISEASE INCIDENCE IN THE U.S. VIRGIN ISLANDS FOLLOWING HURRICANES IRMA AND MARIA, JUNE TO DECEMBER 2017

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Introduction: Hurricanes Irma and Maria made landfall in the U.S. Virgin Islands (VI) in September 2017, causing both short- and long-term damages to resident health and territorial healthcare systems. Decreased access to care, flooding, confinement to disaster shelters, and lost electricity all posed risks to the transmission of infectious disease (ID). In this investigation, we describe the changes in ID incidence in the VI following the hurricanes.

Methods: A retrospective, population-based analysis of ID-related emergency department visits occurring between June and December 2017 at Schneider Regional Medical Center (St. Thomas, VI) was performed using administrative health data. Visits with a documented International Classification of Diseases (ICD-10), Chapter I (A00-B99) primary diagnosis code were included. Date of ED encounter was stratified to respective pre-, inter-, post-hurricane periods (6/1/2017–9/5/2017, 9/6/2017–9/21/2017, and 9/22/2017–12/31/2017) using landfall dates of Hurricanes Irma and Maria. Cumulative incidence of ID-related diagnoses were calculated for each hurricane period. Risk ratios (R.R.) were calculated to measure associations between disease incidence and hurricane period.

Results: Of 10,716 diagnoses, 2.6% (n=277) related to ID. Median age of ID patients was 17 years, 52% were female, and 76% were black. Pre-hurricane, ID comprised 2.8% (n=134) of all diagnoses, inter-hurricane 2.0% (n=19), and post-hurricane 2.5% (n=124). 'Viral infection, unspecified' was the most frequently diagnosed morbidity for all infectious disease diagnoses, with 12.4 per 1,000 diagnoses. 'Enteroviral vesicular stomatitis with exanthem' (Hand, Foot, & Mouth Disease, B08.3) followed at 3.3 and 'viral intestinal infection, unspecified' (A08.4) at 2.3 per 1,000 diagnoses. There were 38 unique ICD-10 diagnoses of infectious disease during all periods. From pre-hurricane to inter-hurricane, the incidence of 21 diagnoses decreased, 9 increased, and 8 did not change; only 'viral infection, unspecified' (B34.9) significantly changed, decreasing from 16.4 to 6.3 per 1,000 diagnoses (R.R.=0.38, 95% CI=0.16, 0.87). From pre-hurricane to post-hurricane, the incidence of 15 diagnoses decreased, 12 increased, and 11 did not change; HFMD had the greatest incidence increase (R.R.=15.51, 95%CI=3.72, 64.7), and 'viral infection, unspecified' had the greatest incidence decrease (R.R.=0.58, 95%CI= 0.41, 0.84).

Discussion: These results indicate that ID presented a modest but preventable share of the morbidity after Hurricanes Irma and Maria. The high incidence of HFMD post-hurricane, a disease transmitted by physical contact, may reflect resident confinement to crowed spaces such as a shelter. We recommend to stakeholders integrate public health messaging about ID transmission into hurricane preparedness campaigns.

137. CUMULATIVE HIV VIREMIA COPY-YEARS AND HYPERTENSION AMONG PERSONS LIVING WITH HIV

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Background: The association between HIV viral load (VL) and hypertension examined by using cross-sectional VL measures (e.g. peak VL, the most VL, and viral suppression) has been controversial. Systemic inflammation and immune activation may be caused or perpetuated by ongoing replication of HIV. The inconsistent findings may be resultant from a failure of cross-sectional VL measures in capturing cumulative plasma HIV burden. In this study, we examined the association between HIV and hypertension by estimating the cumulative plasma HIV burden.

Methods: We included 686 people living with HIV (PLWH) who completed the baseline investigation of Florida Cohort Study. Hypertension diagnosis was extracted from their medical records. Individual HIV VL during 5 years prior to baseline investigation was obtained from eHARS. Five-year viremia copy-years (CVY), a time-varying measure of cumulative plasma HIV exposure, was determined for each patient using the area under the VL curve during the five years. Multivariable logistic models were used to evaluate the association of VCY with hypertension.

Results: A total of 277 (40.4%) participants were recorded with hypertension as defined by objective medical record and self-reported hypertension. The median VCY was 4.4 log10 copy ×

years/mL (interquartile range: 3.0 - 5.4 log10 copy × years /mL). When controlling for age, gender, race and other potential confounders, 2.7 - 3.7, 3.8 - 4.7, 4.8 - 5.7, and >=5.7 log10 copy × years/mL were associated with 1.91 [95%CI= 1.11, 3.29], 1.91 [95%CI=1.03, 3.53], 2.27 [95%CI=1.29, 3.99], and 1.25 [95%CI=0.65, 2.42] times odds of having hypertension than VCY less than 2.7 log10 copy × years/mL, respectively. No association with hypertension was observed for cross-sectional measurements, including peak VL, the most recent VL or viral suppression (the most recent VL>=200 copies/mL) after controlling for potential confounders (>0.05). Further, the association of viremia copy-years with hypertension was independent of the cross-sectional measurements.

Conclusion: Cumulative HIV burden was associated with hypertension, independent of cross-sectional VL measures. A persistently low level of HIV viremia could be a significant strategy for hypertension prevention.

138. DETERMINING THE LETHAL CONCENTRATION VALUES FOR FIPRONIL IN AMBLYOMMA AMERICANUM

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Of the ticks species found in Florida, Amblyomma americanum is the most abundant due in part to its high fecundity and ability to parasitize a variety of vertebrates. White-tailed deer are the main host for adult A. americanum, however immature stages prefer to

feed on a variety of smaller animals such as raccoons, squirrels, and even domestic pets. Fipronil is commonly utilized as a flea and tick preventative on companion animals in products such as Frontline®. Because of their ubiquitous presence in North and Central Florida, and diverse feeding habits, A. americanum can potentially be exposed to fipronil. The risk of resistance development is always possible under high selection pressures presented on treated domestic pets. The aim of this study was to calculate the lethal concentration (LC) and discriminating concentration (DC) values in a susceptible population of A. americanum to provide baseline data for potential resistance research that could arise due to wide-spread usage of fipronil. Engorged female A. americanum were held in a humidity chamber to promote oviposition. Concentration-finding experiments were conducted using the Food and Agriculture Organization (FAO) larval packet test (LPT) to obtain the mortality data needed to calculate the LC and DC values. Larvae were placed in chromatography paper packets that were treated with a range of fipronil concentrations that produced between 25% and 100% mortality. The number of live and dead larvae were counted after 24 hours of exposure. The LC50, LC90, and LC99 values were calculated using PoloPlus software and were determined to be 0.00454%, 0.00719%, and 0.0104%, respectively. The DC is calculated as two times the LC99, which was 0.0208% for fipronil. These values can be used to screen tick populations for potential resistance in future studies and for routine surveillance.

139. DEVELOPING THE CHIRONOMID MIDGES AS A NATURAL HOST MODEL TO STUDY HOST-MICROBE INTERACTIONS

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Chironomids are ubiquitous in aquatic environments, and they play an important role in the ecosystem as a primary food source for birds, amphibians, fishes and other aquatic invertebrates. The goal of this project is to develop the chironomid midges as a natural host model to study host-microbe interactions. We have developed a novel method to manipulate the microbiome of the chironomid larvae. Using this method, we found that axenic (microbe-free) chironomids did not develop to adulthood, compared with the non-sterilized counterpart. We transplanted the microbiome using homogenate of conventional chironomids, fruit flies, and mosquitoes, all of which were able to rescue axenic chironomid development. Strikingly, we were able to rescue axenic chironomid development by inoculating with a single strain of an aquatic bacterium Vibrio cholerae, also notorious as a human diarrheal pathogen. Our results suggest that the chironomids rely on microbes to complete the life cycle, but such host-microbe relationship appears to be flexible in the context of microbial identity. With this method, we will be able to manipulate the chironomid microbiome to study the host-microbial interaction. We are also looking into targeting the chironomid and its documented, natural association with V. cholera to develop management strategies to control the transmission of cholera.

140. DEVELOPMENT OF BIOINFORMATICS SERVICES IN THE LIBRARY: UNDERSTANDING RESEARCHER'S BIOINFORMATICS SUPPORT NEEDS

Joe Wu - University of Florida; **Hannah Norton** - University of Florida; **Mary Edwards** - University of Florida; **Michele Tennant** - University of Florida

University of Florida Health Science Center Library (HSCL) is committed to offering quality bioinformatics training services. Here, we seek insights on the bioinformatics training needs of life sciences researchers across campus to help guide our efforts to improve existing or develop new services. A survey was created in Qualtrics and distributed to life sciences researchers in the academic health center as well as those in relevant interdisciplinary institutes, and the departments in the College of Liberal Arts & Sciences and the College of Agricultural & Life Sciences. These units represent those in which a knowledge of bioinformatics tools could enhance the work of their researchers. Survey results indicate a demand for bioinformatics training. Bioinformatics tasks such as genomic data analysis, development of data analysis pipelines, and molecular biology network analysis received the most training interest. We observed that graduate students were less likely to rate their proficiency at the expert level as compared to faculty for tasks involving the search and retrieval of molecular biology information from databases. Further, a higher percentage of graduate student respondents showed interest for training in these bioinformatics tasks as compared to faculty. Finally, classes, individual in-person consultations, and online video tutorials ranked among the top three preferred modes of training. The HSCL is already taking steps to fulfill bioinformatics training needs. We offer workshops as well as a credit-bearing course (GMS5909) that address use of databases (NCBI, Ensembl, UCSC, etc.) to retrieve molecular biology information such as gene sequences or reference genomes, which may be used in downstream tasks such as phylogenetics or high-throughput sequencing analysis. The HSCL also offers workshops and consultations on the use of genomic data analysis tools that are

available on HiPerGator and invites bioinformatics experts on campus to conduct training. Notably, we recently coordinated with UF's Interdisciplinary Center for Biotechnology Research (ICBR) to conduct a four-session next generation sequencing workshop series that covers the experimental aspects as well as analysis using HiPerGator tools. Next generation sequencing analysis workshops conducted by ICBR were video recorded and these are available online for life sciences researchers across campus. Development of new workshops such as RNA seq analysis, ChIP seq analysis, and molecular biology network analysis are underway.

141. DMSO PREFERENTIALLY INCREASES TOXICITY OF HYDROPHOBIC COMPOUNDS ON BOTH AEDES AEGYPTI AND ANOPHELES GAMBIAE

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Mosquitoes are the main vectors of many significant human diseases (e.g., malaria, yellow fever, dengue, and zika) and insecticides remain an efficient way to limit the size of mosquito populations. In this regard, new active compounds are required to overcome resistances that develop against the old ones, and to limit the environmental impact of the treatments. When screening for new compounds, there are different factors that may prevent an active molecule in vitro from acting on its target in vivo, such as high vapor pressure, solubility, or the capacity to penetrate the cuticle. When searching for a new lead compound with a bioassay, any of these properties might prevent the detection of a potential lead, and being able to isolate those compounds in screening bioassays is crucial. This work used different vehicles in an effort to help the penetration of commercial or potential lead insecticides through the cuticle, via

topical application, thus reducing the impact of retarding effects and potentially increasing toxicity. For these studies, dimethylsulfoxide (DMSO) was used as potential facilitator of ethanolic solutions of compounds applied to the thorax of Aedes aegypti or Anopheles gambiae. A fatty acid derivative showing good neurophysiological effects in vitro, but with very little toxicity in vivo, 11-dansylamino undecanoic acid (DAUDA), was potentiated in its toxicity. With DMSO co-applied in topical assay, the molecule decreased its LD50 value of >20 µg/mg in ethanol alone down to 2.2 µg/mg when dissolved in ethanol containing 25% DMSO. The action of DMSO was then evaluated on different commercially available molecules. Some showed a good toxicity potentiation (> 3 fold decrease of the LD50 value), such as aldrin, 2S-65465, 5-hydroxydecanoic acid, chlorpyrifos and decanoic acid on Anopheles gambiae and 2S-65465 and decanoic acid on Aedes aegypti, while others did not, such as coumaphos and aldicarb. By analyzing the physical properties of the different molecules, DMSO was generally more effective with the more hydrophobic compounds (except the pyrethroid, permethrin). This observation was the same on both Anopheles gambiae, and Aedes aegypti species. These results suggest the use of 25% DMSO in ethanol as vehicle will improve the screening sensitivity of new lead compounds, with little to no added cost. This work was funded by the Deployed War Fighter Research Program via the USDA (58-0208-0-068 and 58-0208-5-001).

142. ELUCIDATION OF MECHANISMS MEDIATING TOPICAL SOCS1-KIR PEPTIDE INHIBITION OF PSORIATIC PLAQUES INDUCED BY IMIQUIMOD

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Psoriasis is a dermatological disease marked by plaques and erythematosus on the skin. It occurs in roughly 3% of the adults in United States. Inappropriate activation of TLR7/8 at the psoriatic lesion on Dendritic cells and Macrophages is a well-understood mode of pathogenesis in psoriasis. An imiquimod-induced model of psoriasis is an established model to generate plaque-type psoriasis pathology in mice. Imiquimod is a TLR7 agonist that ligates the TLR7 on dendritic cells and macrophages. TLR7 ligation on Macrophage and Dendritic cells leads to secretion of a storm of cytokines primarily including Type I interferons, TNFα, IL-6, and IL-1B. These cytokines can indirectly trigger the secretion of chemokines or chemotactic cytokines by keratinocytes, macrophages, and dendritic cells which cause the migration of both skin resident and peripheral blood T cells and macrophages to the site of trigger. Many cytokines, including type 1 interferons, utilize the JAK/STAT pathway for signal transduction. Although inflammation is critical for the elimination of pathogens and cancers, excess inflammatory signaling drives the pathology present in psoriasis. Suppressor of cytokine signaling-1 is a naturally occurring protein that serves to limit inflammatory processes. By preventing JAK/STAT utilizing cytokines from signaling, the inflammatory phenotype associated with psoriasis can be reduced in patients with mild to moderate psoriasis. We have previously shown that a SOCS1 mimetic peptide (SOCS1-KIR), consisting of the kinase inhibitory region (KIR) of the native SOCS1 protein, effectively limited inflammatory pathways in rodent models of multiple sclerosis, uveitis, and lupus-like pathology. SOCS1-KIR inhibited the kinase activity of Jaks involved in promoting inflammatory pathways promoted by cytokines such as type 1 interferons. In this study, we have shown reduction in cytokine and

chemokine secretion in TLR7-ligated macrophages after peptide administration. The peptide also reduces classical activation in macrophages. This study brings us closer to finding out the therapeutic potential of SOCS1 KIR for patients with mild-to-moderate Psoriasis.

143. ESTABLISHING TISSUE CULTURE CELL LINES FROM REPTILES AND AMPHIBIANS

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Tissue culture cell lines are a critical reagent in the culture and characterization of obligate intracellular pathogens. While a wide variety of cell lines are available from primates, lab animals, and domesticated mammals, cell lines from wildlife species, and particularly reptiles and amphibians, are an extremely scarce commodity. The absence of such cell lines has proven to be a major barrier in the identification and research of intracellular pathogens of these hosts, as well as the potential role such hosts may play in transmission cycles of zoonotic pathogens. As part of an ongoing project to increase the availability and access to reagents for wildlife conservation and disease research, cell lines are being established from reptile and amphibian species to address this need. Cell lines were derived from primary tissues collected at the time of necropsy from freshly dead or euthanized reptiles and amphibians submitted to the Anatomic Pathology service at University of Florida's College of Veterinary Medicine. Continuously dividing cells were selected from primary cell growth of a variety of tissues, including heart, spleen, kidney, skeletal muscle, and liver. Cells were maintained in conditions determined to be ideal for their respective classes, and continued selection for homogenous cellular morphology through passage was performed. Aliquots of growing cells were routinely archived in liquid nitrogen, and a cell line was considered stable after 10-12 successful passages of a single, homogenous morphology. Cell

lines have been successfully established of various tissues from a variety of reptile species, including snakes (n=5), turtles/tortoises (n=3), crocodilians (n=1), and a lizard (n=1). Additional cell lines from other species of snakes, turtles, lizards, and crocodilians and a single species of frog are currently in various stages of development. A database is under development for cataloging the established cell lines. A virtual component of the database will be publicly accessible to permit and promote the dissemination of these and potentially other reagents to scientists with the intent of ultimately providing benefits to both animal and human health.

144. FACTORS ASSOCIATED WITH SUBOPTIMAL ART ADHERENCE AMONG PEOPLE LIVING WITH HIV REPORTING PAIN

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Background: Adherence to antiretroviral therapy (ART) has been proven to be an effective method of reducing HIV transmission from HIV positive individuals to uninfected partners. Previous studies noted individuals reporting pain are less likely to report optimal ART adherence (≥ 95%) compared to individuals without pain. The aim of this analysis is to identify mental health and substance use factors associated with optimal ART adherence among individuals reporting pain.

Methods: HIV+ adults (N=370) recruited across Florida from community health centers completed questionnaires collecting demographics, pain, ART adherence, substance use, and mental health information. Mild/moderate (1-6) and severe pain (≥ 7) were recorded using the Brief Pain Inventory Short Form. A logistic regression was conducted to assess the relationships between

mental health and substance use covariates and suboptimal ART adherence.

Results: Overall, 80% (N=296) of participants reported mild/moderate pain while 20% (N=74) of participants reported severe pain. After controlling for selected covariates, the odds of suboptimal ART adherence among hazardous drinkers were 2.06 (CI=1.18, 3.60) times the odds among individuals not reporting hazardous drinking. Additionally, the odds of suboptimal ART adherence were 1.87 (CI=1.08, 3.25) times the odds of compared to non-users.

Conclusions: Our study identified marijuana use and hazardous drinking as significant predictors of suboptimal ART adherence among PLWH reporting pain. Therefore, strategies seeking to reduce HIV transmission by improving ART adherence should target substance use factors.

145. FIRE ANTS, INVADERS TO THE ENCHANTED ISLANDS: THE GALAPAGOS

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Invasive species pose major threats to public health, natural and agriculture environments resulting in billions of dollars in economic losses annually and are a major concern for oceanic archipelagos, such as the Galapagos. This world heritage site has been affected by >1,579 alien terrestrial and marine species, including 545 terrestrial insects. The tropical fire ant (Solenopsis geminata), one of the most invasive insects, has reached these enchanted Islands. Thus, in our study the goal was to determine the putative source population(s) of four nests found in the island of Santa Cruz, the most human

populated island in the Galapagos. First, we determined the microsatellite genotype based on 43 loci of one worker from each nest. Then we sampled 45 S. geminata nests from several sites in Ecuador and obtained their genotypic profile. We combined this newly generated data with a database previously obtained in our lab that includes genotypic information from almost 200 tropical fire ants from around the world. Preliminary analysis suggests that Ecuador is source of the nests found in the Galapagos. The port city of Guayaquil (Ecuador) is the closest continental site to the islands and would be a likely source, it is part of the main transport routes with most flights originating in Quito with a stopover in Guayaquil, and cargo boats travel monthly from Guayaquil to the Galapagos. More detailed analyses are needed to confirm these results and narrow the geographic origin of the tropical fire ants from the Galapagos within Ecuador. Towards that goal, we conducted a recent sampling in two other islands: Isabela and San Cristobal. Future sampling is planned in Floreana and Santa Cruz. Our ultimate goal, it is to use these genetic data to pinpoint putative source populations and help in the development of biological controls against the tropical fire ants in the Galapagos.

146. GENETIC COMPARISON OF PINEAPPLE HEART ROT ISOLATES FROM COSTA RICA AND ECUADOR

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Phytophthora, meaning plant destroyer, is a genus of oomycetes that causes environmental and economic damage across the globe. It is comprised of over 100 known species and hundreds more are believed to exist. Phytophthora nicotianae, known for its broad host range, can be found in various habitats such as watersheds, vegetables, herbs, forest trees, mountain ecosystems, and recycled irrigation water. Costa Rica, a global leader in tropical agriculture, and Ecuador export a majority of their pineapples to the United States and European markets. In both countries, heart rot disease in pineapples is caused by P. nicotianae. This disease has led to reduced crop yields as well as increasing pest management costs. The objectives of this study were to genotype P. nicotianae isolated from pineapple plants with heart rot disease from Costa Rica and to determine the genetic variation between P. nicotianae causing pineapple heart rot disease in Ecuador and Costa Rica. Due to a history of exporting pineapples from Costa Rica to Ecuador, we hypothesized that the genotypes of the pathogens from the two countries would be similar. Our sample included thirty isolates from pineapple in Ecuador and thirteen from pineapple in Costa Rica. We sequenced two mitochondrial loci, cox2+spacer and trnG-rns, and genotyped nine SSR loci. While the isolates from Ecuador appeared clonal, we observed more genetic variation in our smaller sample from Costa Rica. The isolates from Ecuador had a mitochondrial haplotype that was not observed in the Costa Rica isolates. Our

results indicate that the population structure of P. nicotianae on pineapple differs between Costa Rica and Ecuador. At this time, we did not find support for our hypothesis that the pineapple pathogen in Ecuador originated from Costa Rica, but a larger sample from Costa Rica is required given the apparent genetic diversity in this population.

147. HOUSE FLIES AS A VECTOR FOR FARMING ASSOCIATED ANTIBIOTIC RESISTANT BACTERIA

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In the agricultural industry, the introduction of antibiotics was a major breakthrough in terms of improving productivity through reducing disease prevalence in farm animals. However, the rise in antibiotic resistance is becoming a global issue due to the prolonged and excessive use of antibiotics. House flies, the most abundant insect associated with livestock, poultry and other animals are also a notorious mechanistic vector for multiple pathogens in humans and other mammals. In this project we ask the question of whether the house fly bears significant antibiotic-resistant bacteria in the field and the potential for the dissemination of these bacteria. Over the course of several months, we sampled house flies from a livestock farm owned by the University of Florida College of Veterinary Medicine. The prevalence and abundance of antibiotic resistant bacteria isolated from these flies were assayed by plating homogenate of individual fly whole bodies and guts on selective antibiotic media. Morphologically distinct colonies were isolated and identified by Sanger sequencing the full 16S rRNA gene. Our results showed that of the antibiotic resistant bacteria identified, enterococcal species were the most prevalent. Notably, Enterococcus faecium which over the past two decades has been

marked as a leading cause of multi-drug resistant enterococcal infections through horizontal gene transfer of antibiotic resistant genes. In conclusion, our project helps characterize the concern for widespread use of antibiotics in the agricultural industry with respect to the potential of horizontal gene transfer of various antibiotic resistant bacteria.

148. MICROBIOTA OF CANCEROUS AND HEALTHY BREAST TISSUES

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Hypothesis: We hypothesize that the microbial profiles of cancerous breast tissue differ in both abundance and composition from that of healthy, non-cancerous breast tissue. Furthermore, we hypothesize that there will be different microbial profiles in the left and right breast tissues of healthy women.

Background: Microbial communities of various complexities inhabit many sites of the human body and are thought to contribute to overall health. Interest in the role of the microbiome in association with various health outcomes, including cancers, has been increasing over the past decade. In fact, the host microbiome has been implicated in cancers of various sites such as colorectal, liver, and breast. Cancer is a complex disease with a largely undetermined

etiology. Though several risk factors have been identified, they do not fully explain the majority of cases. While fecal microbiota have previously been studied in relation to breast cancer (BC), the role of the microbiota within the breast tissue itself has only been of recent interest concerning the risk for development and progression of BC. In this study, we aimed to evaluate the microbial profiles of healthy and cancerous breast tissues to identify specific microbial characteristics that might be correlated with risk of BC.

Methods: A total of 10 BC tissue samples were obtained from the UF Biorepository. Healthy breast tissue samples were obtained from the UF Department of Plastic Surgery from ten women undergoing reduction mammoplasty. Two pairs of tissue samples from a similar area were collected from each breast. Following assessment by the UF Department of Pathology, 3-5 grams of each sample were snap frozen and sent to our lab for analysis. After DNA extraction, 16S rRNA genes were amplified via PCR and then sequenced. Data were analyzed and evaluated using QIIME and Phyloseq softwares.

Results & Conclusions: Multiple DNA extraction methods were tested to enrich bacterial DNA while minimizing co-extraction of human DNA. A tissue DNA kit yielded both bacterial DNA and PCR product. A blank control also yielded PCR product, indicating concerns for contamination during the procedure that we are currently investigating. Measures of alpha diversity were similar for cancer and healthy tissues. We did not detect clustering by individuals or within each breast. However, some clustering was observed for cancer tissue, but not healthy tissue. In conclusion, while there appear to be differences in microbial diversity between cancerous and healthy tissues, current protocols must be optimized for future unequivocal analysis.

149. RESEARCH TO PRACTICE DISCUSSIONS ON HEAT RELATED ILLNESS

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Heat related illness (HRI) and climate change are contentious issues in which multiple stakeholders have an interest including organizations, academic scientists, individuals, community leaders and specific interest groups. Research surrounding these issues cuts across several academic areas and informal partnerships of academics may come together to share information and educate/communicate scientific findings. These informal partnerships often start communication efforts with research reported and shared among partners and colleagues. This initial first step may provide information for informed decision making and broader dissemination through additional mediated and non-mediated communication channels. The Southeastern Coastal Center for Agricultural Health and Safety (SCCAHS) put together a slate of esteemed speakers on the topic of heat related illness, showcasing research at the intersections of HRI and climate change as it relates to the health and safety of outdoor workers and farmworkers, as well as athletes and military personnel. This crosscutting meeting brought together researchers from various fields to present current findings and pave the way for developing future research collaborations on these topics. The SCCAHS HRI State of the Science Meeting took place October 25-26 at the Don CeSar hotel in St. Petersburg, Florida, with HRI poster session occurring on October 25. Speakers' panel included scientists from environmental and occupational health, exercise science, athletic training, social sciences, nursing, primary health care, atmospheric sciences, and biological sciences. A facilitated panel discussion regarding future steps for HRI research occurred following speaker presentations. Major themes from the discussion included developing

collaborations among scientists and stakeholders, breaking down barriers to get information and working with hard to reach audiences. These themes will serve as foundations for further research with the SCCAHS.

150. SALIVARY MICRORNA AS NOVEL BIOMARKERS

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Background: MicroRNAs (miRNA) have grown in popularity as biomarkers for disease diagnosis as they are abundantly and stably expressed in both tissues and bodily fluids. Plasma miRNA have been linked with AIDS progression. Saliva has been reported as possible source of biomarkers as it contains proteins, mRNA, miRNA, and metabolites. Similarity of plasma and saliva biomarkers suggests a possible role for exosomes in the transport of salivary biomarkers from blood to saliva. Saliva testing is relatively inexpensive and an easily accessible specimen, and because profiles in plasma and saliva often overlap, saliva is an appropriate solution for both systemic and local screening. We hypothesize that plasma miRNA identified as markers of HIV/AIDS disease progression and HIV-associated neurocognitive disorders are also found in saliva.

Methods: Saliva and plasma samples of were obtained from willing HIV-negative participants according to the study IRB. Before exosomes separation, cells and bacteria and cell debris are separated by centrifugation. Exosomes were concentrated performing two ultracentrifuge steps. To confirm presence of exosomes, negative stain transmission electron microscopy was performed. MiRNA

purifications were performed exactly as described in miRNeasy Serum/Plasma Kit. RNA samples, including miRNAs, were eluted into RNase-free water and preserved at -80 ºC or used immediately in cDNA preparation. For qPCR assay, the resulting miR-Amp product of each sample were subjected to ten unique miRNA assays as well as an endogenous control (miR-16) and a negative control (cel-miR-19) as it is endogenous of C. elegans. Data were analyzed using Bio-Rad CFX Manager 3.1 and Prism v6.0.

Results: Presence of exosomes was confirmed by negative stain transmission electron microscopy. Evidence of expression of miRNA obtained from exosomes isolated from saliva and plasma from HIV-negative individuals. Overall all miRNA tested were found in both saliva and plasma, with miR-27a, miR-29a, miR-146a showing higher expression (7-fold and above) in saliva as compared to plasma. These results indicate that miRNA expression in saliva and plasma is overlapping, in line with the underlying hypothesis of a possible exosomal cross-talk between the plasma and saliva, and revealed that saliva is a useful non-invasive specimen and can be used for miRNA targeted biomarker detection in HIV clinical practice.

Conclusions: This is the first step towards identifying new biomarkers that can be used to help guide treatment decisions, evaluate interventions, and/or help with prognosis of HIV/AIDS disease and progression.

151. SUPPLY CHAIN ANALYSIS FOR THE PESTE DES PETITS RUMINANTS VACCINE IN THE KARAMOJA REGION TO IMPROVE VACCINE AVAILABILITY AND REDUCE LOGISTICAL COSTS

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Peste des Petits Ruminants (PPR) is a viral disease that affects small ruminants such as sheep and goats. PPR is currently targeted for eradication by 2030 as it is one of the causes of food insecurity and economical loss among pastoralist communities and livestock owners. Challenges in the eradication program are of interdisciplinary nature and wide in range such as: different social and gender dynamics of livestock owners among different cultures, achieving sustainability of the project by providing vaccines to livestock owners at a price they are willing to pay, and logistical challenges such as poor infrastructure and remoteness. The Livestock Systems Innovation Lab at UF has an ongoing project for PPR vaccination in Uganda and Kenya where approximately one million goats are to be vaccinated. A supply chain analysis of the distribution methods was conducted to improve the vaccination coverage and reduce the cost, thus making it more accessible for livestock owners and improving the efficacy of vaccination programs. The PPR eradication strategy faces many challenges and this project focused on the following: Remoteness: Karamoja is a remote area in northern Uganda with poor infrastructure. Strategic location of distribution centers, storage facilities and other infrastructure in the distribution network is key in order to increase availability and reduce costs. Constraints such as the lack of a reliable electrical grid and security must be taken into consideration. This information would also be used to generate a transportation model to improve delivery. Poor availability of the vaccine: Uncertainty on the demand for the vaccine makes forecasting difficult. Without adequate forecasting methods there is a higher risk of holding a sub-optimal amount of inventory, which leads to increased costs and poor availability. Gathering data to determine optimal inventory at

each stage of the supply chain is one of the objectives that could help improve availability. Coordination in the Supply Chain: The communication and data sharing between supply chain actors is key for optimizing the distribution process. Identifying ways to improve communication channels will guarantee reliable data from all levels in the supply chain. This would also allow to design a model of the supply chain in order to propose a redesign if needed. Such models have been previously used to propose improvements in the vaccine supply chain of African countries.

152. SUPPRESSION OF BIPOLARIS SPP. BY THE SAPROPHYTIC FUNGUS CLADOSPORIUM PSEUDOCLADOSPORIOIDES.

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Bipolaris species are important plant pathogens that cause leaf spots, blight, crown rot and melting out diseases of primarily grass hosts. Cladosporium is a fungal genus which has a primarily saprophytic lifestyle, but some species have been reported to have antagonistic effects to biotrophic fungi, including rusts pathogens. Recently, we co-isolated Bipolaris and Cladosporium from necrotic lesions on native and invasive understory grasses in Indiana forests. Initial observations suggested that the Cladosporium isolates were parasitic to Bipolaris conidia and appeared to reduce the likelihood of successful Bipolaris isolation. Multi-locus sequencing identified Cladiosporium isolates as C. pseudocladosporioides. We examined the antagonistic effect of C. pseudocladosporioides on Bipolaris in media assays. Hyphae of C. pseudocladosporioides were able to invade and outcompete Bipolaris colonies. We characterized the

interaction between the fungi using light microscopy to look for appressoria and other evidence of pathogenesis. We propose that C. pseudocladospoiroides may mediate the interaction between Bipolaris pathogens and grass hosts.

153. THE BOVINE MECONIUM MICROBIOTA VARIES WITH BIRTHWEIGHT AND INFLUENCES THE GUT MICROBIOTA ESTABLISHMENT DURING EARLY LIFE

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The “sterile womb” dogma has been greatly challenged by the “utero colonization” hypothesis recently. Here, we confirmed that the meconium of newborn calves are not sterile using metagenomics analysis of 268 meconium samples from newborn calves. Bacteria were detected in the meconium of 68% (182 samples) of newborn calves. Interestingly, the newborn calves with bacterial DNA detected had lower birth weight compared to others ($P = 0.033$). In addition, we found that newborn calves in low birthweight group harbored more diverse bacteria compared to those in high birthweight group with greater Shannon index ($P = 0.034$). The meconium microbiota structure of newborn calves among birthweight groups was significantly different ($P = 0.046$). Notably, the relative abundance of pathogenic bacteria including Pseudomonas, Legionella, and Campylobacter in the low-birthweight group was 20% higher than the high-birthweight group. Higher prevalence of bacteria in the meconium and the association between meconium microbiota composition and birthweight suggest that the bacterial colonization before birth have impact on early fetal

development. To further explore the influence of the meconium microbiota on the development of gut microbiota, we analyzed the gut microbiota 3 months after birth from the cohort calves. Positive correlations in the relative abundance of phyla Firmicutes (P = 0.006) and Bacteroidetes (P = 0.014) between meconium and 3-month feces were detected, indicating the long-term influence of meconium microbiota on gut microbiota structure. This study provides insights into bacterial colonization in fetal development and suggests the role of early-established microbiota on animal development before and after birth.

154. THE EFFECT OF HUMAN GUT MICROBIOTA ON THE ORGANISMAL PROTEOSTASIS

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The human gut microbiota (HGM) is a complex "organ" that harbors an estimated 150x more genes than are found in the human genome. Bacterial populations within the human gut synthesize metabolites, peptides, hormones, signaling molecules, antibiotics, and other molecules that maintain proper function of the human body. As such, the HGM has been implicated in physiological and pathophysiological processes, including aging and neurodegeneration. Conditions that induce dysbiosis in the HGM, such as infections, antibiotic use, and aging, have been shown to exacerbate neurodegenerative diseases; however, due to the of the polymicrobial communities in the gut, the molecular basis of the host-bacteria interactions and their consequences on human health

are not well-understood. Consequently, we are using C. elegans to study the influence of the human gut microbiota on organismal biology. C. elegans is an established model organism that has been successfully utilized in the study of microbial colonization, pathogenicity, aging, and protein conformational diseases. Its intestine shares many similarities with human intestinal epithelium and has been shown to be colonized by anaerobic bacteria, providing an ideal environment in which to study the obligate anaerobes that colonize the human gut. Utilizing the intestine as a test tube, we are characterizing the effect of microbial colonization on the protein-folding environment across C. elegans tissues. Our preliminary data show that colonization of the C. elegans intestine by monocultures of bacteria found in the human gut enhances localized protein misfolding and aggregation. These results suggest that gut bacteria directly affect protein homeostasis (proteostasis).

155. THE EFFECT OF SOCIOECONOMIC STATUS ON AEDES ALBOPICTUS SIZE

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Mosquitoes in the genus Aedes are a major public health concern as vectors of diseases including dengue, chikungunya, and Zika. Aedes albopictus were introduced to the United States from Asia in the 1980s and have since spread as far north as Connecticut. Ae. albopictus are characterized as anthropophilic, aggressive biters and container breeders. These adaptations result in greater population density in areas of lower socioeconomic status, where discarded containers and access to humans are common. Adult mosquito size is correlated with fitness and their effectiveness as a vector of

disease. In Ae. albopictus, wing length has been found to be an indicator of body size and fecundity. In this study, we used a collection of Ae. albopictus from Charlotte, NC to identify spatiotemporal patterns of adult mosquito size across socioeconomic status. These samples were collected from June to August 2017 from 90 sites that were stratified by socioeconomic status. We measured wing length of 236 adult female specimens with ImageJ. We compiled the wing length measurements by neighborhood class and collection month. In the statistical program R, we used an ANOVA and a Tukey test to search for differences in wing lengths in each socioeconomic neighborhood class over time. We found a statistically significant difference in adult wing length between June and August across all pooled classes, with longer wing lengths in June. There was not a statistically significant difference in wing length between June and July nor July and August. We found no significant differences between individual classes within specific months. The temporal dynamics in wing length may be linked to similar trends in vector fecundity and disease transmission potential.

156. THE MOLECULAR PHYSIOLOGY OF CARBON DIOXIDE IN THE LARVAL MOSQUITO TRACHEAL SYSTEM

Cherie Saffold - Department of Anatomy and Cell Biology, Whitney Laboratory for Marine Bioscience, Whitney Laboratory for Marine Bioscience, College of Medicine, University of Florida; **Paul Linser** - Department of Anatomy and Cell Biology, Whitney Laboratory for Marine Bioscience, Whitney Laboratory for Marine Bioscience, College of Medicine, University of Florida

The Molecular Physiology of Carbon Dioxide in the Larval Mosquito Tracheal System Cherie E. Saffold and Paul J. Linser, UF Whitney Laboratory The physiology of carbon dioxide elimination in the larval mosquito, a potential target for controlling the animals that cause more than 1 million deaths each year, is poorly understood. It is known that one method of carbon dioxide removal is by direct diffusion through the larva's cuticle. However, the molecular components that propagate this transcuticular diffusion are unknown. Previous study has shown that carbonic anhydrases 9 and 10, the anion exchanger AE1, and Na+/K+ ATPase play critical roles in pH regulation of the alimentary canal by ionizing carbon dioxide and transporting its ionic derivative, bicarbonate (Linser et al. 2009). The purpose of this study is to determine where these three molecular components are located in the larval mosquito's tracheal system. Paraffin sectioning, whole mount preparation, antibody labeling, confocal microscopy, and protein analysis by SDS- page western blot were used to achieve these goals. The immunohistochemical data strongly suggests that all three components are present in their predicted locations. The western blot suggests that carbonic anhydrase 9 is present in the tracheal epithelium, but its presence in the hemolymph is inconclusive. The hypothesized molecular physiology of each component is supported by the data.

157. THE RESURGENCE OF VACCINE-PREVENTABLE DISEASES IN VENEZUELA: A THREAT TO REGIONAL PUBLIC HEALTH IN THE AMERICAS

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Venezuela's tumbling economy and authoritarian rule have led to an unprecedented humanitarian crisis. There is a massive exodus of biomedical scientists and qualified healthcare professionals. Venezuelans are struggling to survive in a country with the world's highest annual inflation rate of 46,305% and a minimum wage of USD$1.79 (5.196.000 Bolivars) per month. Sixty-six percent of the population lives in extreme poverty and more than 280,000 children are at risk of death from malnutrition. According to the the last official nation-wide epidemiological bulletin (published in 2016), infant and maternal mortality had risen by 30% and 65%, respectively, over the previous year. Since then, no further national epidemiological records have been released. Long-term shortages of essential medicines and medical supplies, interruption of epidemiological surveillance systems, weakening of immunization programs, and an unprecedented exodus of trained medical personnel have set the stage for the resurgence of vector-borne and vaccine-preventable infections. We discuss the ongoing epidemics of diphtheria and measles and their disproportionate impact on indigenous populations. We also discuss the ongoing malaria

epidemic which is now approaching half a million cases per year, and continuing to increase at rates exceeding those previously reported anywhere in the world. The return of measles and other vaccine-preventable childhood infections in Venezuela has been recognized by the World Health Organization-Pan American Health Organization (WHO/PAHO), which has also emphasized the risk of expansion beyond Venezuelan borders. For example, in Colombia, 45 cases of imported measles in migrants from Venezuela had been recorded as of August, 2018. As Venezuelans flee their country en masse – ca. 2 million since 2014 (11), not only to Colombia (> 820,000 migrants), but also to Ecuador (>450,000) and Brazil (>57,000) (11-13) – there is continued risk that vaccine-preventable diseases will be carried with them. The UNHCR has launched a supplementary appeal for funding of these affected countries. Simultaneously, the spread of vaccine-preventable diseases must urgently be tackled within Venezuela, with an emphasis on local outbreaks, as well as at critical border areas.

158. UF'S SOUTHEASTERN COASTAL NIOSH AG CENTER: SURVEILLANCE STUDIES TO SUPPORT SEAFOOD WORKER HEALTH AND SAFETY IN GULF COAST COMMUNITIES

Andy Kane - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Robert Myers** - Kentucky State University; **Melvin Myers** - Emory University

Surveillance studies with Gulf coast fishers, crabbers, shrimpers, and oyster and clam harvesters are underway to identify risk factors associated with fatal and non-fatal injuries where the majority of workers are self-employed and uninsured. Community partnerships highlight the importance of engaging with seafood workers to implement an in-person questionnaire tool supplemented with workplace observations on harvesting and fishing vessels. Falls overboard and winch injuries are associated with many of the fatalities and severe injuries reported. Musculoskeletal injuries, cuts and lacerations, bites, spine punctures, vibriosis, and heat and sun exposure are also concerns for these workers. Conditions associated

with unstable work platforms in harsh settings, coupled with declining fisheries – related in part to climate and environmental change – appear to increase risk of onboard incidents, drug use and mental health issues. Surveillance data will guide the development of interventions and outreach tools to support Gulf coast seafood worker health and safety.

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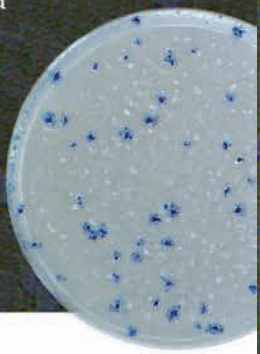
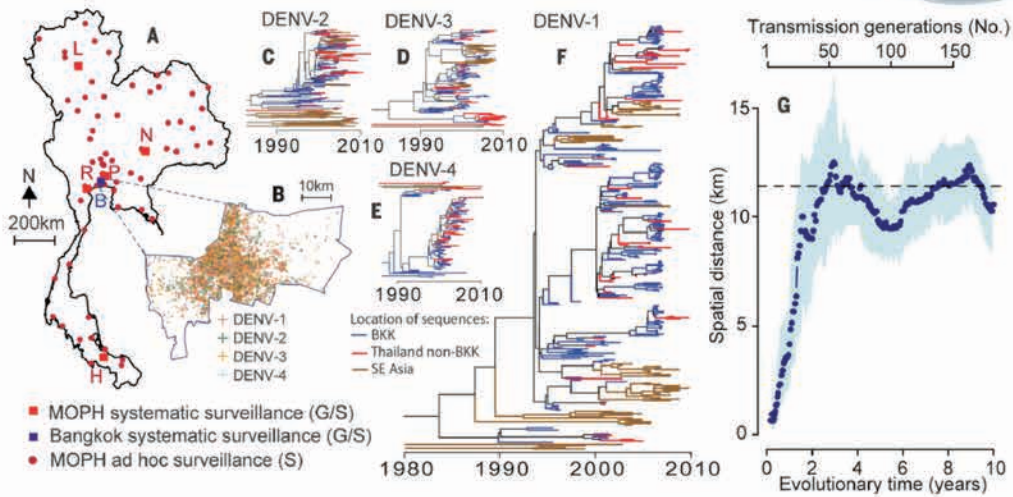
The Cummings lab is conducting a study of human and avian influenza in southern China to understand the spatial dynamics of influenza transmission and the accumulation of immunity to human and non-human influenza viruses (avian and porcine) over a life time. The study seeks to identify the immune landscape that dictates patterns of influenza spread. The Cummings group has followed this cohort, called the Fluscape cohort, continuously since 2009. The group uses a number of serological techniques to quantify immunity to influenza and other pathogens. The photo from the front cover shows a bird market from Guangdong Province, an area where H5N1 avian influenza and the SARS virus emerged. The image on the back cover shows a neutralization test characterizing dengue immunity, another pathogen the lab works on.





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