The Tuanyok Laboratory is investigating the impacts of melioidosis in Southeast Asia. *Burkholderia pseudomallei*, a soil bacterium, is the causative agent of the neglected tropical disease melioidosis. Tuanyok Laboratory has implemented a "One Health" approach to investigate the burdens of melioidosis in humans and animals, as well as the occurrence of *B. pseudomallei* in soil and water throughout Southeast Asia. The image on the cover depicts both dry and wrinkle colonies of *B. pseudomallei* among other bacteria grown on Ashdown's agar from a soil sample collected from a goat farm in southern Thailand.

For more information, please visit
http://www.vetmed.ufl.edu/about-the-college
/faculty-directory/apichai-tuanyok/

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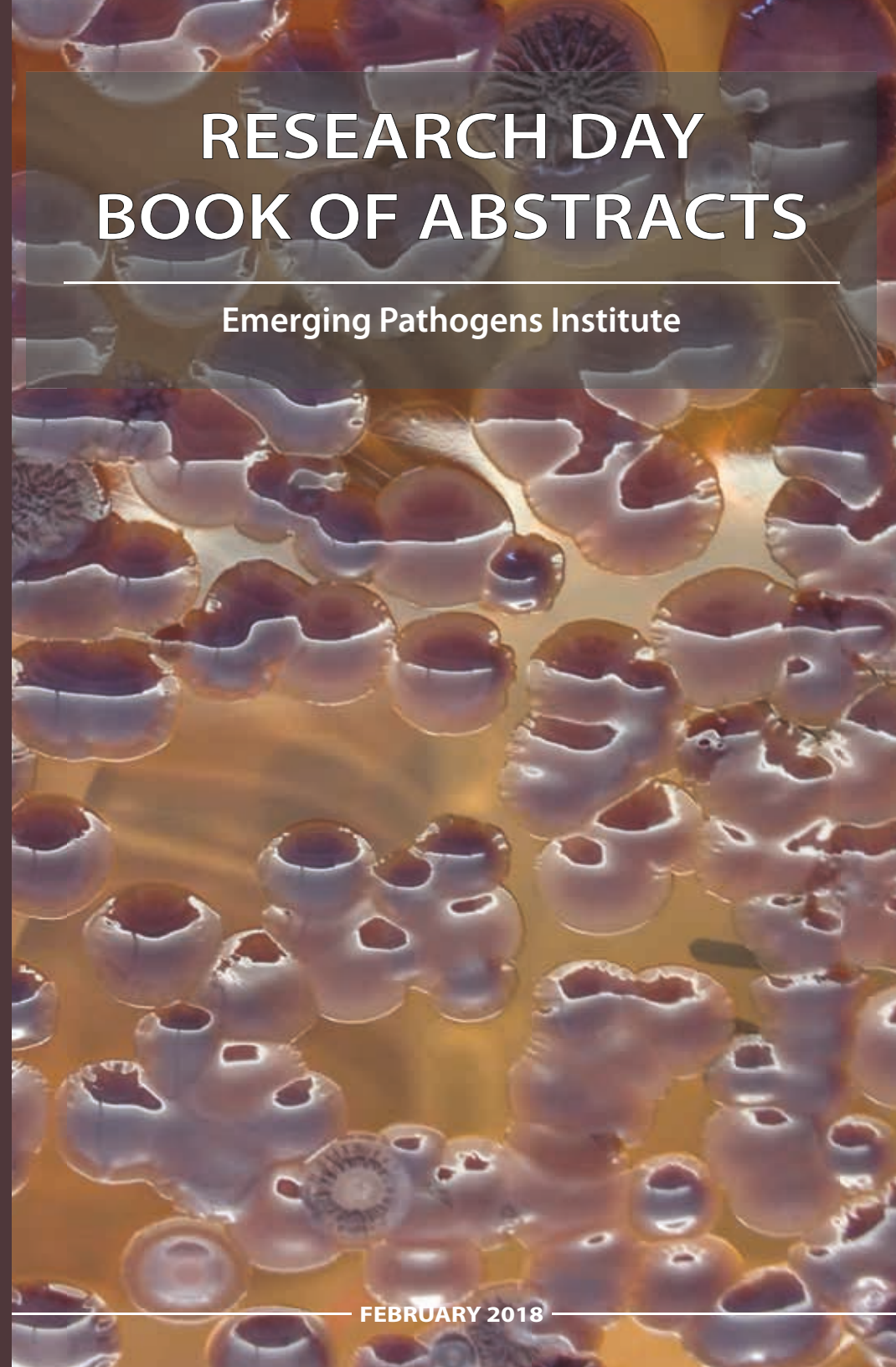
EPI RESEARCH DAY BOOK OF ABSTRACTS 2018

RESEARCH DAY BOOK OF ABSTRACTS

Emerging Pathogens Institute



FEBRUARY 2018

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Letter from the Director

Welcome to the eleventh 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 pathogen-related research conducted by EPI members and collaborators. We are particularly pleased to welcome investigators from outside of UF, including investigators from the Florida Department of Health and other collaborating universities.

This year we have the honor of introducing you to two outstanding investigators visiting us to give speeches during our afternoon session.

Peter M. Small, M.D., is the founding director of the Stony Brook University Global Health Institute. His expertise includes tuberculosis (TB) and global health. For more than a decade Dr. Small was responsible for building and running the innovative TB program at the Bill & Melinda Gates Foundation. He has done seminal work on the clinical, epidemiologic, evolutionary, and genetic aspects of tuberculosis. He has deep expertise in translating cutting edge science into drugs, diagnostic methods and vaccines as well as in the business and public health processes to get innovative tools to those in need. He is joined by Dr. Andrew Pekosz, co-director of the Johns Hopkins Center for Excellence in Influenza Research and Surveillance and director of the Center for Emerging Viral Infectious Diseases. Dr. Pekosz has served on a number of National Institute of Health scientific and policy review boards focused on biosafety and biocontainment and is an expert on the topics of influenza, biosafety, emerging infectious diseases and pandemic preparedness.

Please visit our website, www.epi.ufl.edu, to join our listservs 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. Jack M. Payne, Senior VP for Agriculture and Natural Resources Head of the Institute of Food and Agricultural Sciences (IFAS)* 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)
Dr. Peter Small
Founding Director and Jim and Robin Hernstein Chair of the Global Health Institute at Stony Brook University

"Disrupting TB Care and Control"

(2:10-3:10)
Dr. Andrew Pekosz
Professor of Molecular Microbiology and Immunology and Director of the Center for Emerging Viral Infectious Diseases at the Johns Hopkins Bloomberg School of Public Health

"Surveillance for Human Influenza Virus Infections: More Than Just Choosing Vaccine Strains"

01. A MECHANISTIC BACTERIAL TRANSPORT MODEL TO INFORM FOOD SAFETY MANAGEMENT OF AGRICULTURAL POND WATER

Kathleen Vazquez - Department of Agricultural and Biological Engineering, University of Florida Water Institute, College of Agricultural and Life Sciences, University of Florida; **Arie Havelaar** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Rafael Muñoz-Carpena** - Department of Agricultural and Biological Engineering, College of Agricultural and Life Sciences, University of Florida

Irrigation water is considered a major pathway to fresh produce for food-borne illness related pathogens. The Food Safety Modernization Act (FSMA) specifies sampling-based methods using Escherichia coli as an indicator organism and microbial criteria, geometric mean (GM) and statistical threshold value (STV), to regulate agricultural water. These regulations lack preventive measures, important to controlling the processes that introduce bacterial contamination into water sources. To inform FSMA regulations concerning agricultural water, this study develops a simple mechanistic model to predict the microbial quality of agricultural water. This model proved useful to simulate data from a highly variable surface water irrigation pond in West Central Florida. The performance of the model was similar or superior to existing pathogen transport models, with a Nash-Sutcliffe efficiency of 0.455 when incorporating observed value uncertainty. Global sensitivity analysis was used to reveal the most important processes controlling bacterial water quality criteria: aquatic removal rate and transport dynamics of bacteria for GM, and bacterial source and transport dynamics for STV. It was also found that large peak E. coli concentration events were mechanistically driven by rainfall/runoff processes. From these findings, we suggest improved sampling schedules that focus on peak event timing and practices that exclude wildlife and other potential bacterial sources. Vegetative filter strips, when properly designed and maintained, also provide an

opportunity to mitigate bacterial transfer into the agricultural waters by reducing runoff flow and promoting pollutant settling.

02. ACTUATION OF CHITOSAN-APTAMER NANOBRUSH BORDERS FOR PATHOGEN SENSING

Eric McLamore - Department of Agricultural and Biological Engineering, College of Agricultural and Life Sciences, University of Florida; **Carmen Gomes** - Department of Mechanical and Aerospace Engineering, Iowa State University; **Nick Cavallaro** - Department of Agricultural and Biological Engineering, College of Engineering, University of Florida

We demonstrate a sensing mechanism for rapid detection of Listeria monocytogenes in food samples using the actuation of chitosan-aptamer nanobrush borders. The bio-inspired soft material and sensing strategy mimic natural symbiotic systems, where low levels of bacteria are selectively captured from complex matrices. To engineer this biomimetic system, we first develop reduced graphene oxide/nanoplatinum (rGO-nPt) electrodes, and characterize the fundamental electrochemical behavior in the presence and absence of chitosan nanobrushes during actuation (pH-stimulated osmotic swelling). We then characterize the electrochemical behavior of the nanobrush when receptors (antibodies or DNA aptamers) are conjugated to the surface. Finally, we test various techniques to determine the most efficient capture strategy based on nanobrush actuation, and then apply the biosensors in a food product. Maximum cell capture occurs when aptamers conjugated to the nanobrush bind cells in the extended conformation (pH < 6), followed by impedance measurement in the collapsed nanobrush conformation (pH > 6). The aptamer-nanobrush hybrid material was more efficient than the antibody-nanobrush material, which was likely due to the relatively high adsorption capacity for aptamers. The biomimetic material was used to develop a rapid test (17 min) for selectively detecting L. monocytogenes at concentrations ranging from 9 to 10^7 CFU-mL-1 with no pre-concentration, and in the presence of other gram-positive cells (Listeria innocua and Staphylococcus aureus). Use of this bio-inspired material is among

the most efficient for L. monocytogenes biosensing to date, and does not require sample pretreatment, making nanobrush borders a promising new material for rapid pathogen detection in food.

03. AN ALTERNATIVE RISK RANKING METHOD BASED ON LOG TRANSFORMATION FOR RANKING PRODUCE-HAZARD PAIRS

Min Li - Department of Animal Sciences, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Moez Sanaa** - French Agency for Food, Environmental and Occupational Health & Safety; **Barbara Kowalcyk** - Ohio State University; **Kostas Koutsoumanis** - Aristotle University of Thessaloníki; **Arie Havelaar** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Risk ranking approaches can help identify and prioritize foods and/or hazards that may pose greatest risks to public health. The use of semiquantitative risk ranking methods are relatively simple and flexible, but could result in substantial loss of information and limited resolution. The study aimed to compare published semiquantitative risk ranking approaches with an alternative quantitative approach that includes log transformation (either with or without binning) to score individual food hazard pairs across the ranking criteria, using fresh produce as a model system. Data from literature were used to define scoring bins for ranking criteria used in a published risk ranking model. 10,000 food pathogen pairs were randomly generated from uniform distributions over realistic ranges of the criteria using standard risk assessment methods to define a reference set, and these random variables were then transformed and aggregated according to the different ranking methods.The semiquantitative method used bins to assign each criterion to an arbitrarily defined number, and the alternative methods used log transformed risk scores on a scale between 0 and 1 with or without binning. Individual criteria scores were then summed to derive a final risk score for each produce pathogen pair. The ranking methods were compared to the reference set using scattergrams and Kendall's rank correlation coefficient. The alternative quantitative

methods had markedly higher correlation coefficients than those of the semiquantitative method. The log transformation without binning provides the best ranking relative to the reference method, and the log transformation with binning performs almost the same. The results indicated that use of a quantitative model allows for a higher resolution and reduction in the loss of information and better alignment with sound mathematical principles. A fully quantitative risk ranking method provides a useful approach to prioritize produce pathogen pairs and support risk based decision making.

04. APPLICATION OF RESUSCITATION PROMOTING FACTOR (RPF) TO FACILITATE DETECTION AND RECOVERY OF SALMONELLA FROM FOOD PRODUCTS

Chase Labiste - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Nicolette Duong** - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Andy Le** - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Karla Sanchez** - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Amanda Marika Macarenhas** - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Anita Wright** - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Douglas Archer** - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

The ability to detect pathogenic microorganisms through culturing techniques is critical to food safety; however, pathogenic bacteria, such as Salmonella enterica, can enter into a viable but non-culturable (VBNC) state in which they are not detected using

standard agar culture (1). VBNC Salmonella pose an enormous public health problem as these pathogens remain capable of resuscitating in a host when ingested and causing disease. The VBNC state is a survival mechanism and can be induced when the bacteria are exposed to starvation or other stressors, such as cold temperature or high salinity. Previous studies by Panutdeporn et. (1) al demonstrated that a resuscitation promoting factor (Rpf) could resuscitate Salmonella Typhimirium from the VBNC state. We surveyed a subset of the GenomTrakr database, and found all strains screened to date have the Rpf gene and show 100% identity. In the present research, induction of VBNC Salmonella was accomplished by exposing 7-8 log CFU/mL to osmotic shock using 0.01 M phosphate buffer with 0.0027 M potassium chloride and 1.2 M sodium chloride. Cultures were maintained at 37C with aeration, and viability was determined microscopically by staining with dyes (BacLight) that differentiate live vs. dead cell. Cultures remained at 41-52% viable despite loss of culturability on standard media agar after 4-5 days. Induction period for VBNC varied with the size of inocula. This model will be used to examine the parameters that may influence the induction and resuscitation from the VBNC state. We will study the efficacy of recombinant Rpf to resuscitate Salmonella across multiple genotypes. Results should provide relevant information on strategies to improve detection of pathogens in food and increase the efficacy of disinfection/sanitation protocols throughout the all stages of food production.

05. COMPARATIVE EVALUATION OF SALMONELLA VACCINES DERIVED FROM UK-1 AND 14028S

Shilpa Sanapala - Department of Infectious Diseases and Immunology, College of Veterinary Medicine, University of Florida; **Leandra Mosca** - Department of Infectious Diseases and Immunology, College of Veterinary Medicine, University of Florida; **Shifeng Wang** - Department of Infectious Diseases and Immunology, College of Veterinary Medicine, University of Florida; **Roy Curtiss III** - Department of Infectious Diseases and Immunology, College of Veterinary Medicine, University of Florida

The initial virulence and invasiveness of a bacterial strain may play an important role in leading to a maximally efficacious attenuated live vaccine. Here we show that χ9909, derived from UK-1 χ3761 (the most virulent Salmonella Typhimurium strain known), is effective in protecting mice against lethal UK-1 and 14028S (less virulent S. Typhimurium strain) challenge. As opposed to this, 14028S- derived vaccine χ12359 offers suboptimal levels of protection, with survival percentages that are significantly lower against lethal UK-1 challenge doses. In addition, χ9909 showed markedly higher colonizing ability in the spleen, liver, and cecum when compared to χ12359. Enumeration of bacteria in fecal pellets also revealed that χ9909 can persist in the host for over 10 days whereas χ12359 titers dropped significantly by day 10. Moreover, competitive infection with parent strains UK-1 χ3761 and 14028S χ8312 resulted in considerably greater colonization by the former in multiple mucosal and gut associated lymphatic tissues. Contrary to the aforementioned superior protection, colonization, and persistence exhibited by χ9909, anti-OMP and anti-LPS antibody responses generated in χ9909 immunized mice were not significantly different from those induced by χ12359. Cytokine ELISAs revealed that significantly greater levels of IFN- and TNF- were secreted by stimulated splenocytes obtained from χ9909 immunized mice than those from mice immunized with χ12359. Together, these results highlight the possibility that attenuated derivatives of the parent strains with higher initial virulence make better vaccines.

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

Zhengxin Ma - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Minyoung Kang** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Shanyu Meng** - Department of Agricultural and Biological Engineering, College of Agricultural and Life Sciences, University of Florida; **Jacqueline Zamora** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Zhaohui Tong** - Department of Agricultural and Biological Engineering, College of Agricultural and Life Sciences, University of Florida; **Kwang Cheol Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Shiga toxin-producing Escherichia coli (STEC) are major foodborne pathogens. Several methods have been tried to reduce these pathogens at the pre-harvest level. However, with the difficulty of controlling high-risk factors contributing the pathogen prevalence in animals, the trials have not been successful in meeting the industry requirements to be applied for reducing pathogens. Chitosan microparticles (CM), derived from chitosan, have shown great broad-spectrum antimicrobial activity against pathogens. Here, we report that conjugation of CM and anti-STEC IgY antibodies kill STEC selectively. Lipopolysaccharides (LPS) of seven STEC serotypes (O26, O45, O103, O111, O121, O145, and O157) were extracted by hot aqueous-phenol extraction and then purified with high performance liquid chromatography. The purified LPS was confirmed by SDS-PAGE. Five laying hens were immunized with each LPS respectively, and IgY antibodies were purified from egg yolk. The sensitivity and specificity of produced IgY were tested by ELISA. The detection limit of the 7 types 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. CM and the antibodies were linked by stable

covalent amide bonds to form CM-IgY conjugates. The CM-IgY conjugates killed STEC specifically in the presence of other serotypes. The results suggested that the CM-IgY conjugates have strong antimicrobial activity, and is great candidate to eliminate pathogens selectively in the gastrointestinal tract of animals. Risk assessment and animal feeding experiments will be conducted further to test the potential of CM-IgY conjugates.

07. EXTENDED-SPECTRUM B-LACTAMASE-PRODUCING ESCHERICHIA COLI FROM MECONIUM OF NEWBORN CALVES

Lin Teng - Department of Animal Sciences, College of Agricultural and Life Sciences, University of Florida; **Peixin Fan** - Department of Animal Sciences, College of Agricultural and Life Sciences, University of Florida; **Amber Ginn** - Department of Animal Sciences, College of Agricultural and Life Sciences, University of Florida; **Nolle Noyes** - Department of Clinical Sciences, Colorado State University; **Sihong Park** - Department of Food Science and Technology, Oregon State University; **Corwin Nelson** - Department of Animal Sciences, College of Agricultural and Life Sciences, University of Florida; **John Driver** - Department of Animal Sciences, College of Agricultural and Life Sciences, University of Florida; **Christina Boucher** - College of Engineering, University of Florida; **Steven Ricke** - Department of Food Science, University of Arkansas; **Kwang Cheol Jeong** - Department of Animal Sciences, College of Agricultural and Life Sciences, University of Florida

Extended-spectrum β-lactamase (ESBL)-producing Escherichia coli has become a great concern to public health primarily because of its resistance to third-generation cephalosporins, which are widely used in human healthcare facilities to treat bacterial infections. Although it is controversial, it is commonly believed that food animals acquire antimicrobial resistant (AMR) bacteria by receiving antibiotic treatments. The purpose of this study was to identify the earliest time when animals are exposed to ESBL-producing E. coli. Meconium samples were collected from the rectal anal junction of 322 newborn calves. ESBL-producing E. coli were identified from the samples by plating on MacConkey agar supplemented with Cefotaxime (4

μg/mL). Isolates were further characterized with ChromAgar E. coli and CTX-M gene typing using PCR. ESBL-producing E. coli was detected in 7.5% (24/322) of meconium samples of newborn calves. Illumina MiSeq platform was employed for Whole Genome Sequencing (WGS) of 37 strains from 24 calves. After assembly, nineteen representative strains were selected, based on their Sequencing Types (STs) and whole genome architecture, for further bioinformatics analyses and antimicrobial susceptibility test. Following WGS, phylogenetic analysis revealed that these strains clustered into 8 clusters that coincided with globally prevalent STs. All the isolates carried a variety of virulence genes and were resistant to multiple antibiotics. Comparative genomics analysis revealed that ESBL-producing E. coli from meconium harbored unique efflux pump genes and higher copy number of antibiotic resistant genes compared to isolates from cows. In particular, we identified hyper-virulent strains of ST117 that carries Shiga toxin-encoding genes (stxAB), which may cause severe human diseases. This is the first study showing the prevalence ESBL-producing E. coli in meconium of newborn calves, indicating animals are even start to be exposed to AMR bacteria in the uterus.

08. GENOMIC EPIDEMIOLOGY OF TOXIGENIC VIBRIO CHOLERAE O1 IN HAITI

Taylor Paisie - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Carla Mavian** - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Taj Azarian** - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **David J. Nolan** - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Afsar Ali** - Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Meer T. Alam** - Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **J. Glenn Morris, Jr.** - Emerging Pathogens Institute, University of Florida; **Marco Salemi** - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida

Vibrio cholerae is the causative agent of the disease cholera. This bacterium is ubiquitous in aquatic environments, with environmental toxigenic V. cholerae O1 strains serving 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. Then in October 2010, cholera appeared in Haiti for the first time in over 150 years. Previous studies show that the early cases of cholera in Haiti are consistent with a single-source introduction of V. cholerae O1 from Nepalese U.N. peacekeeping troops sent after the earthquake. After the initial epidemic waves, cholera may now be endemic in Haiti, showing seasonal outbreak patterns associated with the rainy season. Because of this clonal, single-source introduction of V. cholerae O1, it presents a unique opportunity to study the evolution and the selective pressures acting on this microorganism. By performing phylodynamic analysis with genome-wide single nucleotide polymorphisms (SNP), we are able to

investigate the ongoing cholera epidemics occurring in Haiti and the underlying evolutionary processes and selective pressures at a remarkable resolution. Since the start of the cholera outbreaks in 2010, the dominate serotype of V. cholerae O1 circulating in Haiti was the Ogawa serotype. Then in 2015, Inaba became the most prevalent serotype in Haiti. The main driver for the switch from the Ogawa to the Inaba serotype is by a nucleotide substitution in the wbeT gene, causing a premature stop codon. Though the switch from the Ogawa to the Inaba serotype is a common phenomenon in the genome of V. cholerae O1, if the Ogawa serotype still remains dominate in the population and an outbreak of the Inaba serotype occurred, this could have been caused by a separate introduction into the population. Although previous studies have shown that the Inaba serotype has been present in Haiti since 2012, it has never propagated and become established in the Haitian population. By using whole-genome SNP analysis, we are able to assess other potential evolutionary changes that are occurring in the V. cholerae O1 genome to cause this switch in serotype. Our results suggest that the V. cholerae O1 strains currently circulating in Haiti have evolved from their initial clonal, single-source outbreak of the Ogawa serotype to the new, unintroduced Inaba serotype.

09. INVESTIGATING THE PRODUCTION AND RELEASE OF SHIGA TOXIN FROM RECENTLY EMERGED SHIGELLA FLEXNERI ISOLATES

Natasha Weatherspoon-Griffin - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Miranda Gray**; **Anthony Maurelli** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

The emergence of Shiga toxin (Stx) production by traditionally non-toxin producing bacterial pathogens is cause for concern because Stx is associated with bloody diarrhea and hemolytic uremic syndrome, a severe and life-threatening kidney damaging sequela. Historically, Stx production and its subsequent clinical diagnoses have been associated with Shigella dysenteriae serotype 1 and Shiga toxin-producing enterohemorrhagic Escherichia coli (STEC) strains. Our lab has reported the presence and expression of Stx in S. flexneri, a traditionally non-toxin producing species. At first glance, it would appear that this is just another bacterial species producing the same toxin. However, key differences such as the presence of the Stx encoding genes on an active bacteriophage as well as the intracellular lifestyle of S. flexneri makes these Stx-producing strains remarkably different than its S. dysenteriae and STEC counterparts, respectively. Here, I report, for the first time, the iron-independent production of Stx from S. flexneri, contrary to those reported for S. dysenteriae isolates, as well as the low iron-induced release of Stx via outer membrane vesicles in both S. flexneri and S. dysenteriae.

10. INVOLVEMENT OF ENDOCANNABINOID SYSTEM IN INNATE IMMUNE RESPONSES TO SALMONELLA TYPHIMURIUM INFECTION

Larry Reser - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida; **Austin Sheppe** - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida

Cannabis is most commonly associated with recreational use, though it has played a medicinal role in societies throughout time. In humans, cannabinoids bind two receptors: CB1 and CB2. CB1 receptors are found within the central nervous system, and their activation is primarily responsible for the psychotropic effects associated with cannabinoids. CB2 receptors are present in lymphoid and myeloid cells and have no psychotropic effects when activated. Instead, CB2 receptors are responsible for the physiological effects correlated to cannabis use, some of which may be beneficial to the host. Endogenous cannabinoids (endocannabinoids) are naturally synthesized within the human body; consequently, they produce no psychotropic effects. Agonistic endocannabinoids, like 2-arachidonoylglycerol, activate CB2 receptors and can be used to independently investigate the physiological effects of THC. More specifically, these drugs can give insight to how cannabinoids impact inflammasome regulation and activation. We first analyzed the effects of 2-arachidonoyl glycerol (2-AG) on bacterial clearance and inflammasome activation. We hypothesize that the inflammasome is upregulated by 2-AG, leading to a reduced bacterial load in Salmonella-infected macrophages. We quantified IL-1beta, a pro-inflammatory, to determine the impact of 2-AG on inflammasome regulation. We performed real-time PCR to quantify transcripts of CB1/2 receptors and the enzymes FAAH and DAGLa, which are linked to endocannabinoid degradation. Our preliminary results have shown an upregulation of CB2 and downregulation of endocannabinoid-decomposing enzymes upon Salmonella infection. The implications of this indicate that the endocannabinoid system may play a role in inflammasome activation and enhance pathogen clearance.

11. ISOLATION OF VIBRIO CHOLERAE O1 STRAINS WITH UNPRECEDENTED SEROLOGIC CHARACTERISTICS FROM SURFACE WATER SAMPLES IN HAITI

Meer T. Alam - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Shrestha Sinha Ray** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Md Mahbubul Alam** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Judith Johnson** - Emerging Pathogens Institute, University of Florida; **Valery Madsen Beau De Rochars** - Emerging Pathogens Institute, University of Florida; **Mohammed H Rashid** - Emerging Pathogens Institute, University of Florida; **J. Glenn Morris, Jr.** - Emerging Pathogens Institute, University of Florida; **Afsar Ali** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

Cholera has been endemic in Haiti with toxigenic V. cholerae has established itself in the aquatic reservoirs in that country. In contrast to some previous investigators' claim that there was no environmental and/or water contamination of V. cholerae in Haiti, we have previously reported that, toxigenic V. cholerae in deed persisting in Haiti with rainfall and temperature contributing the environmental bloom of V. cholerae with subsequent spill over to humans. Although cholera has significantly declined in Haiti in 2017 (Only over 13,000 suspected cases reported by PAHO and MSPP), we have isolated five V. cholerae O1 isolates from two major environmental sentinel sites (one in rural Gressier/Leogane region and the other is newly established in GHESKIO region). Of five isolates, three came from Gressier region while two came from GHESKIO with all of them isolated from independent site. Remarkably, three (two from GHESKIO) and one from (Gresssier) showed only positive agglutination with V. cholerae polyvalent serum that is unprecedented and remains to be further investigated.

The remaining two isolates, included (i) an Ogawa serotype with intact cholera toxin gene, and (ii) an Inaba serotype with loss of ctx gene required for virulence. Data presented here clearly show that environment has played a significant role in diversifying serologic characteristics of V. cholerae O1 strain in Haiti. Our data highlight the importance of continued monitoring of environmental V. cholerae that remains a key to predict the ultimate fate of cholera in Haiti in years to come.

12. MODELLING CAMPYLOBACTER INFECTION DYNAMICS IN YOUNG CHILDREN IN LOW-RESOURCE SETTINGS

Nitya Singh - Department of Animal Sciences, Emerging Pathogens Institute, University of Florida; **Arno Swart** - Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM); **James A. Platts-Mills** - School of Medicine, University of Virginia; **Arie Havelaar** - Department of Animal Sciences, Emerging Pathogens Institute, University of Florida

(A)symptomatic infections with thermophilic Campylobacter spp. are highly prevalent among children in developing countries and have recently been implicated as one of the key factors responsible for environmental enteric dysfunction and stunting in young children. Previous efforts of modelling Campylobacter infection dynamics describe the effect of boosting and waning of immunity in exposed populations, and have suggested that force of infection and probability of asymptomatic infection depend on the exposure frequency and dose. In these studies, parameter estimates were based on the only available experimental challenge-rechallenge study. Due to scarcity of experimental data and observational studies in low-income countries, applying these compartmental models to understand Campylobacter dynamics is still in its infancy. The current study uses recently published observational data on Campylobacter prevalence in diarrheic and non-diarrheic children in the MAL-ED study to estimate model parameters in developing countries. Available data have been transformed to represent the different compartments of the model and were plotted for all eight reported countries for comparative analysis of disease dynamics. Estimation

of model parameters was done with maximum likelihood and Bayesian optimization methods. The current model can be used to predict the impact of changing the exposure frequency and dose on the prevalence of Campylobacter in children, to support hygiene interventions in low resource settings. It provides novel opportunities to estimate the incidence of pathogen-specific diarrheal illness in high exposure populations.

13. PREVALENCE AND MOLECULAR CHARACTERISTICS OF EXTENDED-SPECTRUM BETA-LACTAMASE (ESBL)-PRODUCING ESCHERICHIA COLI IN COMMERCIAL CATTLE FARMS

Shinyoung Lee - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Lin Teng** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Kwang Cheol Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Extended-spectrum β-lactamase (ESBL)-producing Escherichia coli (ESBL-E. coli) have been disseminated intercontinentally through complex transmission routes. Clinically relevant ESBL-E. coli strains have been found in food producing animals, indicating food animals are important reservoirs of ESBL-E. coli. However, ESBL-E. coli in beef cattle raised on grass-feeding cow/calf operations are rarely studied, and limited information is available for the occurrence of this antimicrobial resistant microorganisms. We collected 1,096 samples from 17 commercial beef farms in Florida including feces of calves and cows, soil, water, and forage to understand the occurrence of ESBL-E. coli and to characterize the ESBL-E. coli isolates. Eleven farms had ESBL-E. coli in the majority of samples (animals, forage, and soil) except water samples, and the prevalence of ESBL-E. coli ranged between 1.85 to 19.6%. The average prevalence and concentration were 7.42% and 1.56 log CFU/g of feces (95% CI: 1.37-1.74), respectively. To get insights into the potential virulent properties in ESBL-E. coli, we examined the 59 ESBL-E. coli isolates using whole genome sequencing and comparative genomics. CTX-M (66%, 39/59)

gene was the most predominant ESBL gene type and TEM-type ESBL gene was also encoded in 54% (32/59) of the isolates. Results from the antibiotic resistance genes (ARGs) profiling showed that all isolates were multidrug resistant and the functionality of identified ARGs was confirmed by antibiotic susceptibility test, indicating all strains were resistant against at least 4 different antibiotics. Furthermore, we found that all ESBL-E. coli isolates shared virulent factors related to adherence, invasion, ion uptake, and bacterial secretion systems, suggesting pathogenic potentials of ESBLs. Phylogeny analysis based on core genome alignment with 59 ESBL-E. coli strains made non-specific clusters regardless of the farm location, suggesting ESBLs in beef cattle might have introduced outside rather than in-housed-raised. Our results indicate that ESBL-E. coli are prevalent on cow/calf operations regardless of antibiotic use and they are globally transmitting by carriers located nearby farm area.

14. PROSTAGLANDIN E2 ENHANCES INFLAMMASOME ACTIVATION AND REPRESSES M2 MACROPHAGE POLARIZATION DURING INFECTION WITH GRAM-NEGATIVE PATHOGENS

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Cellular metabolites shape the type of immune response, such as inflammatory processes in macrophages. Eicosanoids, including prostaglandins (PGs) and thromboxanes (TxBs), are lipid mediators, some of which have positive while others have adverse effects on inflammation. Certain eicosanoids are suspected to individually act

as molecular sensors for recruitment of neutrophils, while others regulate bacterial uptake. In this study, gene expression analyses indicated that several genes involved in eicosanoid biosynthesis, including COX-1, COX-2, and PLA-2 are upregulated in human macrophages infected with Salmonella enterica Typhimurium or Yersinia enterocolitica. By using a targeted metabolomics approach, we found that the eicosanoid precursor arachidonic acid (AA) and its metabolites, including PGF2α, PGE2/PGD2, and TxB2, are secreted into the cell culture medium of human macrophages infected with these Gram-negative pathogenic bacteria. The magnitude of eicosanoid biosynthesis depends on the virulence of Y. enterocolitica and S. Typhimurium strains, albeit in an opposite way in Y. enterocolitica compared to S. Typhimurium infections. PGE2 pretreatment led to an increased upregulation of IL-1β transcription and secretion upon S. Typhimurium as well as Y. enterocolitica infection which indicates an increased effectiveness of inflammasome activation. Trials with combinations of EP2/EP4 PGE2 receptor agonists and antagonists before S. Typhimurium infection revealed that PGE2 signaling in this infection model works primarily through the EP4 receptor. Downstream of EP4 activation, PGE2 enhances inflammasome activation and represses M2 macrophage polarization. Our studies indicate that PGE2 is a potent enhancer of inflammasome during infection in Gram-negative bacteria, and may ultimately play a role in cell survival.

15. ROTAVIRUS OUTBREAK AMONG DAYCARE ATTENDEES IN POLK COUNTY, FLORIDA, APRIL 2017

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Background: Since the introduction of rotavirus vaccines beginning in 2006, morbidity associated with the virus has declined greatly. However, rotavirus continues to circulate, posing a threat to young children and vulnerable populations. An investigation of a rotavirus outbreak among daycare attendees in Polk County, most of whom had been vaccinated, demonstrates the continuing impact of this pathogen in the post-vaccine era.

Methods: Following notification of the outbreak by the daycare, the Florida Department of Health in Polk County's Epidemiology Program conducted two site visits and collected two stool specimens. A detailed line list of all who were ill was maintained throughout the outbreak period. Medical records were requested for 25 individuals, and case surveys were conducted in both Spanish and English with parents or guardians of affected children. Vaccination histories were obtained through the Florida SHOTS database.

Results: A total of 40 children and 8 adults met the case definition of vomiting or diarrhea in a daycare attendee or staff member from April 6 to April 24. The most commonly reported symptoms were diarrhea (94%), vomiting (29%), and fever (23%). Thirteen children met a severe case definition, which included one or more of the following criteria: symptoms lasting six or more days (12), or including six or more diarrhea or vomiting episodes per day (3), or hospitalization (1). Ninety-seven (92%) children received at least one rotavirus vaccine, with 79 (75%) fully vaccinated. Twenty-seven (68%) of the 40 ill children were fully vaccinated, including eight

(62%) of those who met a severe illness case definition. Of the 106 daycare attendees, all were age eligible to receive one or more doses of the rotavirus vaccine. Both stool specimens collected were positive for rotavirus A, G12P[8], an emerging strain implicated in outbreaks worldwide over recent years.

Conclusions: Numerous studies indicate rotavirus vaccine effectiveness between 80 and 87% against severe rotavirus disease. This investigation demonstrates that even within a highly vaccinated population, rotavirus infection should be considered as a possible cause of diarrheal illness outbreaks, and stool specimens should be tested for the pathogen.

16. SALMONELLA TYPHIMURIUM INFECTED MACROPHAGES LEAD TO THE PRODUCTION OF PRO-INFLAMMATORY EXOSOMES THAT ACTIVATE THE INNATE AND ADAPTIVE IMMUNE RESPONSE IN NAÏVE CELLS

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Salmonella enterica serovar Typhimurium is a Gram-negative intracellular pathogen which invades the macrophages and promotes the secretion of pro-inflammatory cytokines and exosomes. Extracellular vesicles are nano-sized vesicles and include exosomes, microvesicles and apoptotic bodies. Exosomes are characterized by their size (30-180 nm) as well as by their biogenesis. Exosomes are generated through the inward budding of the multivesicular endosome. Here, we focus on exosomes released by S. Typhimurium-infected macrophages and their function in stimulating innate and adaptive immune responses in naïve macrophages. We show that exosomes secreted by macrophages transport cargo, including LPS, therefore playing a critical role in intercellular

communication in response to infection. We demonstrate that infected macrophages produce subpopulations CD63- and CD9-positive exosomes as early as 2 hours post-infection. These exosomes trigger the TLR4-dependent release of pro-inflammatory cytokines from affected naïve macrophages and dendritic cells (DCs), including RANTES and TNF-α. We also demonstrate that doses of exosomes derived from S. Typhimurium-infected macrophages given to mice over 2 month period can lead to stimulation of CD4+ INF-gamma helper T cells as well as a significant production of antibodies against S. Typhimurium.

17. SOURCE ATTRIBUTION OF ILLNESSES TRANSMITTED COMMONLY BY FOOD AND WATER IN THE UNITED STATES USING STRUCTURED EXPERT JUDGMENT

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Background: Illnesses transmitted commonly by food and water are the cause of a major disease burden in the United States. In addition to food and water, these illnesses may be transmitted by other pathways: person-to-person, animal contact, or through the environment. Limited data are available to estimate the proportion of disease attributable to each of these modes of transmission. We report findings from a structured expert judgment (SEJ) study to provide such estimates.

Methods: Forty-eight experts were assigned to one or more of 15 panels based on publication records and self-evaluation of professional interest and experience with each of 33 pathogens. Including subdivisions by serotypes, age, and clinical manifestations for some pathogens, a total of 47 target questions for the annual percentage of domestic illnesses that are transmitted through each pathway were elicited. Before providing estimates, experts attended

on-line and in-person training and answered 14 separate calibration questions from which performance weights were derived. Experts provided estimates representing their judgments of the uncertainty distribution (5th, 50th, and 95th percentile) for each question. Cooke's Classical Model, aggregated with equal and performance based weighting, was used to synthesize the panels' collective judgments. Robustness analysis and out-of-sample validation showed that the panels each comprised a satisfactory number of both accurate and informative experts.

Results: Estimates for Salmonella enterica across the modes of transmission were 66% foodborne, 6% waterborne, 7% person-to-person, 11% animal to person, and 10% environmental. Estimates for norovirus were 19% foodborne, 6% waterborne, 70% person-to-person, 0% animal to person, and 5% environmental. Estimates for Cryptosporidium were 8% foodborne, 43% waterborne, 20% person-to-person, 21% animal to person, and 8% environmental.

Conclusions: This is the first study to estimate attributable proportions of disease in the United States across all modes of transmission. For most pathogens, a lower proportion of disease was attributed to foodborne transmission compared with previous estimates. Rather than considering only one pathway, as in previous studies, our findings may provide a more balanced understanding by allowing internal comparison across modes of transmission. For example, while norovirus is primarily transmitted person-to-person, transmission through food, water, and the environment can still occur. Our findings can be applied to estimates of the incidence and burden caused by these pathogens in the United States and support appropriate targeting of resources to prevent and control these diseases by all pathways.

18. SPATIAL-TEMPORAL ANALYSIS OF CHOLERA DATA IN CAMEROON FAR-NORTH REGION

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Background: Cholera, a waterborne disease caused by pathogenic Vibrio cholerae, is responsible for approximately 2.86 million cases and 91,000 deaths worldwide (Ali et al, 2015.) Most of the disease burden exists in sub-Saharan Africa, where access to improved drinking water and sanitation is lacking (WHO, 2016.) In Cameroon, especially, cholera remains an ongoing public health concern (Ngwa et al, 2016.) Our study aims to explore environmental determinants underlying the 2010 cholera outbreaks on a finer scale by specifically focusing on the Far-North Region of Cameroon.

Methods: Individual case data collected from the field were geo-referenced at the village level. Country border and health district information were obtained via the WHO country office and the Cameroon Ministry of Health. Data on the country's population density came from the WorldPop UK website; data on precipitation and temperature came from WorldClim and ArcGIS Online databases respectively. Four specific ecological zones (or eco-zones) were defined based on hydrological and landscape characteristics, and named as mountain, floodplain, plain, and river. Case count data were then graphed for each eco-zone. Considering the distance between the sequential villages(defined by the onset time of initial case in each village) in each eco-zone, check whether they have different patterns by using nonparametric method. Use Poisson autoregressive model and compare the corresponding transmission pattern in each eco-zone.

Results and Concluding Remarks: A total of 6975 cholera cases were reported in the Far North Region in the 2010 outbreak. Of these, mountain, floodplain, plains, and river eco-zones respectively accounted for 4914 (70.45%), 625 (8.96%), 770 (11.04%), and 666 (9.55%) of cases. Nonparametric test shows that the potential different patterns for the spread of Cholera infection cross villages for four eco-zones. Poisson autoregressive model shows the different effects from the covariates (precipitation and temperature) and different neighborhood effects for each eco-zone.

19. THERMAL AND CHEMICAL INACTIVATION OF HUMAN NOROVIRUS: IMPACTS ON VIRAL CAPSID INTEGRITY

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Human norovirus is the leading cause of acute viral gastroenteritis and food-borne illness. Due to lack of a readily available cell culture system for human norovirus, virucidal efficacy of intervention methods has usually been determined either directly by evaluating viral genome and capsid integrity and/or indirectly by using cultivable surrogates. We investigated direct impact of thermal and chemical intervention techniques on human norovirus capsid integrity, i.e. receptor-binding and capsid structure. Norovirus-like particles (VLPs) corresponding to GII.4 Sydney, the predominant epidemic strain in recent years, were used this study. The suspension assay, in accordance with ASTM protocol E1052−11, served as the standard method to evaluate the impact of the inactivation treatment on viral capsid structure. VLPs were exposed to sodium hypochlorite (household bleach) at 0-1,000 ppm free available chlorine for 2 min prior to neutralization. Physical inactivation

consisted of treating VLPs with heat (21-95 ºC) for 2 min. After treatments, capsid integrity was evaluated using an H Type 2 HBGA-receptor binding assay and Western blot analysis. For heat, human norovirus capsid lost HBGA receptor-binding capability at >72.5 ºC (p<0.05). No noticeable degradation of VLP polypeptide was observed at temperatures as high as 95 ºC; however, large polypeptides (>120 kDa) began to appear at >45 ºC, indicating a cumulative aggregation of virus particles over increased temperature. Chlorine treatment resulted in loss of HBGA receptor-binding at concentrations as low as 1.0 ppm, as evidenced by 2.2 reduction in normalized absorbance at 450 nm (p<0.05). On the other hand, ≥50 ppm chlorine was required to observe degradation in norovirus capsid protein by Western blot. Overall, major structural protein of human norovirus GII.4 Sydney displayed resistance to thermal degradation but was susceptible to the oxidizing nature of sodium hypochlorite. Based on the method used in this study, loss in receptor-binding was observed before capsid protein degradation. The impact of these treatments on virus infectivity remains to be determined. This study provides an improved understanding of changes in norovirus structure and functionality when formulating inactivation strategies.

20. USE OF SYNDROMIC SURVEILLANCE FOR DETECTING LOCAL ROTAVIRUS ACTIVITY, POLK COUNTY, FLORIDA

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Background: Rotavirus is one of the leading global causes of gastrointestinal (GI) illnesses among children, most of whom are infected by the age of five. The Florida Department of Health in Polk County (FDOH-Polk) gauges current local rotavirus activity based on discharge diagnoses (DD) for the disease from emergency department (ED) visits to area hospitals, using Florida's Electronic Surveillance System for the Early Notification of Community-based Epidemics (ESSENCE-FL). For this study, laboratory-confirmed cases from local ED visits identified in ESSENCE-FL were reviewed to determine whether rotavirus surveillance could be improved by identifying additional information to preferentially detect its activity above background levels for all other GI syndrome category visits.

Methods: A line list of laboratory results for patients testing positive for rotavirus between January 2016 and September 2017 was matched with corresponding ED visits identified in ESSENCE-FL by medical record number, and combined with the chief complaint (CC), DD, and demographic data. Epi Info 7 was used to identify frequently used words or terms in the CC and DD fields using the Word Cloud feature, and compared with the frequency with which they appeared by age or age ranges. Pearson correlation coefficients were calculated between the number of positive laboratory results by month and either the corresponding percent monthly visits among all EDs within the county for selected CC terms and age groups, or the corresponding number of selected DDs.

Results: The most common DD among the 161 laboratory-confirmed patients matched to ESSENCE-FL visits was "diarrhea, unspecified" (32.3%); "rotaviral enteritis" was listed for 29.2% of cases. Correlation with number of positive laboratory results by month was highest among percent visits for all ED patients with a CC that

included “diarrhea”, and who were between one and four years of age (r = 0.89), versus r = 0.81 for numbers of visits with “rotaviral enteritis” DD for that age range.

Conclusions: Limiting queries of the CC field for “diarrhea” to an age group ordinarily most affected by rotavirus was similar to searching directly for rotavirus DDs during this timeframe. Because the appearance of discharge diagnoses can lag for several days, the inclusion of an additional chief complaint query can serve as an earlier or adjunct alert for increased rotavirus activity.

21. VALIDATION OF LIQUID CRYSTAL-BASED IMMUNOASSAY FOR RAPID DETECTION OF SALMONELLA

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Salmonella, is the leading cause of bacterial foodborne illness in the United States. Salmonella outbreaks are commonly associated with eggs, meat and poultry, but other foods such as fruits and vegetables have been associated with Salmonella outbreaks. A rapid and sensitive detection method is required in order to effectively monitor food products for the presence of Salmonella and control outbreaks in timely manner. The goal of this study is to evaluate a novel liquid crystal-based detection assay for Salmonella detection. Liquid crystal-based immunoassay is a novel assay using immunomagnetic separation and liquid crystal biosensors to detect target pathogens. If the target is present in a sample, aggregates of target and immunomagnetic beads are formed, which causes distortion of liquid crystal matrix. This distortion can be detected as a positive signal. In this study, serially diluted Salmonella culture samples were tested with or without enrichment for various hours. Liquid crystal immunoassay was able to detect Salmonella at 106 to 107 CFU/mL without any enrichment. When samples were enriched for 12 hrs, the assay could detect Salmonella as low as 1 CFU/mL. The assay was very specific to Salmonella and did not show any cross-reactivity with other pathogens. The novel immunoassay based

on liquid crystal technology shows a great potential as a rapid and sensitive detection method for of Salmonella and can easily applied for other types of pathogens.

22. 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. E. coli O157:H7, the most common STEC serotype, is responsible for approximately 36% of those illnesses. 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. The procedure was optimized for E. coli O157:H7 and applied to other STEC including O121:H19 and O145. Live and dead STEC cells 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 conventional PCR. E. coli O157:H7 live cells were successfully differentiated from dead cells with detection limit of 1.0x10^3 CFU/mL. Under the optimized DNA photo-labeling condition, live STEC (1.0x10^5 CFU/mL) DNA was amplified while dead STEC DNA (1.0x10^5 CFU/mL) was inhibited from amplification. In addition, live STEC (1.0x10^8 CFU/mL) mixed with dead generic E. coli (1.0x10^8 CFU/mL) was successfully

identified. Photo-labeling of dead STEC (1.0x10^6 CFU/mL) mixed with live generic E. coli (1.0x10^6 CFU/mL) showed inhibition of DNA amplification. With 12-h enrichment, as low as 1.0x10^2 CFU/mL of live E. coli O157:H7 was able to be detected in presence of 1.0x10^8 CFU/mL dead E. coli O157:H7. The results from this study suggest that DNA photo-labeling combined with PCR-based detection methods can potentially differentiate live and dead STEC.

23. VIBRIO CHOLERAE C-DI-GMP GLOBAL REGULATORY GENE, ROCS, INVERSELY INVOLVED IN VPS-INDEPENDENT BIOFILM FORMATION IN LAKE WATER

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Vibrio cholerae, the causative agent of watery diarrheal disease cholera, switched to growth-advantage stationary phase (GASP) phenotype. We previously reported that a “GASP” phenotype strain (GASP-700D) sustained a 49-bp mutation in flagellar gene, flrA, following its persistence for 700-days in nutrient-poor filter sterilized lake water (FSLW) microcosm. We reported that FlrA mutation led to increased vps-independent biofilm formation in FSLW, but not in nutrient-rich L-broth, compared to its wild-type N16961S strain. On whole genome sequencing of GASP-700D and comparative genomic analysis with wild-type strain, we revealed that GASP-700D sustained a total of five different mutations, including a 49-bp deletion in VC0653 (rocS) encoding a protein consisting of c-di-GMP synthetase (GGDEF) and phosphodiesterase (EAL) domains. c-di-GMP is a global regulator that positively regulates biofilm formation in V. cholerae in L-broth. To determine if RocS, akin to FlrA, plays any role in biofilm formation in FSLW, we created an identical mutation in rocS gene

(AA259), and an identical double mutations (ΔflrAΔrocS) seen with GASP-700D in N16961S (AA273) strain. Standard quantitative biofilm assay and confocal microscopic analysis confirmed that both the rocS single mutation and ΔflrAΔrocS double mutations promoted vps-independent biofilm formation in FSLW and in artificial seawater (ASW), but not in L-broth. As expected, the double mutant AA273 produced similar level of biofilm formation as GASP-700D. We are the first to report that c-di-GMP negatively regulates vps-independent biofilm formation in nutrient-poor conditions potentially facilitating the persistence of toxigenic V. cholerae in aquatic environment while promoting cholera transmission in humans.

24. A 3D PRINTED POINT-OF-CARE DEVICE FOR NUCLEIC ACID-BASED VIRUS DETECTION

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Introduction: Conventional methods to detect pathogenic bacteria and virus, including plaque culture, enzyme-linked immunosorbent assay and polymerase chain reaction, often require sophisticated infrastructure, stable electric supply, expensive reagents requiring careful transport/storage, long assay period, and highly trained personnel. Point-of-care (POC) tests can deliver quick result directly at the site of analysis. Also, they can be carried out by less-skilled personnel. These advantages make POC tests appropriate for preventing and controlling the epidemic outbreak of infectious disease. Our lab has developed a POC analytical device using 3D printing and lamination technique. Fabricated with low-cost materials such as polylactic acid (PLA), thermoplastic film and paper, our device is capable of performing a sample-to-answer nucleic acid test for RNA virus in less than one hour, without the use of lab equipment and tools.

Materials and Methods: Our device contained three layers. The top two layers, a reagent reservoir layer and a mixing chamber layer, were designed for sample introduction and lysis, and fabricated with 3D printing technique. The bottom layer was designed for RNA

purification and amplification. It contained a piece of laminated chromatography paper which was taped to a machined polycarbonate (PC) holder. The reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) technique was used for detecting RNA from H1N1 virus and Zika virus. SYBR green dye and a LED flashlight was used to read the test result with the naked eye.

Results and Discussion: Our device has been used to detect lab generated H1N1 virus aerosols with a condensation-based virus aerosol collector. Test results illustrate a successful detection of virus aerosols generated from a 106 TCID50/ml H1N1 virus titer in the aerosol generator. In another effort to detect Zika virus from biological fluids, our device detects down to 0.5 PFU Zika virus from 140 µL spiked urine/saliva sample, making its sensitivity comparable with most commercial platforms such as the QIAamp Viral RNA mini Kit.

Conclusion: Our experiments have shown the successfully functioning of our POC device to detect flu virus and Zika virus with colorimetric method. Further development is undergoing for multiplexed and quantitative test.

25. APPLICABILITY OF PREDICTION MODELS IN FORECASTING INFLUENZA SEASON TRENDS, POLK COUNTY, FLORIDA

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Background: Each year, influenza results in significant economic and clinical burden. Models that can incorporate existing surveillance data to predict and interpret influenza seasonality would be helpful in many aspects of annual response, including in anticipating hospital staffing needs and bed space. This study aims to determine the applicability of one forecasting system developed for larger metropolitan areas, the Above Local Elevated Respiratory Illness Threshold (ALERT) algorithm, at a local level in Polk County, Florida.

Methods: Weekly positive Influenza A test data were collected for one local hospital for each season (reporting week 40 through week 20 of the following year) beginning in 2014, and supplemented with Florida Department of Health (FDOH) viral respiratory surveillance data for the same hospital for missing data points. Data were uploaded to the ALERT algorithm's online portal weekly, and resulting predicted seasonal onset thresholds and median durations (measured as those capturing closest to 90% of cases over the season) were recorded. Running case counts and percentages were calculated for each season to determine the reliability of the algorithm in predicting the respective durations. Correlation coefficients were calculated between the weekly collected data, FDOH viral surveillance data, and syndromic surveillance data for influenza-like visits through Florida's Electronic Surveillance System for the Early Notification of Community-based Epidemics (ESSENCE), to determine the applicability of influenza data across those sources.

Results: For the seasons between 2014 to 2017, the ALERT algorithm's predicted durations (89% to 91% of cases captured) ranged from coinciding with the calculated end of the season (2014-2015) to overestimating the duration by seven weeks (2015-2016), and prematurely estimating the end of the 2016-2017 season by

week 10 while it continued beyond week 20. Correlation coefficients were strong between the hospital's data and ESSENCE (r=0.963) and FDOH surveillance data (r=0.957).

Conclusions: The ALERT algorithm has yielded mixed results with predicting the expected duration of influenza seasons once the onset threshold has been reached, and may require adjustments for improving those predictions in locations with smaller populations. However, strong correlations between the data sources demonstrate that they can help ensure those analyses remain consistent.

26. IMPACTS OF SINGLE-WALLED CARBON NANOTUBES ON HOST LIPID METABOLISM AND ITS ROLE IN IMMUNE RESPONSES FOLLOWING INFLUENZA A VIRUS INFECTION

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Respiratory infections, such as those caused by influenza virus, are responsible for significant morbidity and mortality on a global scale, and the economic burden for medical treatment and time lost from work is high- around $40 billion and 40-100 million school and work days lost in the United States annually. Defining co-agents, such as engineered nanoparticles, that alter respiratory viral pathogenicity is a relatively new line of research, as most investigations into health impacts of these materials have focused on pulmonary disease endpoints such as fibrosis, allergic-type reactions, and cancer. Carbon-based nanomaterials, synthetic particles that are being widely used in biomedical applications and consumer and industrial

products, have been previously shown by our group to increase the viral titers of influenza A virus (IAV) in human small airway epithelial cells (SAECs) in vitro and in mouse lungs in vivo, while repressing several antiviral genes such as ifit2 and ifit3. To better understand the molecular mechanisms that contribute to these observations, we investigated how single-walled carbon nanotubes (SWCNTs) modulate the host innate immune response in vitro with a focus on lipid metabolism enzymes, such as sphingosine-1 phosphate lyase (SPL) and sphingosine kinase 1 (SK1), and expression of interferon-stimulated genes (ISGs), specifically interferon-induced proteins with tetratricopeptide repeats (IFITs) and interferon-induced transmembrane proteins (IFITMs). We hypothesized that SWCNTs are inhibiting expression of the lipid metabolism enzyme, SPL, resulting in decreased expression of ISGs, and thus increased viral titers. For our approach, we exposed SAECs to 20 µg/mL pristine SWCNTs for 24 hours, followed by exposure to IAV for 24 hours. We quantified viral infection with viral titers and the mRNA expression of SPL, SK1, IFIT, and IFITM genes via quantitative real-time PCR (qRT-PCR). Gene expression analysis showed that SWCNTs alone did not alter the expression of ISGs, but did significantly repress IAV-induced expression of several specific ISGs. The importance of the balance between lipid metabolism enzymes and the timing of their expression, rather than their individual levels at only one time point over the course of infection was also demonstrated. Future work will focus on the impacts of inhibition or over-expression of SPL and SK1 on the interaction between SWCNTs and IAV in SAECs. These studies highlight the important role that lipids play in the immune response, as well as the importance of assessing pathogen susceptibility when determining the environmental health and safety of nanomaterials.

27. ONLINE SEQUENTIAL MONITORING OF DISEASE INCIDENCE RATES WITH AN APPLICATION TO THE FLORIDA INFLUENZA-LIKE ILLNESS DATA

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Online sequential monitoring of the incidence rates of chronic or infectious diseases is critically important for public health and stability of our society. Governments around the world have invested a great amount of resource in building global, national and regional disease reporting and surveillance systems. In these systems, conventional control charts, such as the cumulative sum (CUSUM) and exponentially weighted moving average (EWMA) charts, are usually included for disease surveillance purpose. However, these charts require many assumptions on the observed data, including the ones of independent and identically normally distributed data when no disease outbreaks are present. These assumptions can hardly be valid in practice, making the results from the conventional control charts unreliable. Motivated by an application to monitor the Florida influenza-like illness data, we develop a new sequential monitoring approach, which can accommodate the dynamic nature of the disease incidence rates, spatio-temporal data correlation, and non-normality. It is shown that the new method is much more reliable to use in practice than the commonly used conventional charts for sequential monitoring of disease incidence rates.

28. ROLE OF OXIDATIVE STRESS IN SWCNT INHIBITED INNATE IMMUNE RESPONSES TO VIRAL INFECTIONS IN VITRO

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Background: Despite of extensive application, nanomaterials has also raised concerns regarding their potential health impacts. Previous research showed that pre-exposure of lung epithelial cells to single-walled carbon nanotubes (SWCNT) modulated expression of several inflammatory and anti-viral genes in concert with increased viral titers following subsequent exposure to influenza A virus H1N1 (IAV). But the mechanisms of increased IAV infectivity by SWCNT remained unclear. Evidences indicated that SWCNT can induce oxidative stress which would have an impact on innate immune signaling pathways. Thus, in the present study, we assessed the effect of oxidative stress induced by SWCNT on retinoic acid-induced gene I (RIG-I) / mitochondrial antiviral signaling (MAVS) signaling pathway in small airway epithelial cells (SAEC).

Methods: Reactive oxygen species (ROS) were measured using a DCFDA method in SAEC exposed to SWCNT (0.2-30 ug/mL) or IAV

(MOI=0.1-5.0) singly and in combination. Mitochondria respiration of SAEC was measured using a Seahorse assay following exposure to SWCNT for 24 hours. Antioxidant N-acetyl-l-cysteine (NAC) was applied alongside the sequential exposures to SWCNT and IAV. ROS production, RNA expression of inflammatory and antiviral genes, virus titers (TCID50), and immunofluorescence of mitochondria, RIG-I and MAVS protein was measured.

Results: A dose-dependent increase of ROS production was observed at 0.2-50 ug/mL of SWCNT while the co-treatment of NAC blocked ROS from SWCNT. Mitochondrial respiration by Seahorse assay showed cell energetics was not affected by SWCNT at the tested dose range. SWCNT significantly inhibited expression of inflammatory and antiviral genes (RIG-I, MDA5, TLR3, IFNβ1, CCL5, IL8, IFIT2, IFIT3) and repressed the signalosome formation of MAVS while increasing IAV virus titers. With co-treatment of NAC, the gene expression levels, MAVS signalosome formation and virus titers in cells treated with SWCNT+IAV showed no significant changes compared with those treated with IAV only.

Conclusion: SWCNT inhibited RIG-I / MAVS signaling and increased IAV infectivity in part through oxidative stress mechanisms.

29. A SYSTEMS APPROACH TO DISSECTING MALARIA TRANSMISSION BIOLOGY

Rhoel Dinglasan - University of Florida

An overview of the Dinglasan Laboratory Research Program on Malaria Transmission.

30. FOLIAR NEMATODES: A POTENTIAL THREAT TO FLORIDA STRAWBERRIES AND A TAXONOMIC CHALLENGE

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In the early 1900´s foliar nematodes were reported in Florida for the first time as damaging parasites of strawberries (Fragaria ananassa), causing a disease called “crimp” or “summer dwarf”. The symptoms include deformation of buds, leaves and flowers, undersized leaves with crinkled edges, tight crowns, reddened and stunted petioles and few flowers and fruits. Those symptoms can be confused with mite or insect damage and suspected plants are rarely diagnosed for nematode presence. Recently foliar nematodes have been reported on strawberries and ornamentals in the United States, Canada, and Europe, and on soybean, cotton and forage grasses in Brazil. Foliar nematodes belong to the genus Aphelenchoides, which are primarily fungal feeders with few exceptions (A. fragariae, A. ritzemabosi and A. besseyi), which can also feed on foliage of many plants. Late November 2016, a farmer in Plant City, FL reported the presence of plants with symptoms that corresponded to foliar nematode infection. Infected plants were originated from a nursery in North Carolina, and also found on a few other farms in Plant City. Foliar nematodes were present, and putatively identified as Aphelenchoides besseyi (R. Inserra, DPI, Gainesville), a species also known from rice and alfalfa. The infected field in Plant City has been continuously monitored since the detection, in order to correctly identify the nematode and study its survival and potential spread.

Leaves and soil samples were extracted in order to obtain nematodes and those with morphological characteristics resembling Aphelenchoides spp. were collected for molecular identification (18s) and multiplied in MS media with alfalfa and fungal plates (Monilinia fructicola). After 30 days, nematodes were collected from media and inoculated on alfalfa and rice in order to determine the ability to re-infect plants. A. fragariae cultured in MS media was used as a control. Nematodes from MS media with alfalfa showed a higher rate of infection in alfalfa (6 nematodes per g of leaves) when compared to A. fragariae (no infection) and nematodes from M. fructicola plates (1 nematode per g of leaves). Morphological analysis and molecular identification showed that the nematodes infecting strawberry plants in Plant City are not A. fragariae. The identification of the correct species is still a challenge since morphologically the specimens collected resemble A. besseyi and A. subtenuis (Inserra, R.), while the molecular identification resembles A. bicaudatus. Correct identification is a first step in understanding and managing this potentially new nematode threat for Florida strawberries.

31. HONEYBEE COMMENSAL NEISSERIACEAE AS A DELIVERY PLATFORM FOR VACCINES AGAINST NEISSERIA GONORRHOEAE.

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Neisseria gonorrhoeae and Neisseria meningitidis, pose major health concerns. The plasticity of their genomes allows them to evade immuno-surveillance and to develop antibiotic resistance at a pace that may make them untreatable in the near future. World-wide reports of new cases are in excess of 100 million and in the tens of thousands for N. gonorrhoeae and N. meningitidis, respectively. Currently there is no vaccine to prevent gonorrhea, whereas a range of robust vaccines is available against virtually all the N. meningitidis groups that infect humans. Recently it has been noticed that meningococcal vaccine MeNZB confers partial cross-protection against gonorrhea. It is believed that the underlying mechanism of cross-protection is based on the homology between N. gonorrhoeae and N. meningitidis antigens. Homology between antigens was also the rationale for generating protective immunity against N. meningitidis by deploying the commensal Neisseria lactamica. However, several lines of evidence make the use of N. lactamica unsuitable for vaccination. In fact, all Neisseria spp. and virtually all mammalian commensals of related genera within the family Neisseriaceae have the potential to become pathogenic in an immunocompromised host. Moreover, there are indications that at least N. lactamica may play a positive role in the development of the immune system. By expanding on this body of knowledge, we hypothesize that the honey bee obligate commensal Snodgrassella alvi, which phylogenetically is a member of the Neisseriaceae family, can be deployed as a live-vaccine scaffold to generate protective immunity against Neisseria gonorrhoeae. Several lines of evidence

strongly support S. alvi as an excellent candidate as a live-vaccine platform. First, S. alvi is expected to be incapable to colonize humans. Second, there has never been a report of S. alvi being associated with any pathological state in mammals or any invertebrates. Third, the overall phylogenetic relatedness between S. alvi and pathogenic Neisseriae extends to proteins presumed to be pivotal in post-translational processing, thus leading to the expectation that heterologous protective antigens can be expressed in S. alvi in a conformational active form. Fourth, the protein homology between S. alvi and pathogenic Neisseriae is in the 40-60% identity range which is potentially sufficient to confer cross-reactivity. Preliminary evidence will be presented in support of our hypothesis.

32. NATIVE TREMATODE PARASITES ALTER INVASIVE RUSTY CRAYFISH BEHAVIOR AND ECOLOGICAL IMPACTS

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Parasites may be transmitted between native and invasive species during biological invasions; however, the importance of this transmission for most invasions is unknown. In the Great Lakes region, a trematode parasite (Microphallus sp.) reduces invasive rusty crayfish (Faxonius rusticus) population growth, alters its behavior, and reduces its per-capita impacts on lower trophic levels. We were interested in whether Microphallus was introduced to the Great Lakes region with rusty crayfish, or if it was transmitted to rusty crayfish from a native congener (F. virilis or F. propinquus). We collected all three congeners from 25 lakes in the Great Lakes region and rusty crayfish from 38 streams in the native range (the Ohio River drainage). We sequenced an ~800 bp region of the mitochondrial COI gene for 182 trematode parasites from collected crayfish. Parasite genotypes were similar among crayfish in the Great Lakes region, and diverged significantly between the Great Lakes region and the Ohio River drainage, suggesting that the species of Microphallus present in the Great Lakes was transmitted to rusty

crayfish after the crayfish was introduced. These results provide new evidence that parasites in an invaded range can be important for invasive species impacts.

33. THE IMPACT OF SANITATION INTERVENTION ON SOIL-TRANSMITTED HELMINTHS (STHS) INFECTIONS AMONG CHILDREN IN URBAN MAPUTO, MOZAMBIQUE: EPIDEMIOLOGICAL CHARACTERISTICS IN THE BASELINE SURVEY

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Background: Helminthiasis is considered one of the most prominent neglected tropical diseases (NTDs) which have infected about two billion population around the world. Children under five make up about 10%-20% of the population who are infected with soil-transmitted helminths (STHs): Ascaris lumbricoides (roundworm), Trichuris trichiura (whipworm), and hookworm. Insufficient water, sanitation and hygiene (WASH) and poverty are closely correlated with the STH infections. A recent two-year non-randomized before-and-after controlled (BAC) trial was conducted to study the sanitation intervention in the peri-urban area of Maputo, Mozambique. Here, we report on the baseline associations between the STH infections and demographic, social-behavioral and environmental factors.

Methods: This study investigated 1,676 children under five of their STHs infection intensities, and surveyed the household and compound sanitation and hygiene information. Stool samples were tested by the Kato-Katz technique. The exploratory analysis was based on the univariate non-spatial logistic regression models by using demographic, environmental and behavioral factors that were associated with the infections of STHs. Significant factors derived from the univariate analysis were further adjusted in the multivariate logistic regression models. We then applied the negative binomial multivariate models for the significant factors identified from the logistic models for infection intensity of STHs and used Moran's I to check for spatial autocorrelation.

Results: The mean age of the children was 22.2 months (SD 13.3 months) old, with age ranging from 29 days to 48 months. The mean height and weight were 70 (SD 26.5) centimeters and 10.5 (SD 10.4) kilograms. Only one positive sample was detected for hookworm. Overall, 37% and 23% of the children were infected with T. trichuira and A. lumbroides at baseline, and the mean worm burden for trichuriasis was 496.3 EPG (SD 2131.4 EPG) and was 2571.2 EPG (SD 9353.8 EPG) for ascariasis. No statistically significant associations were found between the WASH factors and the infection of STHs. We detected marginally significant associations between height, age, and ascariasis (p < 0.1) or strongly significant associations between age, breastfeeding status and trichuriasis (p < 0.05). No significant spatial autocorrelation was detected after checking the Pearson residuals of the fitted models.

Conclusion: In the baseline survey, no significant associations were found for WASH and STHs infection; significant associations were only found between age, height, breastfeeding status and STHs infection. The midline survey will provide more evidence on the associations between sanitation factors and the infection of STHs.

34. GENOMIC EPIDEMIOLOGY OF DRUG RESISTANT MYCOBACTERIUM TUBERCULOSIS IN MEXICO

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Introduction: Multidrug resistant (MDR) and extremely drug resistant (XDR) tuberculosis (TB) have now been reported everywhere in the world and rates continue to increase. In 2016, half a million cases of MDR-TB were reported globally and 10% acquired XDR-TB during therapy. In the Latin American region, Mexico has one of the highest rates of MDR-TB. However, there is limited information on the population genetic of drug resistant Mycobacterium tuberculosis circulating in the country. The goal of this study was to explore the genetic diversity of drug resistant M. tuberculosis strains collected in Veracruz, Mexico and investigate whether resistance is acquired de novo during therapy or is due to transmission using whole genome sequencing.

Methods: From April 2013 to May 2015, 110 drug resistant clinical M. tuberculosis isolates were collected from patients suspected of TB disease by the Veracruz Public Health Laboratory. Genomic DNA extracted from a loop of cultured mycobacteria was sent to our group at EPI for sequencing. In total 41 genomes were successfully sequenced on the illumina MiSeq platform at an average depth of 97.5 x (range: 31.0 – 182.5) coverage to the laboratory strain H37Rv. We used distance-based and maximum-likelihood methods to investigate the evolutionary relationship between the genomes using a FASTA file of high quality SNPs.

Results: Of the 41 genomes analyzed, 15 (36.6%) were MDR, 17 (41.5%) had resistance to at least one drug, 6 (14.6%) had resistance to multiple drugs but did not meet definition for MDR, and 3 (7.3%) were XDR. All the strains belong to the Euro-American global lineage,

except for one Beijing strain resistant to the antibiotic ethambutol. Based on the phylogenetic analyses, we concluded that MDR and XDR-TB in the sample was due both to recent transmission and to de novo acquisition of drug resistance during therapy among patients infected with already resistant strains.

Conclusion: In Mexico, TB treatment is at first empirical with drug resistance at the end of the intensive phase when patients do not respond as expected. These data illustrate the need for drug susceptibility testing before initiation of therapy.

35. GENOTYPING AND DRUG RESISTANT PREVALENCE OF MYCOBACTERIUM TUBERCULOSIS IN A SINGLE LABORATORY IN HAITI

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Tuberculosis (TB) has been a major public health problem throughout the world. According to the WHO, there are 10.4 million of new TB cases with 1.8 million deaths in 2015, most of which are in Asia and Africa. Haiti is one of the poorest and most improvised countries in the Western Hemisphere with 16,431 of TB cases and a high incidence rate in 2015. To investigate the prevalence and drug resistance of Mycobacterium tuberculosis (MTB), a hospital based study was conducted at Signeau Sanitarium, Gressier, Haiti during

2014 (March-October) and 2015 (January-March). Sputum specimens obtained from 664 non-consecutive patients with suspected pulmonary TB based on clinical symptoms were further tested by culture and fluorescent microscopy. Sixty-seven (10.1%) of the sputum samples were smear positive, while Mycobacteria were cultured from 119 (17.9%) of these samples in liquid or solid media. Drug susceptibility tests were performed in randomly selected 102 isolates using solid media with isoniazid (INH; 0.2 µg/mL and 1.0 µg/mL) and rifampin (Rif; 1.0 µg/mL). Twenty(19.6%) out of 102 culture positive samples were confirmed to be resistant to one of the two drugs, 6(5.9%) of which were resistant to both drugs and classified as the multi-drug resistant (MDR) strains. Furthermore, molecular techniques such as line probe assays were used to identify mutations in 81 base-pair of the Rifampicin Resistance Determining Region (RRDR) of rpoB gene, and inhA promoter region and katG gene for INH resistance. Seventy-five out of 119 randomly selected isolates were further tested by these molecular techniques. Eight (10.7%) isolates were resistant to one of the two drugs and 4 (5.3%) strains were MDR. Three different mutation patterns were identified in these resistance strains, while 2 strains had no known mutations in any of these genes. These strains are subjected to further analysis by whole genome sequencing. Furthermore, spoligotyping, a gold standard molecular typing technique for MTB, was conducted of these 119 positive isolates to reveal the pathogens' diversity. Of these, 31 known shared international types (SITs) were identified, while the other 32 spoligotypes were novel. Among these known SITs, the most predominant one was SIT2 (13.1%), followed by SIT764 (11.1%) and SIT42 (7.4%). Phylogenetically, our study has identified that majority of the Haitian isolates are grouped with 3 major clades: H3 (24.7%), H2 (12.4%), and T1 (12.4%) which are also found in four broad geographic areas including Africa, the Americas, Europe, and Asia. This would suggest that the transmission of TB to Haiti was through the European colonization. Collectively, our study has shown that TB remains a public health problem in Haiti, and a better case management, as well as the pathogen characterization for the MDR TB are greatly needed.

36. LIVE RECOMBINANT ATTENUATED SALMONELLA VACCINES (RASVS) AGAINST MYCOBACTERIUM TUBERCULOSIS

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Tuberculosis (TB) is an ancient disease that is yet to be erased. Globally, Mycobacterium tuberculosis (Mtb) is the leading cause of death by a single infectious organism. Increasing numbers of cases caused by multi-drug resistant Mtb strains, their host subversion strategy and co-infection with HIV cumulatively makes it difficult to completely eradicate the pathogen by chemotherapy. The currently available vaccine, Mycobacterium bovis Bacille Calmette-Guerin (BCG) provides limited protection in infants and young children but does not confer long-lasting protection. In recent years, new candidate vaccines have been developed. These vaccines are primarily either recombinant BCG or employ viral carriers to deliver antigens or are subunit vaccines. Most of these vaccines use the common Mtb antigens (Ag85A/B and ESAT-6) to elicit immunity. We have developed and tested Recombinant Attenuated Salmonella Vaccines (RASVs) to deliver Mtb antigens as a strategy to achieve

better humoral and cell mediated immune responses against various pathogens. In this study, we reported the efficacy of improved versions of the RASVs which were made by adding mutations to earlier versions of RASVs. We are testing the candidate RASVs carrying the pYA4891 plasmid, delivering Ag85A, CFP-10 and ESAT-6, in mice, comparing their protective efficacy to that of BCG. We found that three (□1021, □2068 and □2341) out of the four RASVs tested elicited protection similar to that of BCG. We also evaluated five additional Mtb antigens, which are reported to be strong antigens, to be used in combination with Ag85A, CFP-10 and ESAT-6. These studies suggest that FAP (Fibronectin attachment protein) and TB15.3 are promising antigens for further evaluation.

37. PHARMACOKINETICS OF BEDAQUILINE AND DELAMANID IN PATIENTS WITH MDR-TB

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Introduction: Multi-drug resistant tuberculosis (MDR-TB) is a major barrier in controlling TB because of limited treatment options. Novel anti-TB drugs, such as bedaquiline (BDQ) and delamanid (DLM), are promising new treatment options; however, little is known about their pharmacokinetics (PK) under programmatic conditions. In this prospective observational study, we aimed to assess the PK of BDQ and DLM. This ongoing study is being conducted at the National Center for TB and Lung Diseases in Tbilisi, Georgia.

Methods: The study included patients ages 16 years or older with MDR, pre-extensively drug-resistant (XDR), or XDR tuberculosis. The

treatment included bedaquiline or delamanid along with other second-line drugs. BDQ was dosed at 400 mg once daily for the first two weeks, then 200 mg thrice weekly for 6 months. DLM was dosed at 100 mg twice daily. Blood samples were collected after 4 to 6 weeks of initiating therapy at 0, 2, 6, 10-14, and 24 hours post dose. Samples were shipped to the Infectious Disease Pharmacokinetics Laboratory at the University of Florida and stored at -80°C until assayed, using a validated LC-MS/MS assay. The plasma standard curves ranged from 0.01 to 2.00 mcg/mL for both BDQ and DLM assays. A non-compartmental analysis was conducted using Phoenix v7.0 software. Mann–Whitney test was used to compare the medians among subgroups, using JMP® Pro v13.0 (SAS Institute, Cary, NC). IRB approvals were obtained from all participating sites.

Results: There were 33 BDQ and 31 DLM patients analyzed to date. The median value for the maximum concentration (Cmax) and area under the concentration-time curve (AUC0-48h) for BDQ were 2.51 mg/L (range, 0.41 – 5.90) and 54.7 mg*h/L (range, 18.2 to 122.8). The median Cmax and AUC0-12h for DLM were 0.31 mg/L (0.09 to 0.75) and 2.88 mg*h/L (0.81 to 6.57). Males, smokers, and alcohol consumers had significantly lower DLM Cmax compared to their opposite counterparts (0.29 vs 0.45, 0.26 vs 0.41, and 0.22 vs 0.41 mg/L, respectively). The same significant trend was observed with DLM AUC0-12h.

Conclusions: These preliminary analyses described the PK of BDQ and DLM. The observed BDQ and DLM concentrations are slightly lower than what have been reported in the literature in healthy volunteers. Gender, smoking, and alcohol use were found to be associated with DLM plasma concentrations. Further analysis is needed to adequately describe the effect of covariates and how they inform dosing decisions.

38. POPULATION PHARMACOKINETICS OF CYCLOSERINE

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Background: Cycloserine is commonly used for the treatment of multidrug-resistant tuberculosis (MDR-TB). However, limited information exists with regards to its PK/PD in TB patients. Our objective is to estimate the population PK of cycloserine and later use the model for further PK-PD analysis.

Methods: Four data sets were used for the analysis. The first was healthy volunteers given cycloserine 500 mg as a single dose. They were intensively sampled over 48 hours. The second data set comes from US patients with MDR-TB. Two to four samples were collected from each patient at 2, 6, 12 and/or 24 hours. The third set represents patients with MDR-TB from Tbilisi, Georgia who were enrolled in a prospective observational study and had up to 6 serum samples collected over a 24 hour period approximately 4-6 weeks after initiating treatment. The fourth represents MDR-TB patients from Kibong'oto, Tanzania that were enrolled as part of a multi-country prospective observational study that collected serum at 1, 2, 6 and 12 hours after medication administration at 2 weeks after MDR-TB treatment initiation and at 2 and 6 hours after administration at 4 weeks and 8 weeks after initiation. The combined data were modeled using Pmetrics nonparametric population PK software.

Results: There were 142 subjects (12 healthy volunteers and 130 MDR-TB patients) and 751 observations. The mean bodyweight was 64 kg (14.4). Cycloserine data were adequately described by a one-compartment model with delayed (Tlag) and first-order (Ka) absorption. The individual predictions matched well with the

observations (R2 = 0.89, Bias = -0.169, slope 1.02, intercept = 1.16). Allometric scaling by bodyweight normalized to 70 kg was used for apparent clearance (Cl/F) and volume (V/F). The parameter estimates (CV%) for Ka, Tlag, V/F and Cl/F were 5.4 hrs-1 (39%), 0.46 hours (98%), 30.6 L (30%), 1.25 L/hr (63%). Mean Cl/F was 2.32 L/hr for healthy volunteers and 1.16 L/hr for TB-MDR patients (P <0.01).

Conclusion: Our model adequately described the pharmacokinetics of cycloserine, and it shows that MDR-TB patients have a lower Cl/F compared to healthy volunteers. This model will be used for further PK-PD analysis, and will be enriched by ongoing data collection from diverse cohorts in Georgia, Tanzania, Uganda, Bangladesh and the Russian Federation.

39. WHOLE-GENOME SEQUENCING ANALYSIS OF INTRA-HOST GENETIC VARIABILITY OF MYCOBACTERIUM TUBERCULOSIS

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Introduction: Genetic fingerprinting of Mycobacterium tuberculosis (Mtb) is essential for the surveillance and control of tuberculosis (TB) disease. Mtb was previously thought to be a clonal organism, with little to no genetic diversity within the species. However, with the advent of molecular typing tools, much diversity has been revealed. Particularly, it has been estimated that the genetic diversity within one patient may be greater than the variability between any two epidemiologically linked hosts. In this study, we investigated intra-host genetic variability of Mtb within a patient with advanced pulmonary TB and a confirmed secondary case.

Methods: We sequenced the complete genomes of 20 Mtb isolates collected at serial biweekly intervals from the same patient during standard TB therapy, and one isolate from an epidemiologically confirmed secondary case. Sequencing was done using the Illumina MiSeq platform. Short-reads were trimmed, filtered, and high-quality SNPs (hqSNPs) called in reference to the H37Rv laboratory strain. We investigated the phylogenetic relationships between the genomes using distance-based and maximum likelihood methods. The genetic distance between sequential genomes (measured in SNPs) was used to determine whether the detected variability was a result of intra-host microevolution or mixed infection.

Results: Of the 20 original within-patient isolates, we discarded nine due to significant Nontuberculosis mycobacteria (NTM) contamination. We had an average of 86.4 x coverage (range: 48.9 – 149.1) across the 12 genomes. Overall, 7 SNPs differentiated the genomes, with 5 SNPs recorded within the index case. Pairwise SNP comparisons revealed no variation among the serially sampled genomes within the index case or between the index and secondary cases from days 0 to 30 of therapy. However, days 31 to 60 saw a steady SNP accumulation (~2 SNPs per sampling interval).

Conclusions: The low level of diversity among isolates led us to conclude that intra-host variability was due to microevolution rather than mixed infection. Our findings provide further evidence in support of intra-host genetic variability of Mtb. More analyses are needed to elucidate the mechanism and functionality of this variability.

40. A HITCHHIKER'S GUIDE TO TRANSMISSION: LONG-DISTANCE MOVEMENTS OF MALARIAL PARASITES IN A MIGRATORY BIRD

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Migratory birds have been implicated in the spread of multiple pathogens, yet the transmission dynamics within and among avian species remains understudied. Here, we evaluate the transmission dynamics of avian malaria and other haemosporidian parasites in the north-central Great Plains because this region serves as sympatric breeding ground for a diverse community of resident and migratory avifauna. Our aims were: (1) examine variations in haemosporidia burden between migratory Upland Sandpipers and resident Greater Prairie-Chickens, (2) evaluate potential for cross-species transmission, (3) infer phylogeographic transmission patterns from parasites recovered from Upland Sandpipers. We found that while the haemosporidia prevalence was not significantly different between the species, Upland Sandpipers harbored more diverse and divergent parasite lineages than Greater Prairie-Chicken. This demonstrates that migratory habit can increase the diversity of disease agents. Furthermore, we found evidence of cross-species transmission at sympatric breeding grounds. Upland Sandpipers were infected with a grassland endemic Plasmodium lineage (P4) that frequently infects Greater Prairie-Chickens and other species. P4 has also recently been recovered in the Galapagos avifauna, suggesting not only a recent translocation from the Great Plains but a high invasion potential of this parasite. Our results illustrate that movements of migratory birds such as the Upland Sandpiper have consequences on the spread of infectious disease agents.

41. A SEMI-PARAMETRIC BAYESIAN FRAMEWORK FOR FLEXIBLY MODELING COUNT DATA AND PERFORMING MODEL SELECTION

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Count data commonly arise in medical entomology and epidemiology but adequately modeling them is challenging given that these data are often zero-inflated and/or over-dispersed. Multiple modeling approaches have been put forward to properly accommodate these data characteristics but unfortunately different model choices lead to substantially different inference and yet it is not straightforward to determine which model one should adopt. Here, we propose a semi-parametric multinomial regression model (MN model) that can be used as a default model for count data, helping to avoid this conundrum. This model is extended to allow for automatic model selection (MN-MS model), a feature that is often lacking in more sophisticated statistical models developed to deal with count data. Using simulations, we show that the proposed MN model is able to fit data that arise from several types of parametric distributions, helping to circumvent the problem of selecting the most appropriate discrete distribution to use for the likelihood. Furthermore, a comparison with Poisson regression, with and without AIC model selection, reveals that the MN-MS model has superior performance in identifying which covariates are relevant and which are not. We illustrate the use of this model on a case study which examines the environmental drivers of malaria risk in the Peruvian Amazon. More specifically, we model the human biting rate of six different mosquito species known to be able to transmit malaria as a function of land-use land-cover (LULC) and rainfall. We find that An. darlingi, the species widely regarded to be the primary malaria vector in the region, is strongly associated with highly anthropized landscapes, closely following the primary road in the

region. On the other hand, all the other mosquito species tended to have higher mean biting rates in landscapes with a lower fraction of exposed soil and urban area, revealing a striking shift in species composition. Ultimately, we find that areas with an intermediate level of disturbance, far from the primary road and from forests, tend to have higher overall mean mosquito biting rate.

42. ALGORITHMS TO PREDICT ARBOVIRAL INFECTIONS USING CLINICAL AND DEMOGRAPHIC INFORMATION FROM FEBRILE PATIENTS IN AN AREA WITH COINCIDENT OUTBREAKS OF DENGUE, CHIKUNGUNYA AND ZIKA

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The accurate differential diagnosis of arboviruses during coincident outbreaks of dengue, chikungunya and Zika viruses is of paramount importance in order to ensure both proper clinical management as well as timely and accurate surveillance reporting. We trained and validated logistic regression, decision tree and random forest models for each of the three pathogens, including subtypes for dengue, to

clinical and demographic data from a cohort of school children from the Gressier region of Haiti. Prediction statistics including the area under the receiver operating curve, sensitivity and specificity were estimated and averaged over 100 validation sets. CHIKV was the only model that was able to efficiently distinguish between infection with 72% sensitivity and 63% specificity. The logistic regression model performed as well as the random forest model, suggesting that a linear combination of variables may be sufficient to aid clinicians in differentially diagnosing CHIKV. The most important clinical variables in this classification were arthralgia, inferior member pain, wrist pain and temperature. In the cluster analysis, we found that CHIKV has a very diverse clinical spectrum of disease. We were able to effectively distinguish between CHIKV and non-CHIKV cases based on patient demographics and clinical symptoms. These results can aid clinicians in the safe prescribing pain management drugs as well as follow-up care to patients with suspected arboviral infections during coincident outbreaks of CHIKV, ZIKV and DENV.

43. ALPHAVIRUS LIFECYCLE IN THE MOSQUITO HOST

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Arthropod-borne-viruses (arboviruses) are etiologic agents of both medical and veterinary diseases. Variants of the Alphavirus Sindbis (SINV) are being used to map the in vivo process of arbovirus infection in Aedine and Culex adult female mosquitoes. Percent and timing of dissemination differs between SVHR, AR339, TR339 and eGFP-TR339-TaV (SINV variants) suggesting genomic effects in crossing the midgut. Organ infection is relatively pantropic; head ganglion, fat body/hemolymph, gut and thoracic muscles, tracheoles and salivary glands are permissive, while malpighian tubules and ovaries are refractory. While this infection is not ubiquitous; most organs have receptors and undergo productive infection, other cells in the organism do not replicate virus. Differences in temporal responses exist; head ganglia clears infection, midgut muscles & salivary glands are transiently infected, tracheoles, hindgut muscles and fat bodies/hemolymph remain persistently infected. Organ-associated visceral muscles respond differentially to virus; midgut muscles clear virus, hindgut muscles remain persistently infected and ovarian muscles are refractory to SINV. Because visceral muscles are all composed of the same tissue type, differential virus phenotypic expression may reflect organ function, different molecular milieu or variations in cell ultrastructure. SVHR-associated pathology observed in the lateral lobes of the salivary glands, co-localizes with cellular apoptosis and the presence of heparan sulfate (purported SINV receptor), localized in lateral lobe duct cells. Salivary gland median lobes remain unaffected by SINV and challenging mosquitoes with AR339 resulted in lateral lobe pathology similar to that observed following SVHR infection. Pretreatment of cultured mosquito cells (C710) with lactoferrin, a molecule that binds heparan

sulfate, inhibited infection with AR339 greater than TR339 suggesting differences in variant-receptor interaction. Confocal analysis of SINV dissemination using eGFP-TR339-Tav reporter virus, has revealed earlier dissemination and intricacies of virus foci. The virus lifecycle in the mosquito involves a multitude of host cell interactions that represent links in a chain…links that scientists hope to break in efforts halt the cycle of disease transmission.

44. ARYL TRIFLUOROMETHYL OXIMES AS POTENTIAL NEW MOSQUITOCIDES

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Fluorinated methylketones have been identified as potential mosquitocides, suspected of acting as reaction coordinate analogues that inhibit acetylcholinesterase (AChE) thereby leading to paralysis and eventual death. Previous work has explored trifluoro-, difluoro-, and fluoromethylketone analogs with pyrazole substituted trifluoromethylketone (TFK) derivatives exhibiting some of the highest inhibition and mosquito selectivity. Although TFKs were found to be potent inhibitors of Anopheles gambiae AChE, poor topical toxicity was observed, and this is believed to be due in part to high volatility and metabolism issues. Use of an oxime moiety to mask the ketone and offer a more metabolically robust derivative improves topical toxicity to some degree but did not afford the toxicity expected from the previously observed high AChE inhibition from TFKs. Hypothetically, the oxime should readily undergo hydrolysis to the TFK at acidic pH, but it is possible that the TFK oxime does not fully hydrolyze within the mosquito. We also suspect

that the low cLogP value (calculated as 2.12) of the TFK oxime limits compound uptake at the mosquito cuticle and potentially limits CNS penetration. To this end, we synthesized a series of derivatives in which the oxime hydroxyl group was converted to the less polar oxime ether or oxime ester. We also investigated the effects of electron donating and withdrawing groups to determine if the electron density about the oxime ester/ether oxygen had an effect on lability through hydrolysis susceptibility. Although none of the synthesized compounds exhibited 100% mortality through topical application of 1 μg/mg of mosquito, a few trends were observed. The introduction of electron donating groups (14-19) gave less favorable results than those with electron withdrawing groups (8-13) suggesting that electron withdrawing groups might increase the nucleophilicity of the compound. It was also seen that an increase in the functional group size (1-7) led to an increase in mortality, and it is possible that this portion of the molecule is interacting with the hydrophobic residues of the acyl pocket within AChE. Because none of the oxime ethers or oxime esters exhibited topical toxicities better or equal to the previously made oximes, this suggest that the oxime does not act as a pro-drug by hydrolyzing to the TFK. Further derivatization of TFK analogs will investigate substitution on the pyrazole ring to potentially decrease volatility and metabolism.

45. ASSESSMENT OF PHENOTYPIC INSECTICIDE RESISTANCE ON THE VECTOR COMPETENCE OF AN AEDES AEGYPTI MOSQUITO POPULATION FROM KEY WEST, FLORIDA

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Understanding the interaction of the resistance phenotype in mosquitoes and vector ability is important in the control of mosquito-borne diseases. Insecticide resistance is known to be energetically costly, resulting in stressed out mosquitoes with altered ability to vector a pathogen. Although chemical control remains the best method for controlling mosquitoes and the pathogens they transmit, resistant mosquitoes that develop a greater ability to transmit pathogens would greatly impact traditional mosquito control programs, resulting in an increase in vector-borne disease transmission. This would greatly hinder mosquito control in their ability to effectively decrease the number of mosquito pests. Therefore, this study determined the impact of phenotypic insecticide resistance on the vector competence of Aedes aegypti for Dengue virus and on their ability to mount an immune response once infected.

46. AVIAN MALARIA PREVALENCE AND TRANSMISSION IN CATTLE EGRETS (BUBULCUS IBIS)

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The emergence of vector-borne diseases has been linked to global climate change. Avian Haemosporidia are widely distributed blood parasites that are transmitted by arthropod vectors and their avian hosts. Cattle Egrets (Bubulcus ibis) are a rapidly expanding avian species, yet little is known about the potential for this host to transport pathogens. The objective of this project is to determine Cattle Egrets' role in the transmission of Avian Haemosporidia parasites: Plasmodium, Haemoproteus, and Leucocytozoon. Live Cattle Egret are trapped and sampled for blood, ticks, feathers, and morphological measurements. Dead Cattle Egret are collected from USDA Bird Air Strike Hazard (BASH) control efforts and sampled for muscle, ticks, feather, spleen, and morphological measurements. PCR and gel electrophoresis are used to determine prevalence of Plasmodium, Haemoproteus, and Leucocytozoon and positive samples are sequenced for genetic analyses. As of October 31, 2017, 425 Cattle Egrets have been sampled from 16 locations around the southeast United States. Of the 425 samples, 133 have been screened and sequenced for Avian Haemosporidia parasites: 29.3% are positive for Plasmodium, 0% are positive for Haemoproteus, 17.3% are positive for Leucocytozoon, and 4.5% are co-infected with Plasmodium and Leucocytozoon. Cattle Egret sample collection, screening, and sequencing for Haemosporidia parasites is ongoing. Avian Haemosporidia may be a good model for investigating the role

of climate change in arbovirus emergence. This project will provide a better understanding of disease transmission in Cattle Egret.

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

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Farmed white-tailed deer (Odocoileus virginianus) breeding operations, commonly referred to as deer farms, are an emerging agricultural industry in Florida. A major challenge to this industry is the high mortality rate in breeding stock, especially fawns and yearlings, caused by various bacterial infections and viral hemorrhagic diseases (HD). Before management can be improved and properly implemented, the cause of death in the farmed herds must be determined. Using the diagnostic program provided by the University of Florida Cervidae Health Research Initiative (CHeRI), we determined the proportion of farmed white-tailed deer that have died from bacterial infections, hemorrhagic disease-causing viruses, or other causes. Participating ranches throughout the state provided deceased, farmed white-tailed deer to be necropsied on-site or sent-in sampled organs to be analyzed throughout the 2017 calendar year. Samples from both collection methods were each tested for hemorrhagic disease using molecular methods, in addition to

microbiological and histopathology methods. Parasite identification was also performed when applicable. A total of 121 deceased farmed white-tailed deer were sampled in the year 2017. Of the animals submitted, 76 were born in the year 2017, 19 were born in 2016, and 25 were born in 2015 or before, with one animal's age being unknown. The cause of death was reported as hemorrhagic disease in 43% (52/121) of the animals, while 42% (51/121) of the deer deaths were attributed to bacterial infection. For the remainder, 10 animals died of other causes, and seven had too little information provided to determine a cause of death. HD and bacterial infections are a significant sources of mortality in farmed white-tailed deer. This data provides farmed white-tailed deer breeder's insight on how to improve management practices that can significantly improve the health of present and future herds.

48. CHARACTERIZING PERSONS UNDER INVESTIGATION (PUIS) FOR ZIKA VIRUS INFECTION AT A COUNTY HEALTH DEPARTMENT, 2016-2017, DUVAL COUNTY, FLORIDA

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Background: The Zika virus emerged into the public sphere in Brazil in 2015 and spread to the Americas and Florida in 2016. The major threat was to pregnant women and their fetuses due to potential severe birth defects associated with infection. Because of the high rate of asymptomatic infections, testing became a priority. In Florida, county health departments helped organize Zika testing requests and responded to inquiries. The Florida Department of Health in Duval County (DOH-Duval) instituted a tracking system to supplement the statewide surveillance system and measure workload related to the Zika response.

Methods: Persons under investigation (PUIs) were recorded in Microsoft Excel and included client demographics, reporting method and reason, and risk factors for Zika infection. Data collected during the height of the international epidemic from July 2016 to November 2017 were analyzed using SAS 9.4 to improve understanding of the

population served at DOH-Duval and aid in planning for future responses.

Results: During the period of interest, DOH-Duval reported eight cases of travel-related Zika virus infection, yet addressed 561 PUIs related to Zika. An average of 32 PUIs were addressed monthly with a maximum of 115 in August 2016. Most PUIs were reported through laboratory test orders (40%, n=213), patient contact (32%, n=169), and provider contact (25%, n=134), with the remainder from partner health departments (3%) and syndromic surveillance (1%). Females (n=424) were reported via patient contact (35%, n=150) more than provider contact (26%, n=111). Males (n=112), however, were reported via provider contact (21%, n=23) more than patient contact (17%, n=19). 93% of all PUIs were Zika testing requests (n=501), with the remainder for guidance (6%), follow-up on transfer patients (1%), and mosquito control requests (1%). Most testing requests were for females (80%, n=407). Of females with known pregnancy status (n=390), 76% were pregnant (n=298). Of pregnant females with known risk factors for Zika infection (n=269), almost one third had no risk factors (29%, n=77).

Conclusions: Aligning with state and national guidelines, one important focus of the DOH-Duval Zika response was providing pregnant women access to Zika testing. The Florida Governor's August 2016 advisory announcing that any pregnant woman regardless of risk could request Zika testing through their health department likely contributed to trends within this group. Patients, particularly females, had a direct role in organizing their Zika testing. A supplemental tracking system proved beneficial to better characterize and estimate the true burden of the Zika response in Duval County.

49. CHECKLIST OF MOSQUITO SPECIES, THEIR DISTRIBUTION AND PATHOGEN TRANSMISSION IN HAITI

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An updated checklist of 53 mosquito species from Haiti is presented in this paper. The number of species from the publications covering 93 years shows a total of 53 mosquito species.. This checklist provides data on distribution of the mosquitoes and potential diseases transmitted by these mosquitoes in Haiti. The 53 species belong to 11 genera: Aedes (12 species), Anopheles (6 species), Culex (14 species), Psorophora (8 species), Deinocerites (1 species), Limatus (1 species), Wyeomyia (3 species), Orthopodomyia (1 species), Toxorhynchites (2 species), Uranotaenia (4 species), Sabethes (1 species). No new species has been identified ever since indicating a lack of investigational studies on mosquito species in Haiti, although it is quite possible that there might be several species that remain undescribed. The presence and abundance of the mosquito species in a given location could be an indicator of suitable environmental features in the mosquito habitats. The knowledge of the distribution of the mosquitoes (and their biology) will aid in understanding mosquito vectors responsible for transmission of pathogens in Haiti.

50. CHIKUNGUNYA VIRUS WITH EVIDENCE OF INCREASED CENTRAL NERVOUS SYSTEM INVOLVEMENT DISEASE IN PAKISTAN

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Chikungunya (CHIKV) has been documented in Pakistan as the cause of a significant outbreak over the winter of 2016-2017. Physicians in Karachi reported an epidemic of patients presenting with a dengue-like illness with severe arthralgia and swelling of the joints in December of 2016. Concomitant with this epidemic, an ongoing cross-sectional study for arboviral diseases which included screening for CHIKV also identified many of these patients as enrollees in this clinical study. Exposure to CHIKV was verified via detection of viral nucleic acids or virus-specific IgM with virus-specific neutralizing antibodies. Patients enrolled under this study were retrospectively evaluated for CHIKV exposure via RT-PCR and PRNT. The data enclosed demonstrate that CHIKV has been circulating in the Sindh region of Pakistan since June 2015. Clinical data indicate that in addition to arthralgia, rash, and fever, clinical manifestations of neuroinvasive disease are present in over 40% of patients with CHIKV exposure. Phylogenetic analysis indicated that the Karachi isolates were most similar to the East Central South African CHIKV lineage and showed sequence homology to isolates obtained in other parts of Pakistan and India. More importantly, two of the CHIKV isolates had a nucleotide substitution in the E1 gene corresponding to an amino acid change at chain F portion of the E1 protein.

51. CLINICAL REGIMENS OF FAVIPIRAVIR INHIBIT ZIKA VIRUS (ZIKV) REPLICATION IN THE HOLLOW FIBER INFECTION MODEL SYSTEM

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Background: Zika virus (ZIKV) infection is associated with serious, long-term neurological manifestations. There are currently no approved antiviral therapies for the treatment or prevention of ZIKV. We have identified favipiravir (FAV), a viral polymerase inhibitor with broad-spectrum activity, as a promising treatment strategy for ZIKV infection. However, our previous studies were conducted in a non-human primate cell line (Vero) under static drug conditions. Here, we aimed to further investigate the clinical potential of FAV by evaluating its antiviral activity in a human cell line as well as assessing the ability of pharmacokinetic (PK) profiles associated with clinically-relevant FAV regimens to inhibit ZIKV production.

Methods: A 2015 human ZIKV (PRVABC59) strain was inoculated onto HUH-7 cells (human liver cell line) in a 6-well plate or in the HFIM system and static concentrations of FAV were administered. Viral supernatants were sampled daily, clarified, and frozen at -80°C. ZIKV burden was quantified by plaque assay on Vero cells. A mechanism-based mathematical model (MBM) was developed to describe ZIKV production in the presence of FAV over time and fit to data generated in the plate assay and HIFM system. The model was employed to predict viral burden when PK profiles associated with the clinically-utilized influenza (low dose) and Ebola (high dose) FAV regimens were simulated. Model predictions were validated experimentally using the HFIM system.

Results: FAV inhibited ZIKV replication in a dose-dependent fashion on HUH-7 cells, resulting in EC50 values of 218.3 uM and 236.5 uM in the plate assay and HFIM system, respectively. The MBM fit both data sets well and predicted that both clinically-relevant FAV regimens inhibit and delay ZIKV replication. The predicted maximal extent of inhibition was 3 log10 PFU/ml for the low dose regimen and 4.8 log10 PFU/ml for the high dose regimen. Importantly, the experimental data generated in the HFIM system validated the model predictions, as measured viral burden values were very similar to those predicted by the model. These findings demonstrate that the model was able to prospectively predict viral burden during drug treatment with different regimens of FAV.

Conclusion: Our findings indicate that clinically-relevant regimens of FAV are able to substantially suppress ZIKV replication. These results indicate that FAV is a promising treatment to combat ZIKV. Due to the risk of embryotoxicity associated with FAV, this treatment would be indicated for infected non-pregnant patients.

52. 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 Bayesian hierarchical model to correct such detection bias for the estimation of the basic reproduction number R0 and age- and sex- relative susceptibility. In this model, the actual case number in each age-sex group is considered latent but linked to the surveillance-reported case number by Binomial distributions. Data augmentation Markov chain Monte Carlo algorithm is constructed to estimate model parameters and the latent case numbers jointly.

Results: The proposed method was shown to provide unbiased estimates in a simulation study, and is then applied to the Zika surveillance data in San Andres, Girardot and Cucuta, Colombia. Assuming the case detection rate was 100% for females in fertility age group (aged 20-40), the case detection rates for other age-sex groups ranged from 50%-60% in the three cities. A female was 54% (95% CI: 48%, 63%) more susceptible than a male. Children (age<20) and older adults (age>40) were 91% (79%, 103%) and 43% (34%, 51%) more susceptible than young adults (aged 20-40), respectively. R0 ranged from 2.7-4.4 for the three cities. Without adjusting for the detection bias, the relative susceptibilities across age and sex groups would be overestimated by 1.9- to 2.7- fold, and the estimates of R0 would be biased by about 20%.

Conclusion: Important epidemiological parameters such as R0 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.

53. DENGUE COHORT IN YUCATAN, MEXICO: BASELINE AND FIRST YEAR FOLLOW-UP

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Dengue is the most prevalent mosquito-borne viral disease of humans and is caused by the four serotypes of dengue virus. To estimate the incidence of dengue and other arboviruses, we analyzed the baseline and first year follow-up of a prospective school-based cohort study and their families in three cities in Yucatan, Mexico. Through enhanced surveillance activities, acute febrile illnesses in the participants were detected and yearly blood samples were collected to evaluate dengue infection incidence. A Cox model was fitted to identify hazard ratios of arboviral infections in the first year of follow-up of the cohort. The incidence of dengue symptomatic infections observed during the first year of follow-up (2015 - 2016) was 3.5 cases per 1,000 person-years (95\% CI: 1.9, 5.9). The incidence of dengue infections was 33.9 infections per 1,000 person-years (95\% CI: 31.7, 48.0). The majority of dengue infections and seroconversions were observed in the younger age groups (≤ 9 years old). Other arboviruses were circulating in Yucatan during the study period. The incidence of symptomatic chikungunya infections was 8.6 per 1,000 person-years (95% CI: 5.8, 12.3) and the incidence of symptomatic Zika infections was 2.3 per 1,000 person-years (95% CI: 0.9, 4.5). Our model shows that having a dengue

infection during the first year of follow-up was significantly associated with being female, living in Ticul or Progreso, and being dengue naïve at baseline. Age was not significantly associated with the outcome; it was confounded by prior immunity to dengue that increases with age. This is the first report of this cohort and provides incidence estimates of the three arboviruses co-circulating in all age groups. This study provides important information for understanding dengue and other arboviruses epidemiology and informing public health policies.

54. DETECTION AND SEQUENCING OF SPONDWENI VIRUS IN FIELD-CAUGHT CULEX QUINQUEFASCIATUS MOSQUITOES, HAITI 2016

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Spondweni virus (SPONV) and Zika virus (ZIKV) are closely related flaviviruses which have a high level of cross-reactivity and cause similar diseases in humans. While both viruses were first described in Africa, only ZIKV has geographically spread, and isolated in Asia and the Americas and the Caribbean. Research on SPONV is sparse, and little is known about pathogenesis, host range, and vector competency – with only six clinical reports of SPONV infection and recent mosquito studies reporting common species (including Aedes aegypti, Aedes albopictus, and Culex quinquefasciatus) are not susceptible to SPONV infection. As part of on-going arbovirus surveillance activity in a semi-rural region in Haiti a total of 1756 mosquitoes were caught using Biogents Sentinel traps, set from May through August 2016. Mosquitoes were pooled and homogenized,

then tested for the presence of Chikungunya virus, Dengue virus, and ZIKV viral RNA by rtRT-PCR. Initial rtRT-PCR screens revealed a pool of seven mixed-sex Cx. quinquefasciatus mosquitoes that were ZIKV vRNA-positive. Following unsuccessful attempts to amplify ZIKV-specific amplicons, a closely related virus was suspected. Random hexamers and published SPONV-specific primers were then tested, and virus-specific amplicons were identified and sequenced by Sanger sequencing. Here we report the first detection of SPONV outside of Africa, from Culex mosquitoes collected in Haiti in 2016, raising questions about the role of SPONV in clinical illness, and misdiagnosis with Zika Fever.

55. DIEL ACTIVITY PATTERNS OF AEDES AEGYPTI AFTER RESURGENCE IN ST. AUGUSTINE, FLORIDA AS COLLECTED BY A MECHANICAL ROTATOR TRAP

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According to surveillance data collected by Anastasia Mosquito Control District of Saint John's County, Aedes aegypti (Ae. aegypti) have recently repopulated the area of Downtown Saint Augustine, Florida. For this reason, there has been a higher public health concern in regards to the risk of diseases spreading such as, Zika, Dengue and Yellow Fever. This study assessed the diel host seeking activity patterns of Ae. aegypti in Downtown Saint Augustine, Florida. Three sites in this area were selected to assess the peak hours of host seeking activity by way of two Johns Hock 8-bottle rotator traps. Mechanical traps provide a safe, standardized alternative for assessing the diel activity of Ae. aegypti. A significant difference in mean capture of Ae. aegypti was detected from 1700h to 1900h and 1900h to 2100h. Less significant was the mean capture from 1500h to 1700h. These results indicate that for this location, the Ae. aegypti population has a peak time period for host-seeking

activity occurring between the hours of 1700h to 2100h. These evening activity periods suggest that Ae. aegypti is not strictly a "day-biting" mosquito, and may instead be opportunistic. Furthermore, these times are a targetable treatment window when managing these urban vectors.

56. ESTABLISHING THE DISCRIMINATING CONCENTRATION OF PERMETHRIN IN ACARICIDE SUSCEPTIBLE LONE STAR TICKS

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The lone star tick, Amblyomma americanum, is known to spread the agents of human monocytic ehrlichiosis and tularemia, and is suspected to be a vector for many other pathogens. Acaricide resistance has become an increasing problem in the battle to control arthropod populations, however is it unknown if A. americanum has developed resistance to acaricides. Determining the discriminating concentration (DC) of acaricides, is vital for successful management efforts. The objective of this study was to characterize the DC of permethrin in A. americanum. Ticks were obtained from an acaricide susceptible laboratory colony (Oklahoma State University). Assays were performed using Food and Agriculture Organization larval packet tests with concentrations of permethrin in a trichloroethylene olive oil diluent applied to each packet. Approximately 100, 14-16 day old larval A. americanum were placed into each packet and then the packets were placed in an incubator for 24 hours. After the holding period, the number of alive and dead ticks in each packet were counted. The offspring from 10 engorged females were tested in 10 separate experiments (>20,000 larvae). The percentage mortality at each concentration was used to conduct a probit

analysis. The lethal concentration to 99% mortality (LC99) was 2.27%. Therefore, the DC, which is two times the LC99 was 4.54%. In the future, the DC will be used to screen field-collected ticks for resistance. Monitoring resistance will assist future pest management efforts for A. americanum. If resistance is detected, then adjustments to application rates and techniques will need to be made to mitigate resistance. This research is being performed under the Centers for Disease Control and Prevention Southeastern Regional Center of Excellence in Vector-borne diseases.

57. EVALUATION OF ACCESS TO CARE AND PREGNANCY OUTCOMES OF PREGNANT ZIKA CASES REPORTED TO THE FLORIDA DEPARTMENT OF HEALTH, JANUARY 2016 – DECEMBER 2017

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Background: Exposure to Zika virus (ZIKV) during pregnancy can result in fetal abnormalities. In the continental U.S., Florida has one of the highest numbers of pregnant women exposed to ZIKV either through travel or local exposure. Due to the potential impact on fetal health, it is essential that these women are aware and have access as needed to pre- and post-natal care programs, such as Healthy Start and Early Steps.

Methods: Data were obtained from the Florida Department of Health (FDOH) Merlin reportable disease system. ZIKV infection cases involving pregnant women or their infants were identified between January 1, 2016 and December 12, 2017 using national criteria developed by the Centers for Disease Control and Prevention and the Council of State and Territorial Epidemiologists. Processes to ensure connection with care were developed by FDOH and tracked in Merlin.

Results: Four-hundred twenty pregnant women were identified as ZIKV infection cases in Florida. Of those pregnant women, 348 (83%) were connected to prenatal care and 335 (80%) were connected to Healthy Start. Thirty-four (8%) cases were not referred to any additional services, due to lost to follow-up (29) or patient refusal (5). As of December 12, there were 371 (88%) live births, 15 (4%) fetal losses, 17 (4%) ongoing pregnancies, and 17 (4%) lost to follow-up. Eighty-four percent of infants were tested for ZIKV. Of the travel-associated cases, 8 out of 286 (2.8%) had liveborn infants with laboratory evidence of congenital ZIKV infection compared to 1 out of 30 cases (3.3%) with local ZIKV exposure. Fifty-five livebirths were born to women with ZIKV exposure that could have occurred in Florida or outside of the continental U.S. None of these women had infants with laboratory evidence of congenital ZIKV infection. Of the nine infants with demonstrated congenital ZIKV infection, seven had congenital symptoms such as microcephaly (6), limb anomalies (2), and intracranial calcifications (2). All nine congenitally infected infants were referred to the Early Steps program. Additionally, three fetal losses, delivered to women with travel-associated exposure, tested positive for ZIKV.

Conclusion: Nearly all ZIKV positive pregnant women (92%) were advised of local pre- and post-natal care programs and most chose to use these types of services. Fortunately, more than 96% of all liveborn infants appeared healthy and tested negative for ZIKV at birth. Ensuring families are informed of local resources will help facilitate identification and management of any delayed congenital symptoms in ZIKV positive infants.

58. EVIDENCE OF ZIKA VIRUS RNA FRAGMENTS IN AEDES ALBOPICTUS (DIPTERA: CULICIDAE) FIELD COLLECTED EGGS FROM CAMAÇARI, BAHIA, BRAZIL

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A major mosquito-borne viral disease outbreak caused by Zika virus (ZIKV) occurred in Bahia, Brazil, in 2015 largely due to transmission by the mosquito, Aedes aegypti (L.). Detecting ZIKV in field samples of Ae. aegypti has proven problematic in some locations suggesting other mosquito species might be contributing to the spread of ZIKV. In this study, several (5) adult Aedes albopictus (Skuse) mosquitoes that emerged from a 2015 field collection of eggs from Camaçari, Bahia, Brazil, were positive for ZIKV RNA, however attempts to isolate live virus were not successful. Results from this study suggest that field-collected Ae. albopictus eggs may contain ZIKV RNA that require further tests for infectious ZIKV. There is a need to investigate the role of Ae. albopictus in the ZIKV infection process in Brazil and to study the potential presence of vertical and/or sexual transmission of ZIKV in this species.

59. EXTRINSIC INCUBATION PERIOD OF ZIKA VIRUS IN FLORIDA AEDES AEGYPTI AND AE. ALBOPICTUS

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Zika virus (ZIKV) is a mosquito-borne Flavivirus first identified in Africa in 1947. The Asian lineage of ZIKV emerged in Brazil in 2015 and subsequently spread throughout the Americas. The continental U.S. received the first imported case of ZIKV infection in December 2015. In July 2016, Florida experienced the first locally acquired ZIKV infection in the continental U.S. Although most often asymptomatic, ZIKV infection may be associated with severe illness including microcephaly and Guillain Barré syndrome. Concerns about these potential risks from ZIKV infection have increased the need to investigate the interactions between potential vectors and ZIKV. The extrinsic incubation period for potential mosquito vectors in Florida is unknown and is a critical parameter in determining risk of transmission. To address this gap in our understanding of ZIKV epidemiology, we orally exposed Florida Ae. aegypti and Ae. albopictus to ZIKV infected blood meals and held them at a constant incubation temperature of 28°C. The saliva was collected from cohorts of mosquitoes and tested for the presence and titer of ZIKV at two day intervals over a period of 24 days to evaluate the extrinsic incubation period of ZIKV originating from Puerto Rico. Results of this study will be presented.

60. FATTY ACIDS AND RELATED KV2 CHANNEL BLOCKERS: ELECTROPHYSIOLOGY AND TOXICITY ON MOSQUITOES

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Ligand-gated ion channels form an important superfamily of proteins involved in many biological processes. Among them, the potassium channels constitute a very diverse group involved in neural signaling, neuronal activity and action potential. Among the different types of channel activation, voltage-gated potassium channels (Kv) are sensitive to membrane polarization. They are homotetramers, and each of the four subunits composed of six transmembrane α-helices: S1 to S4 form the voltage-sensor domain; S5 and S6 constitute the pore domain. Between S5 and S6, a highly conserved loop forms the selectivity filter within the pore. In this work, we tested diverse compounds that were reported as potassium channel blockers, such as TRAM-34 or 5-Hydroxydecanoate (5-HDC) (Wang et al. 2007, Notsu et al. 1992) but their effects have not been explored on voltage-gated Kv channels. Other compounds such as fatty acids (FA), due to their structures, might fit in the lumen of the pore and block the channel activity. To test this hypothesis, we performed patch-clamp recordings of engineered HEK cells expressing Kv2.1 channels. As possible Kv channel blockers, these compounds could represent a lead for novel pesticides. Thus, we tested these compounds for insecticidal effects through topical application on adult mosquitoes (Anopheles gambiae and Aedes aegypti), and through insect preparations (An. gambiae intact and headless larvae

assay and Drosophila central nervous system recordings). Electrophysiological recordings revealed that fatty acid compounds without groups on the main hydrocarbon chain, e.g. Decanoate, 11-dansylamino-undecanoic acid (DAUDA) yield a more potent action on Kv2.1 currents than fatty acids with long-chain groups (e.g. 5-HDC, a hydroxy group in position 5 in the chain). Also, in comparison to PRC1358, a known Kv channel blocker, Decanoate and DAUDA yield a similar potential to block potassium current in vivo. Results obtained through headless larvae assays were very similar to those obtained through patch-clamp recordings. It indicates a continuum between cell level and organ/organism level. Yet, when these compounds (DAUDA and Decanoate) were tested on adult mosquitoes, even though they knocked down insects, they were not as lethal as PRC1358. One possible reason for this difference is compound penetration through the mosquito cuticle. To solve this, we are currently working on new non-polar compounds that could bypass the cuticle barrier. The other potential K+ channel blockers, namely TRAM-34 and Rolipram, did not yield any promising effect on mosquitoes.

61. FEASIBILITY OF A NOVEL OVINE MODEL TO STUDY VERTICAL TRANSMISSION OF ZIKA VIRUS

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Four pregnant, first trimester, Polypay ewes were inoculated with 1 X 10^6 pfu of Zika virus (ZIKV) for two consecutive days (intravenous on day 1, subcutaneous on day 2) and subsequently followed for 45 days post-infection. Two control ewes, one pregnant and one non-pregnant, were inoculated with a sham consisting of PBS. Animals were monitored daily for signs of clinical disease with physical and reproductive exams; fetal development was monitored via weekly transabdominal ultrasonography. Urine, serum, and whole blood were collected daily for the first week post-infection, and weekly thereafter. Quantitative real-time PCR (RT-PCR), plaque reduction neutralization tests (PRNT), viral culture, and flow cytometry (FACS) were performed to detect virus infection, monitor the immune response, and develop cellular assays to characterize responses of PBMC's to ZIKV. All animals were sacrificed after 45 days post-infection and gross necropsies were performed on all ewes and fetuses. All infected ewes seroconverted by PRNT with 85% neutralization titers from 8 to ≥64 by week 7, indicating successful infection. Although weekly fetal ultrasonography showed a presumed reduction in total fetal numbers, post-mortem morphometric data revealed no obvious signs of gross malformation

or growth retardation. We were unable to detect replicating virus in serum, plasma, or fetal tissues (brain, liver, lung, kidney) by RT-PCR or by viral culture of maternal and fetal tissue in Vero-76 and HEK-293 cells. Additionally, FACS was used to characterize the changes in PBMC levels amongst our sheep during the course of infection, and we successfully developed a FACS-based method for identifying extracellularly and intracellularly bound ZIKV. Initial data indicates that there was a decreasing trend in CD3+ T lymphocytes by day 3 post-infection for all infected ewes. Further studies are underway to continue to develop FACS-based methods to detect single cell ZIKV infection of PBMCs. This pilot study served as a proof of concept, indicating that ewes are susceptible to infection. Although we did not detect replicating virus, the detection of a serological and cellular immune response, and subsequent inability to detect viable virus in bodily fluids and tissue, mirrors a commonly encountered scenario with clinical samples from human ZIKV-infected patients. Additional trials of serial infections of young animals are planned to adapt the virus to sheep as we continue to develop this novel outbred model of ZIKV infection.

62. FIRST DETECTION OF CHIKUNGUNYA VIRUS WITH A 3' UTR INSERT IN A HAITIAN PATIENT

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Chikungunya virus (CHIKV) is an alphavirus that is capable of causing severe febrile disease in humans. We are performing arbovirus surveillance work at selected University of Florida study sites in Haiti, and have been analyzing the CHIKV genomes of viruses associated with an epidemic of Chikungunya fever in Haiti in 2014. Previously, we reported sequence conservation among 10 CHIKV isolates that appeared to replicate rapidly in mammalian (Vero E6) cell cultures. The genomes of the faster replicating viruses were essentially identical to those of a predominant Asian CHIKV lineage that swept the Caribbean in 2014. After next-generation system sequencing confirmed by Sanger sequencing of the complete virus genome, we now report that a slower growing year 2014 CHIKV isolate from Haiti has a 177 nt duplicated sequence within the 3' untranslated region (UTR). Remarkably, this type of CHIKV 3' UTR has just been reported by others analyzing the genomes of CHIKV in other parts of the Caribbean. The research group that first made that finding proposes that the 177 nt insert may be a dominant mutation that imparts fitness for adaptation of the Asian CHIKV strains to Aedes aegypti mosquitoes of the Caribbean, which are thought to be the dominant arthropod vector of the virus in that geographical area. To our knowledge, this is the first detection of CHIKV genome with a 177 nt insert in Haiti. This finding merits further research: (a) Does it signify

independent establishment of a lineage-specific CHIKV in Haiti, independent of introduction of the 'standard' Asian CHIKV lineage? and (b) What are the outcomes of Chikungunya fever by CHIKV with the evolved 3' UTR?

63. FIRST DETECTION OF OROPOUCHE VIRUS IN A HUMAN IN HAITI

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First Detection of Oropouche Virus in a Haitian Patient Oropouche virus (OROV) is a bunyavirus that is an important cause of arbovirus illness termed Oropouche fever in Latin American countries, especially in the Amazon region of Brazil, Venezuela and Peru, and in other countries such as Panama. The virus was first isolated from the blood of a febrile forest worker in Trinidad and Tobago in 1955, and since then, has been estimated to have affected at least 500,000 people. However, the exact number of cases is difficult to determine because the infection is underreported due to the similarity of symptoms Oropouche fever with Dengue -, Zika -, Chikungunya -, and Mayaro fevers. We are performing arbovirus surveillance work at selected University of Florida study sites in Haiti. For that work, plasma from patients with suspected arbovirus infections are tested for arbovirus genomic RNA (vRNA) by RT-PCR methods. During the course of investigations of the causes of serial arbovirus outbreaks that occurred in Haiti in 2014 after a Chikungunya fever epidemic, we noted that some outbreaks involved more than one arbovirus. We now report that detection of OROV in the plasma of a Haitian patient who has tested negative for Chikungunya virus vRNA by RT-PCR during the midst of a Chikungunya fever outbreak. Since the

quantity of virions in venous blood is high during acute infections by OROV, it was possible to directly sequence the complete tripartite virus genome of OROV from the vRNA purified from a patient with Oropouche fever. Sequence analyses indicate that the three genomes of the virus we detected have 99% identity with the corresponding genomes of year 2009 OROV from Brazil. To our knowledge, this is the first time OROV has been detected in Haiti.

64. FUNCTIONAL AND MECHANISTIC CHARACTERIZATION OF O-FUCOSYLATION IN MALARIA PARASITES

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Protein glycosylation is one of the largest and most diverse classes of post-translational modifications (PTM). Of these modifications, O-fucosylation represents a relatively small but important glycosylation modality that plays a role in protein trafficking, cell movement/adhesion, and protein secretion. In Eukaryotes, the enzyme O-fucosyltransferase 2 (PoFUT2) is responsible for the specific O-fucosylation of a serine/threonine residue on thrombospondin type I repeat (TSR) protein domains. The TSR domains of several key proteins of the malarial parasite facilitate attachment to mammalian and mosquito host cells, and it was recently shown that Plasmodium sporozoite surface proteins containing TSR domains are fucosylated. We hypothesize that Plasmodium modifies these domains via the putatively identified

parasite PoFUT2 homolog and that this PTM influences ookinete and sporozoite invasion of mosquito and human host cells. We describe biochemically the activity of PfPoFUT2 in modifying TSR-domain containing proteins, such as the circumsporozoite protein (CSP), and evaluate its biological significance during parasite transmission through the mosquito. We characterized by mass spectrometry (MS) the TSR domain of CSP, as well as full length CSP, and show that both can be fucosylated by human and Plasmodium PoFUT2 enzymes. We generated a PoFUT2 null mutant of P. falciparum and P. berghei and compared the growth and survival of parasites throughout the life cycle. We observed that the PoFUT2 null mutant is viable, without any notable defects during development in blood-stage culture and mosquitoes; but P. falciparum salivary gland sporozoites are compromised in their motility in the null mutant compared to wild-type, potentially affecting hepatocyte invasion. Our MS data indicate that CSP lacks this fucose residue in the PoFUT2 null mutant sporozoites. We further compared introduction of null-mutant and wild-type sporozoites to mice by IV inoculation and mosquito bite by monitoring liver and blood stage infection.

65. FUNCTIONAL CHARACTERIZATION OF A PUTATIVE SEX SPECIFIC BIOMARKER IN PLASMODIUM FALCIPARUM

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Plasmodium falciparum is an obligate parasite that completes its asexual and sexual life cycles in vertebrate and invertebrate hosts. Mature gametocytes are the sexual stages of the parasite present in the vertebrate microvasculature that are ingested by Anopheles mosquitoes during blood meal. The mature gametocytes are responsible for parasite transmission to the Anopheles vector where gametogenesis, fertilization, and reproduction occur. While the male and female gametocytes are morphologically distinguishable in Giemsa-stained thin blood smears, little is known about the mechanism and key players of sexual dimorphism in P. falciparum. Identification and functional characterization of sex-specific biomarkers would aid in exploring their biology and help differentiate the parasite “gender”. Our previous systematic subtractive bioinformatic analyses compared the sex partitioned proteins in P. berghei and P. falciparum. This study led to the identification of conserved putative female and male specific gametocyte protein biomarkers, but the function of these putative sex specific proteins remains unknown. This study focuses on the functional characterization of a putative conserved protein that may serve as a biomarker for mature female gametocytes and play a role in sex partitioning in P. falciparum. This putative female-specific gene was cloned and expressed using a heterologous bacterial expression

system. The spatio-temporal expression profile of this gene over a 12 day development period was determined by immunofluorescence, real time PCR, and quantitative Single Reaction Monitoring Mass Spectrometry. Furthermore, antibodies against this sex-specific protein were assessed for their potential to block the parasite transmission to the mosquitoes by standard membrane feeding assays. Our body of work has shed light on a novel gene in the context of gametocyte development biology and hints at the utility of such a target for transmission-blocking interventions.

66. GENETIC DIVERSITY OF WEST NILE VIRUS IN HORSE BRAIN AND PLASMA

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In 1999, a highly virulent West Nile virus (WNV) was introduced to North America resulting in more than 28,012 equine infections. Genetic diversification of the virus allows adaptation to new local transmission cycles. A prime mechanism for this diversification is likely through the production of new viral variants which are able to replicate in new climates and hosts. These variants then exhibit new intrahost tissue or cellular tropisms adding to its robustness. WNV has been shown to be genetically diverse within the host in nature. These genetic variations are essential in the adaptation of flaviviruses in a new and changing environment and host. A genetically diverse virus population would seem to have an

advantage on adaptation because the virus has pre-existing variants which are better adapted to a new and changing environment. The presence of WNV antigen in the brain which is characterized by accumulation of glial nodules and perivascular cuffing, are mostly found in thalamus, pons, and medulla of the horse. Five brain tissues and plasma of two horses were sequenced using Pacific Biosciences (PacBio) sequencing to investigate genetic variation in the NS4B gene of the WNV. The NS4B gene was chosen because of the size, signal noise, and conserved sites. The mean read quality of the WNV NS4B gene sequences obtained from PacBio sequencing was 99.18% with 22 average passes. DNA sequencing showed significant difference in sequence variation and reads in different days post infection. This preliminary finding indicated that WNV could adapt in different tissues and generated more variation overtime in the horses. Phylodynamic analysis will be conducted to further analyze the WNV diversity in horse brain and plasma.

67. HEALTH CARE PROVIDER PRESCRIPTION PRACTICES OF TREATMENT FOR CONFIRMED AND PROBABLE RICKETTSIAL DISEASES REPORTED TO THE FLORIDA DEPARTMENT OF HEALTH, 2015-2016

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Background: Rickettsial diseases are a group of bacterial infections that can lead to severe medical complications and ultimately death if not treated promptly. Thus, the Centers for Disease Control and Prevention (CDC) recommends treatment with doxycycline upon suspicion of a rickettsial infection, rather than waiting until test results are reported. Three tickborne rickettsial diseases, anaplasmosis, ehrlichiosis, and Rocky Mountain spotted fever/spotted fever rickettsioses (RMSF/SFR) are reportable in the state of Florida. Florida is a low-incidence state for these diseases; therefore, health care providers in Florida often do not see many cases and may be unfamiliar with CDC treatment recommendations.

This study analyzes health care provider treatment practices for rickettsial illnesses reported to the Florida Department of Health.

Methods: Confirmed and probable cases of anaplasmosis, ehrlichiosis, and RMSF/SFR reported between January 1, 2015 and December 31, 2016 were reviewed in Merlin, Florida's reportable disease database. Case classification was based on national case definitions published by the Council of State and Territorial Epidemiologists. Data collected included antibiotic recommended, prescription date, date of sample collection for rickettsial disease testing, number of health care visits during illness, and the state in which treatment was recommended and provided.

Results: Eighty-seven cases of rickettsial diseases were reported; 53 cases (61%) were treated in Florida and had laboratory and treatment information. Fifty-one cases (96%) were prescribed doxycycline, one (2%) was prescribed other antibiotics due to doxycycline allergy, and one (2%) was not prescribed antibiotics. Of the 53 cases, six (11%) were treated after laboratory results were reported. These cases were treated an average of 3.5 days after seeking care. In addition, nine cases (17%) were not treated at the first health care visit, and consulted with two to four health care providers before rickettsiosis was considered. Eight of these nine cases were hospitalized before receiving appropriate treatment. One of the 53 cases reviewed died, however treatment delay was attributed to the case not seeking care immediately.

Conclusion: The majority of rickettsiosis cases were prescribed doxycycline before lab results were reported and on the first health care visit. However, nearly 30% of cases experienced delays in diagnosis and treatment. In eight cases (15%), these delays likely resulted in more severe illness and hospitalization. Additional outreach to providers is planned to increase awareness of tickborne rickettsiosis and CDC treatment recommendations, particularly in counties where delays in diagnosis and treatment were reported.

68. HUMAN WEST NILE VIRUS DISEASE OUTBREAK IN PAKISTAN: 2015-2016

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Like most of the world, Pakistan has seen an increase in mosquito-transmitted diseases in recent years. The magnitude and distribution of these diseases are poorly understood as Pakistan does not have a nation-wide system for reporting disease. A cross-sectional study to determine which flaviviruses were the cause of arboviral disease in Pakistan was instituted. West Nile Virus (WNV) is a cause of seasonal fever with neurotropic findings in countries that share borders with Pakistan. Here we describe the active and persistent circulation of WNV in humans in the southern region of Pakistan. This is the first report of WNV causing neurological disease in human patients in this country. Out of 997 enrolled patients presenting with clinical features suggestive of arboviral disease, 105 were positive for WNV IgM antibodies and 71 of these patients possessed WNV-specific neutralizing antibodies. Cross-reactivity of WNV IgM antibodies with Japanese Encephalitis Virus (JEV) occurred with 75 of these 105 patients. WNV co-infections with Dengue viruses were not a contributing factor for the severity of disease. Nor did prior exposure to dengue virus contribute to incidence of neurological involvement in WNV infected patients. Patients with WNV infections were more likely to present with altered mental status, seizures, and reduced Glasgow Coma scores when compared with JEV infected patients. Human WNV cases and vector numbers exhibited a temporal correlation with climate.

69. IDENTIFICATION OF INSECTICIDAL PRINCIPALS FROM CUCUMBER SEED OIL AGAINST THE YELLOW FEVER MOSQUITO, AEDES AEGYPTI

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The yellow fever mosquito, Aedes aegypti, is one of the most medically important mosquito species due to its ability to spread viruses of yellow fever, dengue fever and Zika in humans. In this study, the insecticidal activity of seventeen plant essential oils was evaluated via topical application against two strains of Ae. aegypti mosquito, Orlando (insecticide-susceptible) and Puerto Rico (pyrethroid-resistant). Among the oils tested, cucumber seed oil produced complete mortality (100%) at 10 ug/mosquito, and sandalwood and thyme oils also showed notable 1h knock-down effect, as well as high mortality (>80%) at 24 h in the susceptible strain. In contrast, sandalwood and thyme oils displayed relatively high mortality against the resistant strain, with resistance ratios of 2.1 and 1.4, respectively. Cucumber seed oil showed significantly lower activity with a resistance ratio of 44.5. Bioactivity-guided fractionation via flash-column chromatography produced eleven fractions, and fractions 1, 4, 5, and 6 showed significant mortality at the LD95 (2.3 ug/mosquito) of whole cucumber seed oil, but only fraction 5 showed comparable activity to the crude oil when the factions were applied at the equivalent amount that they are present in the oil (by considering their % yields). Surprisingly, the active fractions, 4, 5, and 6 contained 0.40, 0.48, and 0.29% chlorpyrifos, respectively, an organophosphate insecticide, and in non-active fractions, no trace of chlorpyrifos was observed in gas-

chromatography/mass spectrometry (GC-MS) analysis. A further GC-MS analysis identified benzyl acetate, cyclamal, and linoleic acid as the major constituents of fraction 5, but neither individual compounds nor an artificial mixture of those three compounds produced similar activity of the fraction itself. Chlorpyrifos alone or in a mixture with the remaining three compounds showed significantly increased mortality, indicating the insecticidal activity of cucumber seed oil is probably due to the presence of insecticide. This contamination could happen during the cultivation of cucumber in the field, either via deliberate treatment, or spray drift from nearby fields. For future studies of natural products, particularly botanical insecticide research, the contamination of products with synthetic pesticides or other xenobiotics during cultivation, harvest, or processing steps must be thoroughly monitored and avoided.

70. INSECT NEUROTOXICITY AND PHYSIOLOGICAL MODE OF ACTION OF BASIC AMINES

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The basic amines 1-methylpiperazine and 1-methylpyrrolidine have been reported to interfere with Aedes aegypti and other biting fly host-seeking behavior through a possible anosmia, or narcotizing effect. In this study, the behavioral, toxicological, and electrophysiological effects of these amines and the related basic amine, triethylamine (an active ingredient in Flynap) were investigated. Glass tube vapor phase assays of 1-methylpiperazine and 1-methylpyrrolidine on Ae. aegypti adult females exhibited an initial repellency, followed by narcosis (ability to stand, but loss of

propensity to walk or fly), knock down (inability to stand), and paralysis/death when exposed to 200 nL of the tested chemicals. At this concentration, triethylamine produced a quick knockdown effect (within 1 min) without any repellent effect, and the knockdown effect was reversible, unlike that of the other two amines. All three compounds increased the discharge frequency of mechanoreceptor neurons following topical application to American cockroach (Periplaneta americana) tarsus, and decreased the nerve firing of the Drosophila melanogaster larval central nervous system. Electroantennographic study showed a significant antennal response of Ae. aegypti to the three basic amines, and the responses were not affected by prior anesthesia from triethylamine exposure or ice. These observations suggest that the anosmic effect caused by basic amines was not due to simple block of antennal sensory neuron firing. Because of their structural similarity to the potassium channel blockers, 4-aminpyridine and tetraethylammonium, further studies using patch clamp assays are underway to investigate their effects on potassium channels.

71. INTERACTIONS BETWEEN DENGUE IMMUNITY AND ZIKA VIRUS INFECTION

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There is conflicting laboratory evidence regarding whether prior dengue exposure might provide a cross-protective or enhancing effect on subsequent Zika infections. In this study, we characterize dengue incidence before, during and after the recent Zika virus epidemic in Latin America. We test whether there are detectable changes in state-level dengue dynamics in Brazil and Colombia by comparing the performance of autoregressive models throughout time. We also theoretically investigate a range of possible interactions between dengue immunity and Zika virus infection, including both cross-protection and cross-enhancement. We use compartmental models to characterize Zika outbreaks when the virus is introduced in a community where four dengue serotypes circulate endemically. We assessed multiple models that hypothesized multiple alternative immune mediated interactions between dengue and Zika viruses. We characterized return times of Zika epidemics and endemic dengue dynamics among these different models. For each set of assumptions, we further characterized Zika outbreaks in terms of whether a reduction or increase in the peak number of infections was observed compared to the case assuming no immune mediated interactions. We compared the magnitude of change between models and evaluated differences in dengue

dynamics based on changes in the peak number of infections and duration of time between peaks. Simulations that incorporate a protective effect of Zika immunity against dengue infection display a trough in dengue prevalence.

72. INVESTIGATING THE REPELLENCY OF HALOGENATED AROMATIC AMIDE DERIVATES AGAINST AEDES AEGYPTI

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The purpose of this research is to discover new repellents by evaluating their efficacy for personal protection against mosquitoes. Efficacy was determined by the minimum effective dosage (MED), which is the minimum concentration of a repellent that prevents ≥99% of mosquito bites through treated cloth on the forearms of human volunteers. Over the past decade, fluorine containing chemicals have been developed as effective insecticides and fungicides for the control of agriculture pests. Adding fluorines to an aryl ring results in increased lipophilicity and dipole moment of the parent scaffold, which likely contributes to the biological activity of the compounds. Previous work within our group identified trifluoromethylphenyl amide derivates as potential repellents. In this work, we further investigated the repellency of 27 halogenated aromatic amide derivates (15 of which were chemically novel) against adult female Aedes aegypti. DEET (N, N-Diethyl-meta-toluamide) was included as the standard repellent for comparative purposes. There were 3 human volunteers that participated in the

repellency bioassay against Aedes aegypti (IRB Project #201602334). Each volunteer wore a latex glove on their hand, a knee-high stocking over their arm, and a plastic sleeve over their entire forearm with a small rectangular cut-out area (4cm x 8 cm). This area allowed for odors from the volunteer's skin surface to escape and it was the area where the treated cloth was placed. Experimental compounds were dissolved in acetone and serially diluted (from the highest concentration of 1.5mg/cm2). Cloth patches (5cm x 10 cm) were saturated with each solution and allowed to dry before starting the bioassay. Individual patches were affixed over the rectangular window on each subject's arm, and then the arm was inserted into a screened cage (30cm x 30cm x 30cm) containing approximately 500 female mosquitoes. If the treated cloth allowed for 0-4 bites in the one-minute assays, it was considered to have passed. If ≥5 bites were received, then the treatment was recorded as having failed to repel. None of the halogenated aromatic amide derivates repelled Aedes aegypti as efficaciously as DEET. However, compounds 3A (0.031mg/cm2), 6C (0.013mg/cm2), and 6D (0.047mg/cm2) had MED values that were closest to DEET (0.005-0.011mg/cm2). Our results show that halogenated aromatic amide derivates are weak repellents of mosquitoes and it may be possible to synthesize additional compounds with the intention of improving upon the repellency of this class of chemicals.

73. INVESTIGATING TICK PATHOGEN DISTRIBUTION IN FLORIDA: A FOCUS ON BORRELIA

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Ticks are important vectors of pathogens relevant to wildlife, veterinary and public health. Ticks vector the most commonly reported arthropod borne disease in the United States, Lyme disease. The range of Ixodes tick populations has changed in the past 2 decades expanding into the North-East, North-Central and Mid-Atlantic states, due, in part, to climate change. In Florida, 70 cases of locally acquired Lyme disease were reported in 2015 and 2016. Borrelia burgdorferi is the bacterial pathogen responsible for the majority of Lyme disease cases in North America. In the southeastern U.S., Borrelia burgdorferi is vectored by the ticks Ixodes scapularis, I. minor and I. affinis which are present in Florida. In addition, other Borrelia genospecies have been identified in wildlife and people in Florida including B. americana, B. andersonii, and B. lonestari. The distribution of Borrelia burgdorferi and other genospecies has not been thoroughly investigated in Florida. To investigate the distribution of ticks and Borrelia in Florida, we collected ticks by flagging at 33 sites in 2016 and 2017. Field sites were visited 4 to 5 times during the months of March through December. Ticks collected were identified to species, and DNA was extracted from each tick. A previously established nested PCR protocol targeting a region of the flagellin b gene of Borrelia spp. was used to identify the presence of genospecies. One hundred and sixty four Ixodes ticks were collected at 9/33 sites. Positive PCR results were sent for sequencing. Preliminary results for 40 samples have been processed.

One I. minor was positive for B.burgdorferi in Flagler County. On the same date and site as the positive sample, 4 I. scapularis were collected but were negative. I. minor is thought to be an important vector in maintaining B. burgdorferi transmission. Additional laboratory analyses are currently being processed for B. burgdorferi, and other bacterial and viral pathogens.

74. MADARIAGA VIRUS: IDENTIFICATION OF A LINEAGE III STRAIN IN A VENEZUELAN CHILD WITH ACUTE UNDIFFERENTIATED FEBRILE ILLNESS, IN THE SETTING OF A POSSIBLE EQUINE EPIZOOTIC

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Recent genetic studies of Eastern Equine Encephalitis virus (EEEV) have demonstrated clear separation between North and South American EEEV strains: North American EEEV cluster in a single genetic lineage (lineage I), and South American EEEV strains (now

known as Madariaga virus [MADV]) cluster in EEEV lineages II, III, and IV. While there is reasonable understanding of clinical and epidemiologic features of North American EEEV, little is known about MADV, which can cause outbreaks in horses, and infects a variety of mammals including rats and bats. In humans, MADV infections have been associated with encephalitis. We now report identification of MADV in plasma and urine samples from a child with acute undifferentiated febrile illness in Venezuela, and determination of the complete genome sequence of the virus. Our data document the occurrence of a milder MADV infection (i.e., without encephalitis), with a symptom complex that resembles that seen with other arbovirus infections, including those caused by dengue and zika viruses. The patient was ill at a time period wherein there were reports of an outbreak of equine encephalitis at a region the patient had recently travelled to, with veterinarians there reporting horses with fever, lethargy, extreme weakness, unstable gait, muscle twitches, and fatalities. There were also sporadic reports of equine illness in neighboring states. Illness in the horses was only evaluated clinically; diagnostic tests were not performed to confirm etiology. Based on analyses of the complete genome sequence, the MADV strain we detected clusters within EEEV linage III.

75. MAYARO VIRAL REPLICATION KINETICS AND IN VITRO CYTOPATHOGENICITY IN HUMAN DERMAL FIBROBLASTS

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Mayaro virus (genus Alphavirus, family Togaviridae) is an emerging arthropod-borne virus transmitted by Haemagogus mosquitoes in sylvatic regions of Central and South America. Similar to chikungunya virus, Mayaro virus (MAYV) infection leads to fevers, maculopapular rash and arthralgia. Many aspects of its transmission and pathogenicity remain unknown in human cells. Since the virus is injected into the skin by mosquitoes, cells residing in the skin are of particular interest in understanding early infection and antiviral immune responses. Here, we examine viral replication kinetics and cytopathogenicity of MAYV in human dermal fibroblasts, as well as humoral immune responses. Intracellular MAYV infection was visualized by immunofluorescence microscopy, and further quantified by flow cytometry. IF staining revealed that 74% of cells stained positive for intracellular antigen at 24 hours, and 88% staining positive at 72 hours using an MOI of 1. By flow cytometry, 49% of cells stained positive for viral antigen at 20 hours post infection at MOI of 0.1. Upon viral infection of fibroblasts, virus replication was assessed every 6 hours for 72 hours, and extracellular viral titers were quantified by plaque assay in Vero cells. These assays demonstrated that peak levels of extracellular virus release occurred at 20 hours with a multiplicity of infection (MOI) of 1, at which time titers reached 3.7x106 pfu/ml. Cytopathic effect (CPE) in fibroblasts was observed at varying time points (0 to 72 hours post infection). CPE was clearly observable with crystal violet staining, resulting in 78% reduction in adhered cells at 72 hours (MOI=1). Using fibroblasts as target cells, we then conducted antibody-dependent cell cytotoxicity assays with MAYV positive

human sera. This assay revealed no clear trends compared to media controls. Taken together, these findings advance our understanding of initial Mayaro viral infection in a critical cell type, the human dermal fibroblast.

76. METAGENOMIC IDENTIFICATION OF NATURAL WOLBACHIA INFECTION OF FLORIDIAN AEDES AEGYPTI

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Background: Wolbachia is a gram-negative bacterium, belonging to the Rickettsiaceae family, commonly found as an intracellular parasite in arthropods, including several vectors of important human diseases such as Aedes albopictus mosquitoes. In its hosts, Wolbachia causes cytoplasmic incompatibility (CI) and manipulates host reproduction, which allows it to be spread throughout natural populations at a relatively rapid rate. Aedes albopictus is generally superinfected with two different Wolbachia strains, referred to as wAlbB and wAlbA. However, Ae. aegypti—a closely related species to Ae. albopictus and the main vector for Dengue, Zika and Yellow

Fever—is thought to be naturally refractory to Wolbachia. Some mosquito species previously thought to lack such natural infections were found to be naturally infected with the intracellular bacterium. For instance, Wolbachia-infected Anopheles gambiae and An. coluzzii, members of a genus thought to be largely devoid of natural Wolbachia infections, were recently identified in Burkina Faso. Research has shown that when introduced into the Ae. aegypti mosquito, Wolbachia can help to reduce transmission of the aforementioned arboviral diseases that are primarily transmitted by Ae. aegypti. Wolbachia is already being used to replace wild-type populations of Ae. aegypti in order to reduce transmission of Dengue and Zika viruses.

Methods: The identification of Wolbachia infection in Ae. aegypti was achieved through metagenomics analyses, comprising both 16S and unsupervised metagenomics; PCR for Wolbachia endosymbionts; and confirmatory sequencing of obtained PCR products. We collected mosquitoes from 4 different regions near Tampa, FL (Palmetto, Longboat Key, Anna Maria Island and Cortez) in June 2016 and 2017. We pooled 25 mosquitoes, dissected and homogenized their abdomens and extracted both RNA and DNA. Libraries were constructed for both shotgun (DNA and RNA) and 16S libraries, and sequenced with Illumina. Shotgun reads were trimmed using trimmomatic and classified taxonomically with kraken, while the 16S reads were classified using the Brazilian microbiome project (BMP) pipeline. Metagenomics analyses were performed on the HiPerGator2 cluster.

Results: Wolbachia was detected in all four locations in both 2016 and 2017. The Wolbachia genus was represented at only 5% in the Palmetto location in 2016 based on DNA shotgun metagenomics (1% based on 16S metagenomics), while at 1% based on RNA shotgun metagenomics. As for the other locations, Wolbachia was present at lower levels (0.017-0.4), detectable only through shotgun metagenomics in comparison to the 16S method. Moreover, there was a net decrease of Wolbachia from the first year of collections to the second. From the reads (DNA shotgun metagenomics) derived

from mosquitoes collected in Palmetto in 2016, we were able to retrieve fragments for the 16S and 23S ribosomal RNA genes belonging to the novel Wolbachia endosymbionts associated with Ae. aegypti in Florida by reference-mapping against Wolbachia endosymbionts of Culex quinquefasciatus Pel strain wPip. The two fragments that were retrieved for the 16S and 23S genes were 1,546 bp long with a mean coverage of 185.4, and 2,797 bp long with a mean coverage of 146, suggesting that through both shotgun and 16S metagenomics, we are underestimating the real proportion of Wolbachia in the Floridian Ae. aegypti.

Conclusions: During efforts to identify correlates between human arboviral pathogens and discover how the microbiome of Florida A. aegypti wild-type mosquito populations evolve spatiotemporally, we obtained positive results for Wolbachia in the wild population of Ae. aegypti mosquitoes in Florida. Results suggest that Floridian Ae. aegypti are naturally infected with a species of Wolbachia bacterium that is specific to aegypti. We plan to further collect mosquitoes in 2018 in the four locations to determine whether Wolbachia is still present in low levels. Currently, we are trying to establish a Wolbachia-infected Ae. aegypti colony from field collections and will attempt an infection study with the naturally infected mosquito colony.

77. MINIMAL TO NO CHANGE OF ZIKA VIRUS CONSENSUS SEQUENCES AFTER ISOLATION AND LOW PASSAGE IN VERO CELLS

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Zika virus (ZIKV), genus Flavivirus, has a single-stranded positive-sense RNA genome that is approximately 10.8 kb in length, and is the cause of Zika fever. As for many flaviviruses, ZIKV is an arbovirus that cycles between mosquito and mammalian hosts. It has recently caused outbreaks of Zika fever in the Americas, and ZIKV infections of pregnant women can lead to infection of the fetus. The latter can result in the development of severe birth defects such as microcephaly in babies that survive ZIKV infection. Though the ZIKV in circulation in the Americas belong to the Asian clade of the virus, different strains of the virus have quickly evolved. Sequence analyses of ZIKV genomes are thus important and very useful for surveillance efforts designed to track its establishment in new niches, and its spread/circulation among humans and mosquitoes. Moreover, virus

genomic sequence analyses are essential for basic research studies of ZIKV in cultured cells, and for ZIKV used in animal studies. There has been concern that ZIKV isolated in cell culture does not retain the wild-type virus genome sequence, the premise being that nucleotide changes that occur during passage in cultured cells alters the virus phenotype, resulting in either attenuation or enhancement of virulence. We have found this not to be the case when the virus is properly propagated in cell cultures. Here, we reveal reliable methods for preparing accurate ZIKV RT-PCR amplicons for Sanger sequencing, and provide some real-world examples of ZIKV isolation or passage in Vero cells without significant changes within the consensus sequence of the virus genome.

78. MODELLING MALARIA PREVALENCE USING DHS DATA: COMPARISON OF MODEL APPROACHES

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Geospatial statistical models are now widely used in malaria epidemiology to identify risk factors, assessing efficacy of intervention program, and to produce reliable and comprehensive malaria risk maps, which are essential to guide efficient resource allocation for planning and implementation of intervention program. Many modelling approaches have been used in the literatures, e.g. Bayesian geospatial model (BGM), generalized linear model, P-splines regression and Gaussian point process approximation using stochastic partial differential equation (SPDE) approach. The abundance of methods calls for comparison study as it will inform the researchers on selecting the right model. However, such studies are limited and their findings may not be applicable as the geographical scope changes. In this study, we compared the predictive performance of various techniques in modelling malaria prevalence in six African countries separately, using DHS survey data. We found that Bayesian geospatial model is generally the most reliable method for country-level malaria modelling: its performance in these countries was the best or the second best. The General

Additive Model (GAM) and SPDE approach also performed close to that of BGM, and in some countries, GAM outperforms BGM. Given that GAM and SPDE take only a small fraction of time required to run BGM, we think that they are good alternatives to the BGM approach.

79. MODELLING THE DISTRIBUTION OF MAJOR MALARIA ANOPHELES SPP. ACROSS GHANA USING A BAYESIAN APPROACH.

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Understanding the ecology and spatial distribution of dominant malaria vectors is essential to the design of effective and sustainable strategies for malaria control and elimination. However, rarely is spatial distribution of the dominant vectors used in planning at country-level and there have been fewer attempts to understand the link between spatial composition and behavioral bionomics. Previous studies have tried to define ecological niches and vector bionomics, but at larger-scales. These have been important for general understanding of vector dominance and distribution at the continental scale, but they lack the ability to define the density of each species at a finer resolution. In this study, we aim to build on these earlier approaches and assess the determinants of vector abundance, so as to produce better predictive maps of the relative abundance of major vectors of malaria in Ghana. Vector count data were modeled using an over-dispersed Poisson model within a Bayesian framework to evaluate key environmental drivers and to predict spatial distribution. Environmental variables were derived from remote sensing applications. A total of 334 survey sites were collated for the species Anopheles gambiae and An. funestus. Model validation was done on 10% of these locations prior to final predictions. We find that, although mapping of these vector abundance patterns shows promise, there still the need for more systematic and consistent data to be able to help with vector management.

80. MODELLING THE SPATIOTEMPORAL DISTRIBUTION OF AEDES AEGYPTI AND AEDES ALBOPICTUS IN FLORIDA

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The mosquitoes Aedes aegypti and Aedes albopictus are the primary vectors of several important mosquito borne viruses including dengue, Zika and chikungunya. Previous studies characterized the current, and project the trend of, the spatial distribution of Ae. aegypti and Ae. Albopictus by applying boosted regression trees (BRT) to a comprehensive global record of Aedes distribution. These studies, which estimate the distribution of Aedes across the globe are limited because of the minimal amount of data available on mosquito populations that comes from standardized collection. Here, we focus on the performance of these models in Florida, validating with empirical data and increasing the accuracy of model predictions within the state. We obtained routine surveillance data collected from Florida's mosquito control boards on the county level. Each control board or district conducts trapping prior to control efforts, which provided mosquito species, count, and location data. Climate data (i.e. temperature and precipitation) was extracted from the WorldClim. We validated published BRT models by comparing the model prediction and the observed counts of Aedes mosquitoes stratified by county and time. In order to optimize the utility of model predictions, the uncertainty of model prediction associated with various factors will be characterized including geographic scale, climate variation between years, and the interaction between Aedes species. Thus far, geo-located count data for Ae. aegypti and Ae.

albopictus span the 2004-2017 timeframe and six counties of Florida. Ae. albopictus were widely trapped in all six counties, while the occurrence Ae. aegypti were barely recorded in Alachua and Citrus County. This approach should better characterize the distribution of the two Aedes species in Florida and validate and improve model prediction by using mosquito data with finer spatial and temporal resolutions than previously utilized.

81. MOLECULAR DIAGNOSTICS REVEAL WIDESPREAD PYRETHROID RESISTANCE IN AEDES AEGYPTI POPULATIONS OF COLLIER COUNTY FL

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The use of integrated pest management (IPM) is paramount to reducing populations of disease vector mosquitoes, such as Aedes aegypti. IPM approaches should include a comprehensive insecticide resistance monitoring program to guide the use of insecticides for control measures. The CDC bottle bioassay has previously been the preferred method for insecticide resistance testing; however, these processes can be labor intensive and slow to produce results in a timely manner for operational decision making. The presence of allelic variation contributing to pyrethroid resistance has implications for a single molecular diagnostic test for detection of resistance in field populations. In Ae. aegypti, resistance to pyrethroids and dichloro-diphenyl-trichloroethane (DDT) has been attributed to two point mutations (F1534C and V1016I) in the voltage-gated sodium channel. We performed a SNP genotyping screen of four field collected Ae. aegypti populations from Collier County FL and identified high frequency of the F1534C and V1016I pyrethroid resistance alleles. These results correlated to previous CDC bottle bioassays that have revealed Collier County Ae. aegypti resistance to the pyrethroids: deltamethrin, permethrin and sumithrin. We performed CDC bottle bioassay with our resistant Naples Park Ae. aegypti population using four commercially available pyrethroid-

based insecticides for aerial spraying, including: Merus 2.0 (pyrethrins), Anvil 10-10 (sumithrin), Deltagard (deltamethrin) and Zenivex E20 (etofenprox). The botanical-based insecticide, Merus 2.0, performed well with our resistant Naples Park Ae. aegypti stain, with a mortality rate of more than 70% at the CDC diagnostic time of 15 minutes.

82. MONITORING AND MODELING THE DISTRIBUTION OF THE MOSQUITO VECTOR AEDES AEGYPTI IN SOUTHERN NEW MEXICO AND WEST TEXAS

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A current and accurate understanding of the spatial distribution of vector species is vital for countering the threat of mosquito-borne disease. In New Mexico and West Texas, such an understanding is currently lacking, particularly for Aedes aegypti and Aedes albopictus, two species that can vector Zika, chikungunya, and dengue viruses. To address this issue, we completed 236 site visits across twenty-four counties between June 13 and October 31st, 2016. Sampling sites were selected using a clustered, stratified random sampling design, with sites located in six land cover types: urban or built-up land, agricultural land, rangeland, forest land, wetland, and barren land. Mosquitoes were sampled using gravid traps, autocidal gravid ovitraps, backpack aspirators, and larval dipping. Using morphological keys, we identified all mosquitoes to the genus level and all Aedes mosquitoes to the species level. Overall, we collected and identified 1,722 mosquitoes, with 30 sites yielding Aedes aegypti and one site with Aedes albopictus. Aedes aegypti is present in urban areas in southern counties and Aedes albopictus in one eastern county. We then developed species distribution models using logistic regression and MaxEnt to better understand the current distribution of Aedes aegypti. Spatial data representing climatic, environmental, and demographic factors that have been used in similar studies were included as potential explanatory factors. After variable selection and model fitting, variables that were shown to influence the distribution of this

species included winter temperatures, July precipitation, and population density.

83. MOSQUITO DERIVED FACTORS-FARNESYL DIPHOSPHATE AND LIPOPHORIN AND PLASMODIUM PARASITE DEVELOPMENT

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Malaria is a devastating vector borne disease caused by Plasmodium parasites. Plasmodium transmission through Anopheles mosquitoes is obligatory. The molecular mechanisms and pathways utilized by the parasite to undergo the sporogonic development in the mosquito are not yet fully understood. Transmission-blocking approaches that prevent parasite infection of the mosquito and subsequent transmission are touted to be an important tool to control and eliminate malaria. However, in order to develop transmission-blocking approaches, it is critical that we develop a deeper understanding of parasite transmission from humans to mosquito. Our preliminary analyses underscored the importance of mosquito-derived factors to drive the maturation of Plasmodium ookinetes in an in vitro model of sporogonic development. Proteomic and bioinformatic analyses lead to the identification of candidate mosquito host molecules that may influence the maturation during the zygote-to-ookinete transition phase that occurs inside the mosquito midgut. We hypothesize that, two of the identified molecules— (2E, 6E)-farnesyl diphosphate (HMG-CoA reductase pathway intermediate) and lipophorin (lipid-transport protein) play a role in the development of the parasite from zygote-to- ookinete. Farnesyl diphosphate is an essential intermediate in the isoprenoid biosynthesis pathway for the parasite and is also involved in post-translational modifications and activation of proteins such as

Ras in other organisms. The Ras family of proteins are involved in signal transduction in cellular growth and differentiation. A similar activation of analogous proteins could be acting as one of the triggers to maturation of ookinetes. Lipophorin is a mosquito lipoprotein, which is taken up and utilized as a lipid source by oocyst. Using a multi-OMICS approach, we are characterizing the parasitic response to farnesyl diphosphate and lipophorin to capture a global view of the cellular mechanisms driving ookinete maturation. Our study will lead to the improvement of an in vitro culture platform for Plasmodium sporogonic development and a better understanding of the parasite-specific biochemical pathways that can lead to the identification of novel, druggable, transmission-blocking targets.

84. NOVEL FLUORINE-CONTAINING SPATIAL AND CONTACT REPELLENTS

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Mosquito-transmitted diseases such as malaria, dengue, and yellow fever, result in thousands of human deaths yearly. Climate change and global warming can enhance vectorial capacity as well as temporal and spatial distribution of mosquito populations. To find more effective tools for mosquito and disease control, we focus on the development of new repellents and insecticides to prevent mosquito bites and reduce disease risk to humans. We have synthesized 79 trifluoromethylphenyl amides in four successive

stages. A new generation was chosen based on active structures of the previous generation. The compounds were evaluated for repellent activity against female Aedes aegypti. Six compounds were determined to be efficacious contact repellents and up to 10 compounds showed promise as spatial repellents, equal to or exceeding the repellency of DEET. Trends were observed in the correlation of structural features to biological activities of the compounds. For contact repellency, amides with a pentafluoroethyl moiety (-CF2CF3) at a carbonyl carbon were more likely to be better repellents. Adding a chlorine to the para- position of the amide group in 3,4,5-trichlorophenyl amides decreased the repellency compared to the analogous compounds without a chlorine at the para- position (3, 5-dichlorophenyl amides). There was some correlation observed (r2 ~ 0.4) between vapor pressure and the minimum effective dose (MED) for repellency of the amides and no or weak correlation between MED of repellency and lipophilicity or Pka (-log of the acid dissociation constant) values. The LogP (partition coefficient) for most active compounds range from ~ 2.5-4. There was a weaker correlation between vapor pressure vs EC50 (r2 ~ 0.20) for the insecticide-susceptible A. aegypti Orlando strain (wild-type) than the pyrethroid-resistant A. aegypti Puerto Rico strain (r2 ~ 0.45). Salicylic acid esters and 2-trifluoromethylbenzoic acid esters were more active spatial repellents than DEET; derivatives with a shorter alkyl chain were slightly better repellents than those having a longer chain. Four carbamic acid methyl esters were comparable or better than DEET. Four derivatives of 2-trifluoromethyphenyl amides, also, showed better or comparable to DEET activity. These studies report the discovery of novel structures that could lead to new mosquito repellents.

85. PRELIMINARY EVIDENCE OF A WAVE OF ORTHOBUNYAVIRUS INFECTIONS IN HAITI IN 2014 DURING SEQUENTIAL OUTBREAKS OF ARBOVIRUS INFECTIONS

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We are engaged in arbovirus surveillance work at selected University of Florida study sites in Haiti. For that work, plasma and other relevant specimens from patients with suspected arbovirus infections are tested for arbovirus genomic RNA (vRNA) by RT-PCR methods. Though relatively fast and highly sensitive, RT-PCR is only useful for specific targets. Thus, virus isolation is also attempted to cast a wide net whenever possible. These methods are necessary because patients with acute arbovirus infections present with common symptoms that include fever, joint and muscle pains, and a rash, and a specific diagnosis based on overall presentation is unlikely. After an epidemic of Chikungunya Fever swept the country in the first half of 2014, we noted several more serial 'waves' of arbovirus outbreaks. The causative agents included the alphaviruses Chikungunya virus (CHIKV) and Mayaro virus (MAYV), and the flaviviruses Dengue virus 1 (DENV1), Dengue virus 4 (DENV4), and

Zika virus (ZIKV). Among the specimens tested, real time RT-PCR (rtRT-PCR) tests performed on two plasma samples from patients seen in August and November 2014 were negative for CHIKV, MAYV, DENV1, DENV4, and ZIKV vRNAs. Additional tests were negative for Dengue virus-2 and -3 vRNAs. Furthermore, RT-PCR tests using 'universal' primers for the detection of alpha- and flaviviruses were negative. Since levels of arboviruses in viremic blood can be low, and the amount of vRNA in a given rtRT-PCR test too low to be detected, virus isolation in cell culture was attempted. Instead of injecting specimen aliquots into baby mouse or hamster brains for virus isolation, mouse neuroblastoma 2A (Neuro-2A) cells were inoculated with aliquots of plasma from the two 'mystery' patients. Following successful isolation in Neuro-2A cells, the virus isolates were further amplified in Vero E6 cells to produce high titer stocks for further analyses. We now provide preliminary evidence that the mystery virus is an orthobunyavirus, and our finding raises awareness of orthobunyaviruses as a cause of arbovirus disease in Haiti.

86. QUANTIFYING SEASONAL AND DIEL VARIATION IN ANOPHELINE AND CULEX HUMAN BITING RATES IN SOUTHERN ECUADOR

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Quantifying mosquito biting rates enables estimation of mosquito-borne disease risk, and can inform local intervention efforts. Measuring biting itself is fraught with ethical concerns, so the landing rate of mosquitoes on humans is often a proxy measure. Southern coastal Ecuador was historically endemic for malaria (P. falciparum and P. vivax), but control efforts in the 2000s eliminated autochthonous transmission (since 2011). We examined human landing catch (HLC) data for three mosquito taxa during the elimination period: 2 malaria vectors, Anopheles albimanus and Anopheles punctimacula, and grouped Culex spp. Data were collected by the National Vector Control Service of the Ministry of Health over a 5-year time span (2007 – 2012) in five cities in southern coastal Ecuador, at multiple households, in all months of the year, during dusk-dawn (18:00-6:00) hours, often at both indoor and outdoor locations. We used hurdle models to determine if biting activity was fundamentally different among taxa, and to identify spatial and temporal bite rate patterns. Due to a multitude of approaches to studying and quantifying bite rates in the literature, we also created a glossary of terms, to facilitate future comparative studies. Biting varied significantly with species and time. All taxa exhibited exophagic feeding behavior, and outdoor locations increased both the odds and incidence of bites. An. albimanus was most frequently observed biting, with an average of 4.7 bites per hour. The highest and lowest respective months for significant biting activity were March and July for An. albimanus, July and August for An. punctimacula, and February and July for Culex spp. This analysis provides detailed information for targeting vector control and household level behavioral interventions, in particular identifying a timing mismatch with seasonal vector control efforts. These data were part of routine vector surveillance conducted by the Ministry of Health, but have not been collected since elimination. Reinstating surveillance would provide important information in preventing malaria re-emergence.

87. 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 RT-PCR tests for ZIKV genomic RNA. Based on our previous work in Haiti and in Venezuela, we have found that blood is a crucial specimen for differential diagnostic testing of ZIKV and other mosquito-transmitted viruses that cause similar symptoms. As RT-PCR tests require expensive instrumentation and trained laboratory scientists to perform the tests, there are no point-of care tests currently available. Most often, the specimens must be shipped refrigerated or frozen to the testing laboratory; without cold storage, sample integrity deteriorates, leading to false negative results. Furthermore, this requirement for cold-storage adds increased costs related to shipping and handling of cold, infectious material, as well as delays in diagnosis. Alternative low-cost methods for shipping blood have been tested and validated for the shipment of virus-containing blood, such as blotting a drop of patient's blood on sterile filter paper followed by dry storage at room temperature. Dry storage on filter paper facilitates shipment at greatly reduced costs while preserving the integrity of the virus for diagnostic tests. Elimination of cold-chain storage alone substantially reduces costs; however follow-up RT-PCR tests are time-consuming and expensive to perform. Recent advances in the design of diagnostic molecular tests include utilization of RT-LAMP, an innovative, sensitive and effective testing method that can be performed using minimal, inexpensive

instrumentation. In this study, we show that ZIKV in dried blood spots can be rapidly detected using RT-LAMP in a manner that does not require expensive instrumentation. We have implemented a very robust RT-LAMP system that relies on previously described primers and a relatively new commercially available enzyme system. Our results indicate that we can rapidly detect ZIKV in blood and other specimens at a sensitivity that matches or surpasses current procedures. We have also determined that, when compared to other types of filter paper, high-quality chromatography paper is a superior substrate for the preparation of dried blood spots containing ZIKV intended for RT-PCR or RT-LAMP tests. Finally, we show that the suitability of virus nucleic acid extracted from dried blood spots for RT-LAMP or RT-PCR is dependent on the purification method. Lessons learned from this study lead the way to cost-effective, reliable, and fast methods that do not require complex and expensive instruments for the diagnosis of ZIKV infections. Moreover, future developments will lead to systems that may be performed point-of-care by minimally trained personnel.

88. SEROPREVALENCE OF DENGUE IMMUNITY AMONG MULTIPLE SPECIES OF NONHUMAN PRIMATES IN SENEGAL

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Sylvatic strains of dengue virus (DENV) have been shown to readily infect humans without lengthy adaptation processes, providing merit to studying the potential for sylvatic DENV strains to re-emerge into an endemic cycle. However, the importance of particular species of nonhuman primates in the ongoing transmission of dengue in sylvatic settings is unknown. Here, we report age-stratified seroprevalence of DENV-2 antibody among African green monkeys (Chlorocebus sabaeus), patas monkeys (Erythrocebus patas), and Guinea baboons (Papio papio) captured over three years (2010-2012) throughout the Department of Kédougou, Senegal. Serum samples were collected from 219 African green monkeys, 78 patas monkeys, and 440 baboons. The age of each primate was determined either using an algorithm that utilized multiple dental measurements or anthropometric data when dental casts were inadequate. This information was used to estimate the force of infection, λ, the hazard of infection that susceptible individuals experience, of DENV-2 for each species and year. We find that forces of infection of DENV-2 are high in all species, ranging from 0.49 - 5.62 in African green monkeys, 0.37 - 0.68 in patas monkeys and 0.21 - 0.42 in baboons. We also examine heterogeneities by troop and season and investigate spatial patterns of seroprevalence. Our data suggest high-levels of sustained DENV-2 transmission in each of the nonhuman primate species examined.

89. SIMULTANEOUS ON-CHIP DETECTION OF ZIKA VIRUS AND ANTIBODIES USING ARRAY OF NANOWELLS

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Zika virus (ZIKV) infection is often asymptomatic during an incubation period that can range from 3 to 12 days. The serological detection of ZIKV is even more complex due to extensive cross-reactivity between antibodies triggered by various flavivirus infections. The current methods for detecting ZIKV are limited by need for relatively large sample volumes together with low sensitivity and specificity. This hinders both experimental and diagnostic sensitivity for a disease that has low viremia levels, a short incubation period, and a particularly detrimental characteristic of rapid infection via multiple mechanisms. We have developed a nanowell-based method to detect copies of ZIKV transcripts directly from minimal amounts of sample (nanoliter volume) by one-step, single-cell, reverse transcription polymerase chain reaction (RT-PCR). This simple method, when combined with microengraving, identifies ZIKV-specific antibodies in the same nanowell. To test this technology, samples were deposited in a nanochip containing 248,832 wells with 30µm dimension. A slide was incubated with the nanochip to capture only antibodies present in the samples. A fluorescent-labeled ZIKV envelop protein was used to detect ZIKV-specific antibodies. In parallel, RT-PCR master mix containing ZIKV probes and primers were added into the nanochip wells and placed onto a specialize thermocycler for denaturation, annealing, and elongation. The fluorescent intensity of the PCR product was

detected via fluorescent microscopy. Microarrays of the ZIKV-specific antibodies were scanned using GenePix 4400A Microarray scanner and custom-made software was used to correlate data obtained from ZIKV RT-PCR imaging and microarray micrographs. The preliminary data indicate that on-chip detection of ZIKV and ZIKV-specific antibodies is feasible in a high-throughput and nanoliter volume. Further work will be performed to demonstrate reproducibility, specificity, and accuracy of the assay. Our technology will detect ZIKV infection at the early stage when viremia is low. At the same time, it can identify ZIKV-specific antibodies at the later stage of infection. The process requires minimal amount of sample for the diagnosis. The rapid viral and serological detection will help patients be more informed and counseled with their daily activities and travels. Microcephaly in fetus is one of the most devastating consequences of Zika infection, therefore having a timely infection status will better prepare the patients. Our research will provide a necessary and temporary relief to the families and public health concern in the state.

90. SOCIO-ECOLOGICAL FACTORS IMPACTING DENGUE RISK IN HUAQUILLAS, ECUADOR: A BINATIONAL BRIDGE OF HEALTH

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Viral mosquito borne diseases such as Dengue fever, Chikungunya, and Zika represent an increasing burden on the public health of the tropics. These diseases have become hyperendemic in southern coastal Ecuador, where a union of physical, biological, and social factors maintain a resilient cycle of transmission. In Ecuador, the city of Huaquillas is the southernmost coastal urban center with a population of 50,000 people. The city's tropical climate enables the Dengue transmission cycle to continue throughout the year. The climate is hot and semi arid with average monthly temperatures varying between 23°C and 27°C and relative humidity rarely falling below 70%. A wet season spans winter and spring with peak rainfall in March. These wet seasons have coincided with epidemic levels of Dengue fever in the past, especially during El Niño events. Huaquillas is positioned on the Ecuadorian–Peruvian border and is the largest hub of transit and commerce between the two nations. As a result of the city's role as a connecting node, local disease ecology can have considerable influence on regional epidemiology. The pivotal nature of the geography of Huaquillas is recognized by both Peru and Ecuador, who have coordinated programs in vector management and health care. Aedes aegypti and Aedes albopictus are currently significant vectors for viral mosquito borne disease and thrive in urban and peri-urban environments. In urban ecosystems, human activity dictates physical configuration which directly impacts the biological success of disease vectors and consequently disease prevalence. It is imperative to understand the social factors associated with increased vector populations, specifically the abundance of reproductive habitats and larval-pupal presence.

Socio-ecological surveys and accompanying entomological assessments were conducted at the household level in ten localities in Huaquillas. Surveys included questions regarding demographics, utilities, knowledge & perceptions, economic expenditures, and building conditions. Water storage practices, housing conditions, and socioeconomic status are hypothesized to be significant factors contributing to vector population maintenance and disease risk. We aim to analyze survey data using a multi model selection process and explore the spatial distribution of risk at the city scale. An understanding of the social factors contributing to suitable vector habitat and its spatial distribution has immediate utility in mosquito control, public outreach and education, health interventions, and policy decisions. An informed and integrative approach to vector population management could reduce the gross burden of emerging mosquito borne diseases in Huaquillas and the binational area.

91. SPATIAL REPELLENCY SCREENING IN A HIGH-THROUGHPUT APPARATUS WITH AEDES AEGYPTI AND ANOPHELES GAMBIAE.

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Spatial repellents are essential for personal protection against mosquitoes, such as Aedes aegypti and Anopheles gambiae, to reduce annoyance biting and mosquito-borne diseases. The number of safe and effective repellents, including DEET, picaridin, and IR3535, is limited and continuous usage could lead to resistance.

Thus, searching for new spatially-active compounds is necessary for improved disease control. Most current repellent screening methods, such as olfactometers and alternative choice test systems, require complex setup or use of a large amount of compound (mg). The purpose of this study was to test the efficacy of a high-throughput spatial repellent screening apparatus that takes relatively little space and uses small amounts of compounds (typically µg levels). In this setup, compound-treated filter papers were held in caps on both ends of a 5" glass tube, and groups of mosquitoes (n = 16) were prevented from contacting filter paper by nettings. Spatial repellency was quantified by the ratio of mosquitoes on the treated side of the test tube, in which case a value of zero equaled to full repellency. Trifluromethylphenylamides (TFMPAs), anthranilates and other experimental compounds were tested in this apparatus, and their potency was compared to DEET, a standard spatial repellent. DEET had EC50 (half effective repellent concentration on the filter paper) values of 31 (25-39) µg/cm2 and 79 (51-120) µg/cm2 against Ae. aegypti wild-type (OR) and Puerto Rico (PR) resistant strains, respectively, and 39 (29-51) µg/cm2 against the An. gambiae G3 susceptible strain. Screening of TFMPAs identified several compounds (EC50 values in parentheses) that had activity comparable to DEET; 1-4A (25 µg/cm2), 4-2B (15 µg/cm2), 4-3D (17 µg/cm2), and 4-5A (17 µg/cm2) were the most active compounds. Interestingly, 4-3D had an EC50 of 16 (9-26) µg/cm2 against PR strain, ca. 5-fold lower than DEET. Three anthranilate compounds showed strong spatial repellency; ethyl anthranilate was most active, with an EC50 of 6.9 (4-10) µg/cm2 on OR strain, ca. 4-fold lower than DEET. This bioassay system was useful for quantifying both spatial repellency and vapor toxicity; compound CA-12 had knockdown activity at 100 µg/cm2, while all active mosquitoes accumulated in the untreated half. To conclude, this high-throughput screening setup is useful for fast-acting candidate spatial repellents against mosquitoes and potentially ticks, with slight modifications to the size of nettings. Further experiments include testing insecticide-resistant An. gambiae, and quantifying repellent potency of combination treatments.

92. SPATIOTEMPORAL ANALYSIS OF DENGUE IN THE EL ORO PROVINCE OF ECUADOR, 2003-2011

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Dengue virus (DENV) is an endemic disease afflicting southern Ecuador that is primarily transmitted by the vector Aedes aegypti and features seasonal reoccurrence. Our research goal is to identify spatial, temporal, and spatiotemporal clusters of DENV in order to further analyze the related factors that could be attributing to the proliferation of the disease. Using ArcMap and SaTScan, we will perform spatial and temporal analyses of human case data of incidences of DENV at the canton (district) level that have been collected throughout El Oro province by the Ministry of Health of Ecuador. For our analyses in SaTScan, the cases are attributed to the canton in which the incidence of DENV was reported, and then georeferenced to the geographical centroid of that canton. The human case data were plotted to examine visible trends demonstrating the seasonality of the disease in the region and the preliminary results of our analyses using SaTScan indicate that there is both spatial and temporal clustering of DENV cases at the level of canton in El Oro.

93. THE CDC SOUTHEASTERN REGIONAL CENTER OF EXCELLENCE IN VECTOR BORNE DISEASES

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The State of Florida is no longer a hypothesized entry point or transmission site for new vector- borne pathogens. We are “ground zero”. As the gateway for vector-borne diseases (VBD) into the Southeastern United States and routes north, Florida has been at the forefront of applied VBD research with demonstrated excellence in the development of innovative technologies for mosquito surveillance and control, and in defining emergency “surveillance-response” standards. The “CDC Southeastern Center of Excellence (CoE) in Vector Borne Diseases: Gateway Program” is led by investigators from four major universities in Florida (Florida International University, the University of Miami, the University of South Florida and the University of Florida and its associated divisions) with strong vector biology/medical entomology programs. This integrated network carries the full support of the Florida Mosquito Control Association (MCA) representing a vast system of 59 mosquito control districts, the Florida Department of Agriculture and Consumer Services (FDACS), the Florida Department of Health (FDOH), the USDA-ARS Center for Medical, Agricultural & Veterinary Entomology (CMAVE), and the Naval Entomology Center of Excellence (NECE). In the past year, we have expanded the network of applied research collaborations to include colleagues in the Southeastern States (Georgia, Alabama, Louisiana, Tennessee, and North Carolina) and this network is expanding quickly. Objectives | Our center leverages the expertise across a broad remit of vector biology disciplines to achieve the following objectives: (1) Conduct an innovative, applied research program to develop novel control interventions and optimize surveillance paradigms that would allow mosquito control associations throughout Florida and the US to better anticipate and respond to VBD outbreaks; (2) Establish an integrated research and training network between academic

institutions throughout Florida and the Southeast and the local, state, and federal public health agencies to facilitate existing and future efforts in VBD surveillance and control; (3) Expand an effective UF-led training program in basic public health entomology, pathogen diagnostics and advanced molecular vector biology to augment the cohort of personnel who are trained with the requisite knowledge and skills to quickly detect and respond to VBDs; and (4) provide an evidence-based set of recommendations and a tailored template of a "surveillance-response program" to ensure that local mosquito control associations can better predict and address VBD threats in the US. In addition, the CoE is leading four research program projects in collaboration with mosquito and vector control districts/programs, departments of health and agriculture and consumer services throughout Florida and the Southeast:

Project 1: Understanding vector ecology, arbovirus infectious rates & insecticide resistance to optimize mosquito control | The biocomplexity of Aedes vectors of Zika, Dengue, Chikungunya, and other arboviruses in the US remains understudied. We will investigate how fundamental, field-based information on the complexities of vector ecology and adaptation can be used to parameterize local and global models (Project 4) for predicting disease transmission and forecasting the longer-term potential of novel vector control tools in effecting optimized vector control in urban environments.

Project 2: Breaking the transmission of zoonotic arboviruses by mosquitoes | Eastern Equine Encephalitis virus (EEEV) can over-winter in ectothermic hosts. The overall aim of this project is to predict at the focal level where over-wintering is occurring in Florida, and to leverage complementary mathematical models (Project 4) to develop early season intervention strategies to interrupt wintertime EEEV transmission.

Project 3: Ecological and insecticide-resistance models of tick vectors in the Southeast US| The geographic distribution and abundances of tick vectors, their acaricide resistance status and their associated pathogens are ever-changing. The overall aim of the project is two-

fold: (i) Characterize the prevalence and distribution of selected tick-borne bacterial and viral pathogens via high-resolution spatial maps in Florida; and (ii) Understand the mechanisms and extent of insecticide resistance in two vectors of medical and veterinary importance: the brown dog tick and lone star tick.

Project 4: Multi-scale, modular models for Vector-Borne Disease | Models of arbovirus transmission are useful in estimating the burden of Dengue, forecasting areas of risk for the emergence of Dengue, Chikungunya and Zika in non-endemic areas, and in assessing the potential of control interventions. The utility of these models relies on their grounding in empirical data at multiple scales describing the disease transmission process. The aims are to (i) model the dynamics of viruses in their invertebrate and vertebrate hosts as well as the movement of these hosts viz. transmission-risk, (ii) predict the spatial pattern of vector abundance and the probability of introduction of specific arboviruses to particular areas, and (iii) quantify expected impact of control measures to better guide the development of end-user decision-making tools.

94. THE EFFECT OF PERMETHRIN RESISTANCE ON AE. AEGYPTI TRANSCRIPTOME FOLLOWING INGESTION OF ZIKA VIRUS INFECTED BLOOD

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Aedes aegypti is a vector of several arboviruses that affect human health and is of increasing concern because of the re-emergence of dengue, chikungunya, and Zika viruses, including local transmission of these viruses in Florida in recent years. In the continental U.S., a majority of the locally acquired Zika cases have occurred in Florida (280 cases from 2016-2017). Zika virus was first discovered in 1947. The epidemiology of Zika virus has changed in the last 10 years with major human outbreaks reported in the Pacific Islands and the Americas, predominantly attributable to transmission by the peridomestic mosquito Ae. aegypti. Insecticide resistance in Ae. aegypti has been demonstrated in Puerto Rico and several neighboring countries throughout the Caribbean. The resistance status of local populations of Ae. aegypti is of critical importance for effective management by control districts in Florida. According to USDA CMAVE laboratory study, the populations of Ae. aegypti collected from several locations in Florida showed up to 85-fold resistance to permethrin when compared to the susceptible Orlando-1952 strain of Ae. aegypti. We use molecular approaches (RNA-sequencing) to examine differential expression of genes between Key West permethrin resistant selected strain and Orlando-1952 strain of Ae. aegypti in response to Zika virus infection. Global transcriptome analysis showed that there were significant differences between the compared strains after 7-day infection with Zika virus. This study suggested that Key West permethrin resistant selected strain altered global gene expression compared with Orlando-1952 strain of Ae. aegypti in response to Zika virus infection.

95. THE ENVIRONMENTAL IMPACT OF HUMANS AND PRIMATES ON SCHISTOSOMA MANSONI TRANSMISSION USING THE INDIVIDUAL-BASED MODEL.

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Schistosoma mansoni is a water-borne parasite. In 2016, 206.5 million people have schistosomiasis and S. mansoni is major parasite. It is found in Africa, the Middle East, the Caribbean, Brazil, Venezuela and Suriname. S. mansoni is transmitted through water, where fresh water of the genus Biomphalaria act as intermediate hosts. There has a transition from S. mansoni within water environment to human. Then the infected human feces so that it could pollute the water environment. The primates have the same transmission circle. We use individual-based model to simulate the outcome and compare impact from humans and primates to water environment.

96. THE PRACTICAL USE OF MONOCLONAL ANTIBODIES AND PROTOTYPE ISOLATES FOR ZIKA VIRUS RESEARCH AND DIAGNOSTICS

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Background: In the face of a novel teratogenic pathogen, researchers have scrambled to identify reagents to diagnose, treat, or prevent infection. Zika virus (ZIKV) co-circulates with several closely related flaviviruses which exhibit a short viremia that is typically passed by the time a patient exhibits clinical manifestations thus; clinicians often rely on serological techniques for diagnosis. As ZIKV has emerged in new regions, significant genetic changes and new symptoms and pathologies have been described. Strain and serotype-specific neutralization and biology have been described for flaviviruses, and if true for ZIKV, could translate into the need to optimize assays and diagnostics according to which lineage/strain is being evaluated.

Objectives: To identify positive controls for diagnostic and viral characterization assays.

Study Design: Three strains of ZIKV and 4 serotypes of dengue were evaluated for neutralizing and enhancement properties against 3 mAbs, 4 patients with undetermined flavivirus exposure, and 2 patients with ZIKV-only exposure.

Results: ZIKV of African and Asian lineage display unique neutralization and enhancement properties. Moreover, sensitivity of molecular diagnostics varied according to ZIKV lineage.

Conclusions: Variable cross reactivity, neutralization, and enhancement profiles of ZIKV highlights the necessity of

characterizing viral isolates, mAbs, and reagents for diagnostic and in vitro platforms. The variable activity of mAbs in regards to neutralization and enhancement to divergent strains and closely related flaviruses raises serious concerns for use of mAbs as a therapeutic agent.

97. THE SYSTEMS BIOLOGY OF ARBOVIRUS TRANSMISSION

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An overview of the basic science research arm of the CDC Southeastern Center of Excellence in Vector Borne Diseases through the Vector Biology Arbovirus Unit of the Emerging Pathogens Institute.

98. TICK COMMUNITIES IN THREE LAND USE TYPES IN THE LOWVELD OF SWAZILAND

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Ticks are obligate, nonpermanent ectoparasites of terrestrial vertebrates that vector a greater number of pathogens than any other arthropod group. The conversion of natural lands to croplands, pastures, urban areas, and other anthropogenic landscapes results in a suite of environmental impacts. One of these impacts is postulated to be an increase in the number of ticks and therefore the incidence of tick-borne disease in humans, livestock, and wildlife in and nearby converted landscapes. We investigated the role of land cover on tick presence in three land cover types that dominate the Lowveld of Swaziland (savanna, croplands, and mixed croplands). Between June 14 and July 25, 2017, adult, nymph, and larval ticks were collected along six 200m flagging surveys at eight sites using standard dragging techniques for a total of 9,600m surveyed. All ticks observed on the 1m2 drag cloth were collected into ethanol vials and identified to genus or species using standard taxonomic keys. Each site consisted of a habitat interface between savanna and croplands or savanna and mixed croplands. Preliminary analyses show the majority of ticks collected were adults (268/309), and most specimens were collected from savanna land type (294/309). The adult species identified included Haemaphysalis leachi (169), Rhipicephalus simus (56), Rhipicephalus appendiculatus (36), and Rhipicephalus spp (7). The mean number of adult ticks collected in savanna adjacent to sugarcane was significantly greater than the mean number of adult ticks collected in savanna adjacent to communal farms ($p = 0.025$). Ongoing work includes occupancy modeling, spatial analyses, and pathogen screening. This work suggests landscape dependent patterns to tick community

assemblage and motivates further investigation into the mechanisms driving differential land use in ticks.

99. TO SCREEN OR NOT TO SCREEN: AN INTERACTIVE TOOL THAT INTEGRATES COSTS AND SPATIAL HETEROGENEITY TO DETERMINE WHEN MASS-SCREEN-AND-TREAT IS AN EFFECTIVE MALARIA CONTROL STRATEGY

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Resource management is critical to designing effective malaria interventions. The wide availability of rapid diagnostic testing has made pre-screening a viable method to reduce the waste and cost associated with mass drug administration. However, there has been mixed results regarding the effectiveness of mass-screen-and-treat (MSAT) interventions as a means of reducing morbidity and/or interrupting transmission. Two factors that influence intervention efficacy are (1) spatial heterogeneity of disease prevalence and diagnostic performance, and (2) cost of screenings and treatment. We propose a framework for interactively evaluating the cost effectiveness of presumptive treatment versus MSAT interventions. First, we modelled region-specific disease prevalence and diagnostic performances. With the model results, an interactive web application was created using R Shiny, which can incorporate relevant factors (e.g. urban/rural, age, fever history), allows the user to input cost of treatment, screening, and misdiagnoses, and compares the cost efficiency of these interventions in each region of a country. We present an example using DHS data from seven West African countries (Burkina Faso, Cote d'Ivoire, Ghana, Guinea, Mali, Nigeria, and Togo). We believe that this framework can be easily adapted for many contexts and is a useful tool for decision makers to design data-driven, cost-effective interventions.

100. TRANSMISSION POTENTIAL OF MAYARO VIRUS IN FLORIDA AEDES AEGYPTI AND AE. ALBOPICTUS

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Mayaro virus (MAYV) is an emerging mosquito-borne arbovirus present in Central and South America that causes arthralgia and febrile illness. Domestic mosquitoes Aedes aegypti and Ae. albopictus are potential vectors of MAYV that may allow for transmission involving humans in an urban setting. Here we evaluated susceptibility to infection, disseminated infection, and transmission potential of Florida Ae. aegypti and Ae. albopictus for MAYV. Oral infection was significantly higher in Ae. albopictus (85-100%) than Ae. aegypti (67-82%). However, rates of disseminated infection were higher for Ae. aegypti (45-85%) than Ae. albopictus (38-76%), especially later in the infection process. Both mosquito species exhibited substantial barriers to transmission as indicated by low rates of MAYV infection in saliva expectorates. Although Florida potential vectors show the capability to initiate an urban cycle after having fed on higher titers of MAYV infected blood, lower viremia in infected humans is likely to limit transmission potential.

101. UTILITY OF HEMATOLOGICAL ANALYTES AS PREDICTORS OF SURVIVORSHIP TO INFECTION WITH EPIZOOTIC HEMORRHAGIC DISEASE VIRUS IN WHITE-TAILED DEER WEANLINGS

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Epizootic hemorrhagic disease virus (EHDV) is the leading viral cause of mortality in wild and farmed white-tailed deer, and the leading cause of stock and economic loss in farmed deer between three and six months in age. Within Florida, there are three prevalent serotypes of the virus, EHDV-1, -2, and -6. These viruses are transmitted by Culicoides biting midges and active infections typically occur in the fall, corresponding with peak vector activity. This is also the time when fawns are weaned from their mothers, and this age group experiences ~19-40% mortality within 3 months of weaning. In this study, we looked at common hematological analytes of deer at weaning (3 mo. of age) to determine if any of these analytes are predictive of survivorship following EHDV infection. Over the course of 2016 and 2017, a total of 49 individuals were sampled via jugular venipuncture. Complete blood cell count (CBC) with manual differentials, serum biochemistry, and virus neutralization tests for EHDV-1, -2, and -6 were conducted on the samples. A Classification and Regression Tree (CART) analysis was run to determine the predictive ability of 49 analytes for survival of EHDV infection. The CART analysis demonstrated that no single analyte is predictive of survivorship or mortality caused by EHDV infection within 3 months of sampling. This could be due to insufficient sample sizes or an inability of our diagnostics to assess

health outcomes for white-tailed deer fawns. Understanding which animals are predisposed to survivorship or mortality allows farmers to effectively select their stock and mitigate losses with early intervention strategies. However, this preliminary analysis indicates that neither hematological analytes of 3 month old fawns nor maternal antibodies to the virus are useful predictors for EHDV survival during a critical period of susceptibility in young deer.

102. UTILIZATION OF DENGUE IGG FOR NON-TRAVEL RELATED ZIKA VIRUS CASE INVESTIGATIONS

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Background: In July 2016, Florida was the first state to report local cases of Zika virus (ZIKV) infection. While Florida's Bureau of Public Health Laboratories (BPHL) had some ZIKV testing capacity, plaque reduction neutralization testing (PRNT) was unavailable until mid-2017. PRNT testing is costly, takes at least a week to run, and requires specialized training and facilities. Because of limited ZIKV PRNT availability nationwide, testing turn-around times varied, taking several weeks during the time of active local transmission in Miami-Dade County. ZIKV antibodies can cross-react with related flavivirus antibodies such as dengue virus (DENV). To compensate for PRNT testing delays, BPHL performed a two-day DENV IgG antibody test as a proxy for ZIKV IgG. This analysis assesses how useful this proxy was.

Methods: Florida's reportable disease surveillance system was used to retrieve DENV IgG and DENV and ZIKV PRNT assay results for confirmed and probable cases of local ZIKV infection reported from July 1, 2016 to December 13, 2017. BPHL performed DENV IgG testing while PRNT testing was performed by either BPHL or the

Centers for Disease Control and Prevention. DENV IgG enzyme-linked immunosorbent assay results were evaluated for cases with positive ZIKV PRNT results.

Results: Of 33 cases with both positive PRNT and DENV IgG results, 15 (45%) had PRNT titers signifying ZIKV infection (positive ZIKV titer, negative DENV titer). Twelve were symptomatic with samples collected 1-66 days (median 17 days) after onset. Eighteen cases had PRNT titers indicating flavivirus infection (positive ZIKV and DENV titers), with 13 symptomatic and samples collected 0-51 days (median 8 days) after onset. Of 20 cases with no detectable DENV IgG antibodies, 19 (95%) had ZIKV-positive PRNT results and one indicated flavivirus infection. Seventeen cases were symptomatic and samples were collected 1-25 days (median 7 days) after onset. Five DENV IgG seroconversions were identified in individuals with both acute and convalescent samples. Convalescent samples were collected 17-43 days after symptom onset.

Conclusion: While antibodies against DENV IgG were detectable among cases with confirmed and probable ZIKV infection, past flavivirus infections can complicate laboratory result interpretations. For some patients with reduced risk of past flavivirus exposure, detection of DENV IgG antibodies or IgG seroconversion may serve as a proxy for PRNT testing in conjunction with appropriate ZIKV testing and epidemiological risk assessment. This may have particular value for samples collected after two weeks. Use of DENV IgG testing may lessen the burden of time-consuming and expensive laboratory testing.

103. VARIATION IN VECTOR COMPETENCE FOR ZIKA VIRUS AMONG SUBPOPULATIONS OF AEDES AEGYPTI FROM MIAMI-DADE COUNTY, FLORIDA

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Background: Since 1999 three non-endemic, mosquito-borne viruses have emerged in the United States with outcomes ranging from localized outbreaks with limited spread (chikungunya virus, Zika virus) to a nationwide epidemic and the establishment of transmission foci (West Nile virus). A key factor governing arboviral emergence is the efficiency of transmission by local mosquitoes, a population-level property strongly influenced by the average mosquito's vector competence (VC), i.e. its capability of transmitting virus to a new host. VC can be estimated empirically as the proportion of mosquitoes that produce virus in saliva (i.e., bite becomes infectious) following oral exposure and a length of time known as the extrinsic incubation period (EIP). During the EIP, arboviruses must negotiate physiological barriers that include the midgut and salivary-gland epithelia, although infection of other tissues after midgut escape (i.e., a disseminated infection) amplifies the viral load and increases the likelihood that a mosquito's bite will become infectious. We have begun to investigate VC for ZIKV in Ae. aegypti from Florida with the long-term goal of identifying genetic polymorphisms in both the mosquito and viral genomes that influence VC in the field.

Methods: Aedes aegypti eggs were collected from multiple locations in Miami-Dade County in 2016, including Miami Beach (Aeg-MB) and

Little River (Aeg-LR). Eggs were hatched in the laboratory and maintained as distinct subpopulations. Adults from the F2 generation were challenged with multiple strains of 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 and ZIKV from Puerto Rico (strain PRVABC59), plaque assays were used 14-16 days post-feeding to investigate rates of dissemination and transmission (i.e., ZIKV-positive saliva).

Results & Conclusions: Midgut infection rates were highly variable among ZIKV strains for each mosquito subpopulation and Aeg-Orlando. Comparisons among mosquitoes from Miami Beach, Little River, 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%. These data suggest that (i) a relatively high proportion of Ae. aegypti in Miami Beach may be non-competent for ZIKV strain PRVABC59 and (ii) that this non-competent phenotype is likely due to a physiological barrier to salivary-gland infection.

104. WILD PIGS AS SENTINELS FOR HARD TICKS: A CASE STUDY FROM SOUTH-CENTRAL FLORIDA

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As a result of shifts in the habitable range of ticks due to climate change and the ongoing threat of exotic tick species introductions, efficient surveillance tools for these pests and disease vectors are needed. Wild pigs are habitat generalists, distributed throughout most of the United States, and often hunted recreationally or removed as part of management programs, making them potentially useful sentinel hosts for ticks. We compared ticks collected from captured wild pigs and standard tick dragging methods on a south-central Florida cattle ranch from May 2015 – August 2017. Three hundred and sixteen wild pigs were surveyed, and 84 kilometers spanning three habitat types (seminative pasture, improved pasture, and hammock) were dragged. In total, 1,023 adults of four species (Amblyomma auricularium, A. maculatum, Dermacentor variabilis, and Ixodes scapularis) were collected from wild pigs, while 39 adults of three species (A. auricularium, A. maculatum, and I. scapularis) were collected from drags. Only one immature specimen, a nymph, was collected from a pig, while dragging collected 2,808 larvae and 150 nymphs. A. maculatum comprised 96% of adults collected from

pigs, while A. maculatum, I. scapularis, and A. auricularium comprised 38%, 33%, and 28% of adults collected from drags, respectively. Adults of all tick species found on drags were found on pigs, and wild pig surveillance detected adults of an additional species not found on drags. Dragging was far superior for collection of immatures but not for adults of most species found in this study. These findings suggest wild pigs could be used as a sentinel for the detection of tick species. When combined with ongoing wild pig research, hunting, or management, wild pig surveillance can provide an effective method to survey for adult tick presence of some species of interest and may assist range expansion studies.

105. ZIKA MODELS AND UNCERTAIN EPIDEMICS

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Increasingly, epidemiology has embraced the field of disease modeling as an opportunity to not only combat but to predict and prevent pandemics. This paper examines publications related to predictive modeling of the ongoing epidemic of Zika in Latin America in order to explore the social and political consequences of disease models, and to trace uncertainty inherent to this enterprise. First, this paper describes the ways that Zika models obfuscate significant uncertainty within their presentation of statistical representations of reality. It explores how this veneer of objectivity can conceal problems with imperfect scientific knowledge and interdisciplinary communication, as well as health ideologies. Finally, it explores assumptions about the nature of disease that are produced and replicated by disease modelers attempting to examine and predict the spread of Zika. It contends that in their representations of Zika through models, scientists shape ideas of what can “count,” a process that promotes some solutions while obstructing or deprioritizing others.

106. ZIKA VIRUS (ZIKV) REPLICATION IS SUBSTANTIALLY INHIBITED BY NOVEL FAVIPIRAVIR AND INTERFERON-ALPHA COMBINATION REGIMENS

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Zika virus (ZIKV) is a major public health concern due to its overwhelming spread into the Americas. Currently there are neither licensed vaccines nor antiviral therapies available for the treatment of ZIKV. We aimed to identify and rationally optimize effective therapeutic regimens for ZIKV by evaluating the antiviral potential of approved broad-spectrum antiviral agents favipiravir (FAV), interferon-alpha (IFN), and ribavirin (RBV) as single agent and combinations. For these studies, Vero cells were infected with ZIKV in the presence of increasing concentrations of FAV, IFN, or/and RBV for four days. Supernatants were harvested daily and viral burden was quantified by plaque assay on Vero cells. The time-course of viral burden during treatment in vitro was characterized by a novel translational, mechanism-based model which was subsequently used to rationally optimize combination dosage regimens. The combination regimen of FAV plus IFN provided the greatest extent of viral inhibition without cytotoxicity, reducing viral burden by 4.4-log10 plaque forming units/ml at concentrations of 250 µM FAV with 100 IU/ml IFN. Importantly, these concentrations are achievable in man. The translational, mechanism-based model yielded unbiased and reasonably precise curve fits. Simulations with the model predicted that clinically relevant regimens of FAV plus IFN would markedly reduce viral burden in man, resulting in at least a 10,000-

fold reduction in virus during the first four days of treatment. These findings highlight the substantial promise of rationally optimized FAV plus IFN combination dosage regimens which should be further investigated to combat ZIKV.

107. A NEW MEMBER OF THE XOPJ EFFECTOR FAMILY, XOPJ6, PROVIDE INSIGHTS INTO THE VIRULENCE OF XANTHOMONAS PERFORANS IN TOMATO

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Bacterial leaf spot of tomato and pepper is a disease caused by four Xanthomonas species. X. perforans (Xp) is a specific pathogen of tomato, and since early 1990's is the major bacterial spot of tomato pathogen in Florida. Host resistance has been a major goal for breeding programs, however, the durability of plant resistance depends on the ability of pathogens to evolve over time. For Xanthomonas spp., the acquisition or mutation of various effectors have mediated the evolution of its host range and virulence. Previous studies have shown major shifts in Xp populations in Florida, each having different effector content and interaction with host plants. The general objectives of this work were i) to characterize and compare the effector profiles of Xp across different lineages and geographical regions, and ii) to find and characterize specific genes that may change interaction with its host. For this, we analyzed 137 Xp genome sequences from strains collected in Florida, Thailand, China and Nigeria. A tBLASTn analysis revealed the presence of a new member of the XopJ effector family, XopJ6, in 13 out of the 137 strains across the 4 different locations. An homology analysis performed with HMMER showed that this gene belongs to the serine/threonine acetyltransferase XopJ effector family, with a

71.5 % sequence identity, and contains its signature catalytic core (His, Glu and Cys), required to induce resistance mediated host defense responses in resistant plants. The type III effectors AvrBsT (XopJ2) and AvrXv4 (XopJ4), which belong to the XopJ effector family, have been associated with virulence and host range restriction in pepper and tomato. We show, by transferring the gene to a pepper-pathogenic strain, that it induces an AvrBsT-like hypersensitive response in pepper. Our results expand the XopJ effector family and provide new evidence of the role of this family in virulence and as host range restriction, and could have relevant implications for disease management.

108. ANTIBIOTIC RESISTANCE IN BURKHOLDERIA PSEUDOMALLEI

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A challenge for clinicians faced with treatment of Burkholderia pseudomallei (Bp) infections (melioidosis) is the paucity of antibiotics available for therapy due to the bacterium's intrinsic drug resistance. This challenge is augmented by the dearth of information available about the nature of the mechanisms that govern Bp's antibiotic resistance. Understanding resistance mechanisms not only informs optimal treatment in public health settings in melioidosis endemic regions of the world, but also addresses the significant issues of biodefense forensics and warfighter safety posed by a potential bioterrorism agent. Improved molecular techniques such as next generation sequencing accelerated identification of potential

resistance mechanisms that then can be experimentally verified. Here we present a summary of progress made by us on identification and characterization of the molecular mechanisms that govern resistance to clinically significant antibiotics in Bp and how such information benefits informed therapeutic strategies, detection of resistance determinants, and development of new anti-Bp drugs. Efflux is the main multidrug resistance mechanism in Bp. The intrinsic aminoglycoside and macrolide resistance of these bacteria is due to constitutive expression of the AmrAB-OprA efflux pump. Acquired resistance to chloramphenicol, fluoroquinolones, trimethoprim and co-trimoxazole is caused by constitutive expression of the BpeEF-OprC efflux pump due to selection of regulatory mutants. Co-trimoxazole resistance compromises melioidosis eradication phase therapy and Bp post-exposure prophylaxis. β-lactam antibiotics play crucial roles in the treatment of acute Bp infections. Ceftazidime is the most widely used β-lactam antibiotic, but carbapenems are also clinically employed. Four different mechanisms of acquired resistance to ceftazidime have been documented in clinical isolates: deletion of a penicillin binding protein 3 target; increased expression of PenA β-lactamase due to a conserved promoter-up mutation; PenA amino acid sequence changes extending the enzyme's substrate spectrum; and increasing penA copy number due to gene amplification. In some instances, resistance determinant mutations may also compromise the application of selective growth media as diagnostic methods. For instance, increasing numbers of clinical and environmental Bp isolates are being identified that either do not express the AmrAB-OprA efflux pump or express a mutated, inactive pump. Such isolates will be missed when employing the widely used Ashdown's diagnostic medium because it contains gentamicin as selective agent. The main lesson learned is that unlike other Gram-negative bacteria acquired resistance is not due to externally acquired but rather chromosomal resistance determinants.

109. BRUCELLOSIS TRANSMISSION BETWEEN HUMANS AND DOMESTICATED LIVESTOCK IN SOUTHERN KAZAKHSTAN: INFERENCES THROUGH MLVA TYPING

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Brucellosis is world's most prevalent zoonotic disease. Worldwide burden of human cases is represented with an incidence rate of almost 500,000 new cases a year. Kazakhstan represents a hyperendemic hotspot, posing a public health and veterinary threat for itself and surrounding countries, with human incidence rates of 8.49 cases per 100,000 in 2013, representing some of the highest rates in the world. Kazakh Brucellosis control and surveillance strategies are vastly different compared to Western countries, primarily due Kazakhstan's developing nature. For an area like Kazakhstan with multiple co-circulating strains, it is essential to correctly determine the overall spatial structure, for outbreak tracing and determining the estimated species of origin. We estimated the directionality of transmission and genetic relationship with discrete geography, by visualizing and comparing genetic dissimilarity and relationships from a total of 517 veterinary and human Brucella

isolates collected by the Kazak Republican Sanitary-Epidemiological Station (RSES) and the Kazak National Reference Veterinary Center (NRVC) a part of a four year passive surveillance effort. We utilized Non-Metric Multidimensional Scaling (NMDS) plots to visualize and compare genetic dissimilarity, tested the statistical significance of genetic dissimilarity using PERMANOVA tests and built a Minimum Spanning Tree (MST) network to observe phylogenetic relationships using the software R 3.3.3, the R package vegan and PhyloViz V2.0 ,stratified by collection phase, isolate group and Kazakhstan Oblast. Results demonstrated marked separation between animal and human isolates demonstrating a significant difference in genetic diversity and dissimilarity in human and animals visualized by the NMDS plots and MST network and significant PERMANOVA results (R-squared = 0.0059, p<<0.001). Additionally, we encountered significant dissimilarity between the regions of Almaty and Zhambyl oblast (R-squared = 0.043, p<<0.001) indicating an association between the genetic diversity and spatial stratification. The results also postulate the existence of animal to human spillover and a differential spillover effect based on discrete geography. The underlying spatial structure associated with the genetic dissimilarity of Brucella spp. fills in a crucial gap in estimating the transmission directionality of Brucella infections.

110. CHLAMYDIAL MREB HAS DUAL ROLE IN CELL GROWTH AND DIVISION

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Chlamydia trachomatis encodes a putative MreB (CTL0078), but lacks cell division protein FtsZ. MreB is an actin homologue that directs sidewall peptidoglycan (PG) synthesis and is essential in rod shape bacteria. To understand how Chlamydial MreB can support both cell growth and division, we characterized the role of Chlamydial MreB in Escherichia coli for cell division, growth and morphology. Overproduction of Chlamydial MreB in E. coli inhibited growth and altered cell morphology. Bacteria were oval or curved and distended at mid cell similar to overproduction of E. coli MreB. Chlamydial MreB complemented an mreB deletion mutant of E. coli for survival and growth. However, E. coli cells lost their rod shape morphology and grew as rounded cells. Time-lapse monitoring indicated that these spherical cells were not strictly regulated for symmetric cell division and produced daughter cells of unequal size. The cell division initiated constriction appeared on one side of the cell producing a bean shaped intermediate. PG labeling indicated the presence of continuous PG around the spherical cell with a notch or groove on one side of the mid cell. To understand how Chlamydial MreB contributes to PG synthesis in E coli, we utilized specific inhibitors of penicillin-binding protein 2 (PBP2)-dependent sidewall PG synthesis and PBP3-dependent septal PG synthesis. Chlamydial MreB mediated resistance to mecillinam (PBP2 inhibitor) with MIC ≥ 64 µg/ml, but sensitivity toward aztreonam (PBP3 inhibitor) with MIC ≥ 0.06 µg/ml, indicating that Chlamydial MreB supports E. coli growth by directing PBP3-dependent PG synthesis. To understand how Chlamydial MreB directs cell division in E. coli, we overexpressed sulA to block Z ring formation by depolymerizing FtsZ. Surprisingly Chlamydial MreB supported the growth of E. coli even

when division Z ring formation was blocked by SulA. These observations indicate that Chlamydial MreB has a unique dual property to support cell growth and division which otherwise require MreB and FtsZ in E. coli. We are investigating the molecular mechanism of cell division mediated by Chlamydial MreB by protein-protein interactions and by generating point mutations in Chlamydial MreB.

111. CHROMOSOMAL ASYMMETRY, GROWTH KINETICS AND VIRULENCE OF BURKHOLDERIA MALLEI

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Burkholderia mallei is a Gram-negative bacterium that causes glanders, a zoonotic disease, especially in equine populations (e.g. horses, donkeys, mules). B. mallei is considered to be closely related or known as a clone of B. pseudomallei, the cause of melioidosis, on the basis of DNA sequence. B. mallei is a host-adapted pathogen that has undergone genome reduction and mutations. It has two chromosomes (Chr 1 & Chr 2) that contain numerous insertion sequence elements known to be associated with the extensive deletions and rearrangements. GC skew is often used to predict both origin and terminus regions of bacterial replication, showing the two strands (leading and lagging strands) in DNA replication. Although most bacterial species including B. pseudomallei possess compositional symmetries between leading and lagging strands, B mallei shows its asymmetries between the two strands. The aim of this study was to reveal if these asymmetries had any impacts on growth kinetics and/or pathogenicity of B. mallei. We studied GC

skew of 27 B. mallei genomes from NCBI database. GC skew of each genome was obtained by applying the genome sequences to two web-based software tools: CGView Server and GenSkew. We also identified positions of dnaA gene and dif site from each of the sequences. The dnaA gene and dif site have been reportedly considered to be around origin and terminus region of replication in bacterial genome, respectively. Then, we calculated the lengths of both leading and lagging strands in each genome on the basis of two methods: GC skew analysis and positions of both dnaA gene and dif site. The results showed that GC skew analysis did not reveal the origin or terminus of replication of the chromosomes in many strains including a type strain ATCC 23344 as previously reported. In addition, most of B. mallei strains possessed quite different lengths between the leading and lagging strands. For example, strain BMQ had approximately 3.0 Mbp of leading strand and 0.5 Mbp of lagging strand in Chr 1. These different lengths could be seen more often in Chr 1 that contains essential genes for metabolism and cell growth. These chromosomal asymmetries could lead to defects in growth kinetics and virulence in some of B. mallei strains tested in this study. Additional studies such as transcriptomics and proteomics are needed to clarify the relationship between the chromosomal asymmetry, growth, and pathogenicity.

112. COMPLETION OF KOCH'S POSTULATES FOR PANTOEA LEAF BLIGHT OF PONYTAIL PALM

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A plug tray of blighted ponytail palm (Beaucarnea recurvata) seedlings was submitted to the UF Plant Diagnostic Center in September of 2017. Leaf blades displayed translucent water-soaked lesions. Bacterial streaming from the affected tissue was detected under the microscope. Review of disease reports from related species indicate the possibility of Pantoea sp. being the pathogen causing leaf blight. The present study aims to complete Koch's postulates as well as characterize the causal agent of leaf blight on ponytail palm at a biochemical and molecular level. Symptomatic leaf tissue from two independent seedlings was homogenized. The cell suspension was streaked out on nutrient agar to obtain single bacterial colonies. The isolates were gram positive, oxidase negative, urease negative, non-fluorescent, had no pectolytic ability, facultative anaerobes and grew yellow mucoid on yeast dextrose media. The isolates also induced a hypersensitive response on tomato, and tobacco at 108 CFU/ml at 24h and 48h following leaf infiltration. DNA was extracted from both isolates and used as a template for targeted amplification of the following housekeeping genes, fusA, leuS, gyrB, pyrG, rplB, rpoB, and the 16S rDNA region. Amplicons were separated on 1% agarose gel electrophoresis, purified, and submitted for Sanger sequencing. Raw 16S sequences were edited and aligned using MEGA 7.0 software, then compared against the available sequences in the NCBI database using BLAST algorithm. A preliminary pathogenicity test was carried out by cutting off leaf blade tips from healthy ponytail palm seedlings and

dipping them in a bacterial suspension (108 CFU/ml) for 10 seconds. Seedlings were bagged, incubated at room temperature, and monitored for symptom development. Symptomatic tissue was used for re-isolation of bacteria, thus completion of Koch's postulate. MLSA will be performed with the remaining gene sequences to determine species of the pathogen.

113. DETECTION OF MICROORGANISMS RESPONSIBLE FOR POST-RADIATION SINUSITIS

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Background: Sinusitis is a significant cause of morbidity in patients undergoing external beam radiotherapy for sinonasal, nasopharyngeal, or skull base malignancy. A shift in the microorganisms isolated by sinonasal culture has been suspected, and there is a paucity of literature describing the use of gene sequencing techniques to characterize the bacteriology of post-radiation sinusitis.

Objective: To describe and compare the microbial flora involved in acute sinusitis after radiotherapy using both culture and molecular techniques for microbial DNA detection.

Methods: The medical records of patients treated with external beam radiation for sinonasal, nasopharyngeal, or skull base malignancy were reviewed at a tertiary care facility. Patients' sinonasal cavities were swabbed for routine culture or brushed for molecular gene sequencing. Swab specimens were processed for standard microbial culture and brush specimens were sent for gene sequencing and DNA identification at Pathogenius Laboratory (Lubbock, TX).

Results: 22 patients were diagnosed with acute sinusitis during or after radiotherapy. The average time to infection after undergoing radiation therapy was 81.2 weeks. Staphylococcus aureus was the most common organism identified by culture as well as gene

sequencing, followed by Pseudomonas aeruginosa. Several additional organisms were detected by gene sequencing that were not isolated by routine culture techniques. In 63.6% of the cases we examined, the primary organism was different when using gene sequencing as compared to standard culturing techniques.

Conclusion: The bacteriology of post-radiation sinusitis appears to resemble the microorganisms responsible for chronic sinusitis in healthy adults. Next generation gene sequencing techniques may reveal additional organisms responsible for sinusitis and provide complementary results that may impact the medical treatment of post-radiation sinusitis.

114. EFFECTS OF BRUCELLOSIS SEROLOGICAL STATUS ON PHYSIOLOGICAL CONDITIONS AND BEHAVIORAL MECHANISMS OF SOUTHWESTERN MONTANA ELK

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Brucellosis, caused by bacteria in the genus Brucella, is an infectious zoonosis affecting animals and humans worldwide. Free-ranging elk (Cervus canadensis) and bison (Bison bison) in the Greater Yellowstone Ecosystem are the self-sustaining reservoirs of bovine brucellosis (Brucella abortus) in the United States and elk are considered the primary source of livestock infections. It has been hypothesized that Brucella-exposed elk might have different physiological status (i.e. pregnancy rates and body condition) and migration behaviors than healthy elk. Here we tested the effects of brucellosis serological status on pregnancy rates and winter ingesta free body fat (IFBF) of 100 female elk in southwestern Montana. We

also evaluated the effects of serological status on two characteristics of spring migration behavior, migration types (i.e. migrant, mixed-migrant, resident, disperser, nomad, and undetermined type) and timing (i.e. start and end date and duration). The migration behaviors were quantified using a model-driven approach based on the relative net squared displacement (rNSD). We detected a significant difference in pregnancy rates between seropositive and seronegative individuals, with ~30% drop in seropositive elk. However, we did not detect differences in body fat between seropositive and seronegative elk, or differences in either migration type or timing of spring migration. These results confirm that the major pathology of brucellosis in free-ranging elk is associated with reproduction. Given that most elk were identified as migratory animals, significant elk movement in southwestern Montana is expected to happen as early as the beginning of February until the end of July although most elk start migration in early April and end in late May. Knowledge about movement patterns may inform livestock spring grazing practices to reduce the chance of commingling decreasing the risk of elk-livestock Brucella transmission events.

115. FACTORS INVOLVED IN REGULATION OF PENA B-LACTAMASE IN BURKHOLDERIA PSEUDOMALLEI EXPRESSION AND CELLULAR LOCALIZATION

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Therapy of Burkholderia pseudomallei acute infections is largely limited to a few β-lactam antibiotics such as ceftazidime or meropenem. Although relatively rare, resistance emergence during therapy leads to treatment failures with high mortality rates. In the absence of acquired external resistance determinants in B. pseudomallei emergence of β-lactam resistance is invariably caused by mutational modification of genomically-encoded factors. These include the deletion of the ceftazidime target penicillin-binding protein 3 (PBP3) or amino acid changes in Class A PenA β-lactamase that expand its substrate spectrum, as well as penA gene duplication and amplification or its overexpression via transcriptional up-regulation. We previously showed that penA is co-transcribed with the upstream nlpD1 gene, that the transcriptional terminator for nlpD1 serves as a penA attenuator and that 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. Evidence shows binding of an ~22,000 molecular weight protein to the nlpD1-penA intergenic region, which may be indicative of its involvement in regulation of penA transcription. The functional significance, if any, of nlpD1-penA co-transcription remains unknown. Localization experiments with tagged proteins expressed

in E. coli showed that like NDM-1 carbapenemase, PenA and NlpD1 are membrane-bound lipoprotein and evidence points to a localization of both proteins to the outer membrane where NDM-1 is found. Our findings show the complexity of factors involved in regulation of expression of B. pseudomallei PenA β-lactamase and its unique cellular localization.

116. GENOME SEQUENCES REVEAL POLYMORPHISMS IN BACTERIOCIN GENES OF XANTHOMONAS PERFORANS IN FLORIDA

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Bacterial spot is an economically important disease of tomato. Worldwide, this disease is caused by four species of Xanthomonas: X. euvesicatoria, X. gardneri, X. perforans, and X. vesicatoria. In Florida, X. euvesicatoria (Xe) was the sole causal agent of bacterial spot on tomato until 1991 when X. perforans (Xp) was detected. Since it was first detected, Xp quickly replaced populations of Xe in Florida. Previous studies have shown that Xp outcompetes Xe via the production of three bacteriocins (BcnA, BcnB, BcnC). Bacteriocins are proteinaceous toxins produced by bacteria to kill other closely related bacteria. We compared genome sequences of Xp strains collected in Florida from 1991 to 2016 and observed polymorphisms in essential open reading frames of BcnA and BcnB, but observed no variation in BcnC. We investigated the impact of these polymorphisms using inhibition assays on Petri plates and confirmed

a reduction in the ability of Xp strains to inhibit Xe. Xp strains that do not produce BcnA or BcnB remain insensitive to these bacteriocins produced by other Xp strains. However, loss of bacteriocin production could result in Xe becoming re-established on tomato in Florida.

117. MODIFICATION OF BURKHOLDERIA PSEUDOMALLEI LIPID A BY LPXO AND PAGL IS STRAIN SPECIFIC AND TEMPERATURE DEPENDENT

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Burkholderia pseudomallei (Bp) causes melioidosis, a severe tropical disease. High mortality is associated with septicemia triggered by the host response to lipopolysaccharide (LPS). Bp LPS is thought to be a relatively weak inducer of the host immune system compared to well known agonists, thus allowing Bp to become a successful intracellular pathogen. Previous works have shown diversity in the O-antigen of the molecule and the endotoxic lipid A portion, the latter affecting innate immune signaling downstream from the LPS recognition molecule TLR4. The host cell can also recognize intracellular bacteria. While in the cytosol, LPS recognition causes inflammasome activation and cell death. To determine the effect of lipid A modifications on cytotoxicity, LPS was transfected into the cytosol of primed macrophages. Surface plasmon resonance showed that lipid A modifications affect binding kinetics to human LPS binding protein. To investigate whether Bp modulates lipid A composition in response to environmental stimuli like other intracellular bacteria, masses of lipid A isolated from bacteria grown at different temperatures were determined by MALDI-TOF. Acylation and hydroxylation levels of lipid A were modified in type A strain

1026b but unchanged in type B strain, 576a, at the two temperatures. Expression of genes thought to cause deacylation (pagL) and hydroxylation (lpxO) of lipid A were differentially regulated in 1026b at 37˚C but not in the 576a strain, indicating variable regulation of Bp lipid A modification genes within the species in response to temperature. Lipid A profiles from Bp 1026b lpxO and pagL mutants were measured by MALDI-TOF and their immunogenicity in cell culture models was investigated. Taken together, Bp can modify lipid A structures and indicates a patho-adaptive capacity inessential for pathogenesis yet influencing virulence within the species. These data have implications on pathogenesis and vaccine efficacy and design.

118. ONE HEALTH APPROACH AND MOLECULAR EPIDEMIOLOGY OF MELIOIDOSIS IN SOUTHERN THAILAND

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It is well-recognized that melioidosis is endemic in most parts of Thailand. However, the prevalence of melioidosis in humans and animals, and the occurrence of its pathogen, Burkholderia pseudomallei, in natural environment of southern Thailand has not been updated for long time. We used “One Health” approach and multidisciplinary research to investigate epidemiology of melioidosis in southern Thailand. We have been collecting B. pseudomallei isolates from human and animal cases, and soils in Songkhla and

nearby southern provinces since January 2014. All B. pseudomallei isolates identified from patients admitted to the tertiary care hospitals in the region were sent to a collaborative laboratory at Prince of Songkla University for species confirmation. DNA based diagnostics including real-time PCR assays of TTS-1, BTFC&YLF, and LPS genes were used to identify B. pseudomallei. In addition, we have investigated the presence of B. pseudomallei in soils especially in goat farms and a local zoo where animal cases have been reported. We used standard soil culturing techniques with selective media, Ashdown's agar and TBSS-50 broth, for B. pseudomallei isolation. Suspected bacterial colonies grown on Ashdown's agar were subjected to further identification by latex agglutination, lateral flow immunoassay (LFI) and real-time PCR. We have confirmed at least 209 melioidosis cases from humans, as well as, the presence of B. pseudomallei in soils in Songkhla and nearby provinces. The infections were most likely seasonal and associated with rainfall. Genetic analysis using multi-locus sequencing typing (MLST) has indicated that most of these recent isolates had the same STs with those from the Finkelstein's historic collection from southern Thailand a half century ago. Specifically, strains with STs 288 and 84 were frequently found in Songkhla. Interestingly, at least 6 patients were confirmed to be infected by more than one sequence types. This may suggest a high genetic diversity of B. pseudomallei in natural source of the infections. Strains with ST3 were found in human and animal cases, as well as in the environment. Collectively, we believe that implementing the "One Health" approach would provide a current situation of B. pseudomallei infections in humans and animals, as well as its occurrence in the environment in Songkhla and nearby southern provinces that forms an integral part of regional threat assessment of Thailand and Southeast Asia.

119. OPEN PANGENOMES AND RECOMBINATION-GENERATED DIVERSITY IN XANTHOMONAS EUVESICATORIA AND X PERFORANS

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Xanthomonas euvesicatoria (Xe) and X perforans (Xp) are very closely related species- that cause bacterial spot disease on tomato and pepper. Multilocus, core genome, and effector sequence analyses have shown clear divisions between Xe and Xp. However, new genomes also suggest the possibility for gene exchange between these taxa. In this study, we utilized 30 Xe and 35 Xp sequenced genomes, two of which are newly sequenced genomes of Xp and Xe strains from Nigeria, to delineate the pan and core genome evolution of Xe and Xp and estimate the contribution of recombination between strains on evolution. Our results show considerable heterogeneity within taxa, as indicated by bimodal distributions in sequential resampling of strains to delineate core genomes. Pangenome analysis also showed gradual increase in number of genes with each additional genome. For example, the Nigerian Xp strain increased the pangenome size of previously sequenced Florida Xp strains by more than 35 genes. Our analysis also showed that recombination is important in the evolution of these species, but more so in Xp than Xe. We conclude that both Xe and Xp have open pangenomes, with sequencing of new strains expected to increase the gene profiles of new strains that may be important in pathogenicity and fitness.

120. PERFORMANCE OF A CHOLERA RAPID TEST IN THE SETTING OF HIGH LYTIC PHAGE AND ANTIBIOTIC BURDEN: A PROSPECTIVE DIAGNOSTIC STUDY

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Patients present on a daily basis to remote hospitals with primary infections that are impacted by complex secondary host, microbial, and environmental factors. Approaches to reproduce these factors in the laboratory, or effectively study them in the field, are lacking. These problems are critical barriers to make evidence-based clinical decisions and to conduct high-impact translational research, especially during outbreak response. We chose large-scale cholera outbreaks in Bangladesh as a model system to specifically improve data collection, sample collection, and high-throughput sample analysis. We developed (i) a data collection platform (Outbreak Responder) for use at hospital triage that addresses current connectivity limitations, (ii) methods to improve a point-of-care (POC) test for Vibrio cholerae as well as stabilize samples for subsequent analysis, and (iii) scalable assays for the selective pressures of antibiotics (mass spectrometry) and lytic phage (conventional and robotic-assisted qPCR). To validate these methods we conducted a census-based study at a rural and urban hospital during a cholera outbreak in Northern Bangladesh. 961 patients were enrolled and 100,000 data points were successfully collected and visualized geo-spatially in real-time with Outbreak Responder. Samples were collected from 865 patients and immediately tested with the POC test for V. cholerae (2.9% positive) and later by qPCR (6.1% positive) with high-quality DNA extracted from samples stored in RNA-later for up to 6 months at 4 degrees C. The POC sensitivity was low at 40% and specificity was high at 99%. Although 25% of samples from cholera patients harbored the lytic phage ICP1 (12/48), the low POC sensitivity did not correlate with ICP1. The low

sensitivity could not be attributed to antibiotics because all samples tested harbored antibiotics (39/39) despite self-reports of no antibiotic use (30/39). The tools developed in this study demonstrated durability and utility to improve data-collection, field and laboratory diagnostics, and methods to identify key selective pressures. Our goal is to deploy these tools in frontline clinical practice. Scientifically, the next step is to map primary pathogens and associated microbiota as a function of key selective pressures across space and time to better understand transmission dynamics and prioritize interventions.

121. RECOMBINANT ATTENUATED EDWARDSIELLA PISCICIDA VACCINE VECTOR STRAINS AGAINST FISH PATHOGENS

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Aquaculture is the fastest growing food-producing industry in the world than all other food animal producing sectors. However, infectious diseases of bacterial, viral and parasitic have caused most significant destructive impact to aquaculture production and result economic losses in the global aquaculture industry. Edwardsiella piscicida causes edwardsiellosis; is more common fish disease outbreaks in a variety of freshwater and marine fish species. Ichthyophthirius multifiliis (Ich), the causative agent of white spot disease, is a protozoan parasite that causes significant problems to the U.S. catfish industry. Vaccination would be the most effective method to prevent infectious diseases and their associated economic losses. In this study we have successfully designed and constructed a recombinant attenuated Edwardsiella vaccine (RAEV) vector system with regulated delayed attenuation in vivo attributes that synthesizes Ichthyophthirius multifiliis (Ich) protective antigens to enable vaccination of fish susceptible to white spot disease and edwardsiellosis. We designed strains that display features of wild-type virulent strains of Edwardsiella piscicida at the time of immunization to enable strains to effectively colonize lymphoid

tissues and then to exhibit a regulated delayed attenuation in vivo to preclude inducing disease symptoms. To achieve regulated delayed attenuation in vivo that based on the substitution of a tightly regulated araC PBAD cassette for the promoters of the fur and crp genes such that expression of these genes is dependent on arabinose provided during growth. Thus, following colonization of lymphoid tissues, the Fur and Crp proteins cease to be synthesized due to the absence of arabinose such that attenuation is gradually manifested in vivo to preclude induction of disease symptoms. These strains will exhibit a desirable balance between safety and immunogenicity. Our RAEV vaccine will protect teleost fish against multiple bacterial and parasitic infectious diseases.

122. REGULATION OF NOS AND ITS INTERPLAY WITH S. AUREUS METABOLISM

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Staphylococcus aureus is on the forefront of antibiotic resistant bacteria associated in both hospital and community associated infections. The versatility in the metabolism of S. aureus provides many avenues for survival in the host. Previous studies reveal the importance of nitric oxide synthase (nos) in relation to virulence and exogenous oxidative stress. The end product of the NOS reaction, nitric oxide has been shown to react with many cellular components such as lipids, heme cofactors, and iron-sulfur clusters. Using molecular and physiological approaches as well as growth kinetic experiments we characterized the regulation and the importance of nos in antibiotic tolerance, intracellular survival and altered metabolism. The acquisition of data from this study is essential in understanding the interplay between nos regulation and more importantly potential gene targets for new antibiotic therapies.

123. REMOVAL OF MUTANS STREPTOCOCCI FROM SALIVA BY FLOW CYTOMETRY AND CELL SORTING -A FIRST STEP ON EXPLORING A NEW METHOD TO ESTABLISH NON-CARIOGENIC ORAL FLORA-

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As the amount of sugar consumed in our food increases, human beings are faced with painful dental caries, especially in developing countries. The Global Burden of Disease 2010 study estimated that 2.4 billion people were suffering from untreated caries in the world. Thus, dental caries is a major public health problem. Dental caries is a complex bacterial infection including “Keyes triad”: dietary sugar (diet), cariogenic bacteria (dental plaque), and susceptible teeth (host). The main causative bacteria are mutans streptococci, which include Streptococcus mutans and Strep. sobrinus. They are commensal microorganisms in oral flora, and are likely transferred from caregiver’s (mainly mother’s) saliva to children’s mouth at the period of “window of infectivity (19-33 month-old)” when mutans streptococci colonize the mouth. Among many preventive strategies, water fluoridation is recognized as a great prophylaxis of dental caries. While it is very effective, this method is being reconsidered due to the adverse effect. Here we propose a new procedure to prevent dental caries in children using flow cytometry (FCM) and a cell sorting system. This procedure has two steps. At first, mutans streptococci is removed from the caregiver’s (mainly mother’s) oral flora. Second, oral flora without cariogenic bacteria is transplanted into the child’s mouth at the period of “window of infectivity”. Here we studied the initial step on this blueprint. FCM analysis was used to detect Strept. mutans using rabbit anti-Strep. mutans IgG (Ab). It clearly recognized laboratory strains of Strep. mutans and Strep.

sobrinus which were used as a positive control. Oral flora from a volunteer was then analyzed by FCM. This system was able to recognize mutans streptococci living in the oral flora. Similar results were obtained from the oral flora of four additional volunteers. Next, the FCM cell sorting system was used to separate Ab-positive bacteria and -negative ones, After separation of the two fractions, culture, quantitative PCR, and morphological observations were carried out. The FCM cell sorting system provided relatively good separation of Strep. mutans Ab-positive F1 and -negative F2 fractions. Despite some problems, such as cross-reactivity of Ab, FCM cell sorting system will be a good tool to separate mutans streptococci from oral flora. The next direction of this blueprint will involve transplantation of oral flora depleted of mutans streptococci using an animal model.

124. RISK FACTORS FOR STAPHYLOCOCCUS AUREUS INFECTIONS AMONGST COLONIZED INFANTS IN THE NEONATAL INTENSIVE CARE UNIT

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Background: Infants within the neonatal intensive care unit (NICU) represent a high risk population for developing healthcare-acquired infections due to Staphylococcus aureus. Colonization with S. aureus is known to increase an infant’s risk for developing a subsequent

infection. However, there are limited data regarding which colonized infants are at the greatest risk of infection.

Methods: A retrospective chart review was performed on infants admitted to a level IV NICU in Cincinnati, Ohio between April 2015 and March 2016 who had a positive surveillance or clinical culture with S. aureus. Risk factors for developing an infection were identified using multivariate logistic regression. Whole genome sequencing was performed on isolates from infected infants with previous colonization using the Illumina NextSeq 500 platform (San Diego, CA). Analyses were performed using STATA v14.1 (College Station, TX).

Results: 692 at-risk infants were admitted during the study period with 121 (17%) acquiring S. aureus colonization. 20 (3%) infants developed S. aureus infections, 17 of which had previously identified colonization. Infants acquiring S. aureus colonization at an older postnatal age were at increased risk of developing a subsequent infection (odds ratio 1.15, confidence interval 1.06-1.25 per additional week). No other significant differences were noted in demographics or exposure variables, including antibiotic utilization, days intubated, central venous catheterization, or operations performed. Risk of infection did not differ amongst infants colonized with methicillin resistant compared with methicillin susceptible S. aureus. Whole genome sequencing was performed on the paired isolates from 14 of the 17 infants who were colonized and became infected (3 isolates were not available for sequencing). 13 of the 14 paired samples differed by < 40 single nucleotide polymorphisms, suggesting a high degree of genetic relatedness of the infecting isolate to the colonizing isolate.

Conclusions: Older postnatal age at time of colonization was associated with an increased odds of developing a subsequent infection due to S. aureus in our cohort. Older infants have differing care needs and environmental exposures compared with younger infants which may facilitate an increased burden of colonizing bacteria and increased infection risks. Most infants who acquired infections became infected with their colonizing strain of S. aureus

suggests that decreasing colonization rates will decrease overall infection rates. Targeted decolonization of older infants may be an efficient method to decrease infection rates and minimize the emergence of mupirocin resistant S. aureus strains.

125. ROOT-ASSOCIATED "UNCULTURABLE" BACTERIA RECOVERED FROM CITRUS SAMPLES ON LOW CARBON AGAR PLATES

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The rhizosphere is the interface between the root system of a plant and its surrounding soil. It represents one of the most complex terrestrial microbial habitats on Earth with about 10-9 microbial cells per gram roots. Because its key role in plant growth, crop production, and ecosystem health, there is an increased interested in understanding rhizosphere diversity. Culture-independent high-throughput sequencing, mostly of the 16S ribosomal RNA (rRNA) gene, has been use extensively to examine plant-associated and soil bacterial diversity. 16S amplicon sequencing has been optimized for Operational Taxonomic Units (OTUs) identification and diversity measurements, but it has limited sensitivity, as low abundance species can be difficult to detect (depth bias). However, in root-associated microbial communities, species in low relative abundance may play critical functions in ecological interactions, e.g., production of antifungal compounds that protect plants from pathogens. To better capture low abundance taxa in microbiomes, culturing strategies combined with high-throughput sequencing are being developed. In this study, we examined bacterial diversity associated with citrus roots by combining culturing in low nutrient medium plus 16S gene sequencing. We sampled roots and rhizosphere soil over time from 16 trees affected by citrus greening or Huanglongbing (HLB), caused by the α-proteobacterium Candidatus Liberibacter

asiaticus (Las). Trees were injected with penicillin G, which was being examined as a treatment for Las, using application rates of 0, 1000, or 6000 µg/ml. Roots plus rhizosphere were ground and serial dilutions were plated on diluted S medium (approximately 10 µg/ml Carbon), with and without penicillin G. After three weeks, colonies were counted and whole microbial communities were scraped from 86 plates, DNA extracted and subjected to 16S rRNA high-throughput sequencing. A total of 11,907,993 reads were recovered. Bacterial sequences were taxonomically assigned to 40 bacterial phyla, 18 of which are described as unculturable in the literature. Alpha diversity was greatest in samples from untreated control trees with an average of 560 OTUs (s.d. 298 OTUs) described per plate. Principal components analysis based on weighted-UniFrac distances also revealed significant differences between control and treated samples, which were not detected through previous culture-independent direct sequencing. Our work suggests the potential for high throughput culture methods to characterize root-associated microbial diversity of low abundance bacteria isolated on diluted S-medium.

126. SALMONELLA VACCINES AGAINST CHLAMYDIA TRACHOMATIS

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Chlamydia trachomatis (Ct), the most common bacterial sexually-transmitted pathogen, was responsible for an estimated 1.59 million cases in 2016 within the US. A large number of cases are not reported because most people infected with Ct are asymptomatic and do not seek medical help. If left untreated, Ct urogenital infection could lead to severe sequelae, including pelvic inflammatory disease (PID), a leading cause of infertility, ectopic pregnancy and/or chronic pelvic pain. Currently, there is no vaccine against chlamydia. The development of a vaccine to prevent chlamydial urogenital infection can preclude the development of severe clinical manifestations, prevent the spread of infection and incidence of reinfection, pose a cost-effective alternative to antibiotic treatment regimens, and may facilitate future eradication efforts. We recently developed an innovative Salmonella enterica serotype Typhimurium vaccine strain which undergoes triple sugar regulated delayed attenuation, antigen synthesis, and controlled lysis. We used this strain to deliver protective Ct antigens, OmpA and PorB, by Type 2 and Type 3 secretion systems. Immunized mice were challenged intrauterine with Ct serovar D strain D strain UW-3/Cx. Combinational delivery of OmpA and PorB by Type 3 secretion system conferred the most significant protection against Ct intrauterine challenge. Further characterization and improvement of this vaccine is ongoing.

127. SWINE MELIOIDOSIS IN THAILAND: CASE REPORT

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Melioidosis, a neglected tropical disease, is endemic in Southeast Asia and Northern Australia. It is caused by Burkholderia pseudomallei, a Gram-negative bacillus. In endemic areas, the organism is common in the environment and most human and animal cases arise from exposure to contaminated soil and water. Melioidosis has been reported in a wide range of animal species and the most commonly affected livestock are goats, sheep, cattle and pigs. The retrospective study of melioidosis in animals in Thailand during 2006 – 2010 showed that the highest incidence was in goats, followed by incidence in pigs and cattle. Pigs have been reported as less susceptible to disease than sheep or goats. In pigs, melioidosis is frequently asymptomatic, often with lesions detected during routine abattoir inspection. In this study, melioidosis was detected in two pigs during routine farm inspection in the northern part of Thailand in 2016. Gross pathology showed diffuse white-yellowish abscesses in the lungs, liver, and lymph nodes. Histopathology revealed diffuse white blood cells infiltration, hemorrhage, and multiple granulomatous formations in tissues. The abscesses and lesions from all organs were negative on acid-fast stain, but cultured positive for B. pseudomallei. Molecular identification was performed by multiplex SYBR green real-time PCR detecting B. pseudomallei-specific sequences of the TTSS1-orf2 (type III secretion system), btfc-orf18 (the BTFC gene cluster target) and BPSS0120 (the YLF gene cluster target). The investigation has confirmed that the cause of the infection was B. pseudomallei by showing positive to TTSS1-orf2 and YLF genomic group, LPS type A. Multi-locus sequence typing (MLST), a gold standard molecular subtyping technique of B. pseudomallei

was performed by comparison of 7 housekeeping genes. MLST has further revealed that one strain had sequence type 164 (ST164) which had the same ST with the recent human case and soil samples in southern Thailand. This sequence type was also found in human cases in Malaysia. Another strain had sequence type 491 (ST491) which had the same ST with an isolate from a patient in Laos based on MLST database. In summary, the disease manifestations in pigs can range from acute to chronic with subclinical infection being common. Infection can be associated with single or multiple abscesses in lung, liver and associated lymph nodes. Real-time PCR potentially offers a faster and more reliable diagnosis of disease in endemic regions for melioidosis. The present cases showed the risk of zoonotic potential of B. pseudomallei in endemic area. Therefore, veterinarians treating animals from these areas should include melioidosis in differential diagnosis when performing necropsy. Furthermore, herdsmen, veterinarians, or abattoir workers should be aware of this risk of zoonotic transmission. Melioidosis is not currently part of the animal disease control program in Thailand, but its inclusion may now warrant review.

128. THE CONTRIBUTION OF CHLAMYDIA TRACHOMATIS L2 PUTATIVE IRON TRANSPORT SYSTEM TO BACTERIAL GROWTH

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Chlamydia trachomatis is an obligate intracellular, Gram negative pathogen responsible for over 100 million new genital tract infections each year worldwide. For over 20 years it has been known that C. trachomatis requires the essential trace metal iron, for normal development. In low iron conditions, achieved by exposure to iron chelators like 2,2'- bipyridyl (bpdl), C. trachomatis leaves normal development and enters a state known as persistence. In this state, chlamydiae are still viable, increase in size, but cease dividing. Persistence induced in response to iron deprivation is reversible if the chelator is removed and the cultures are replenished with fresh iron-containing media. These data indicate that C. trachomatis is able to transport iron, but to date, no iron transport system has been characterized in Chlamydia. Iron acquisition is a critical activity for all bacteria, thus multiple iron transport systems have been identified in E. coli and the facultative intracellular pathogen, Shigella flexneri. Bioinformatics analysis comparing the S. flexneri sitABCD iron transport system to the C. trachomatis L2 genome revealed a homologous operon comprised of five genes, ctl0323-ctl0327. Previous studies have demonstrated that Ctl0323, also known as YtgA, has iron binding capabilities in vitro. Based on these data, together with the operon organization and protein homology with SitABCD, we hypothesize that Ctl0323-0326 comprise an iron transporter in Chlamydia. To demonstrate that Ctl0323-0326 form an iron transporter, we utilized an iron deficient triple mutant strain of S. flexneri, BS935. Transformation of BS935 with the tetracycline inducible E. coli-C. trachomatis shuttle vector, pREF100 harboring ctl0323-0326, conferred improved growth of the BS935 strain compared to BS935 transformed with the empty vector, even in the

absence of induction. Furthermore, overexpression of ctl0323-0326 in C. trachomatis exposed to low iron conditions produced significantly larger inclusions and improved recovery from bpdl-induced persistence. These data support the hypothesis that ctl0323-0326 encode an iron transport system in C. trachomatis.

129. USING COPPER SULFATE MEDIA TO SCREEN FOR COPPER RESISTANCE IN PLANT PATHOGENIC BACTERIA

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Various species of Xanthomonas bacteria cause disease in many agriculturally important crops. Copper sulfate bactericidal sprays are widely used to control bacterial pathogens in many of these crops. As with any chemical control method, development of resistance is a concern when one method of control is repeatedly used. The purpose of this research is to determine an efficient and reliable method of testing for copper resistance in diagnosed pathogenic bacteria. The protocol has a two facets of confirmation. The first part uses differential media to determine if there is copper resistance. The differential media uses an induction broth and selective agar. The induction broth is an MGY broth amended with 1 mg/L of copper sulfate ($CuSO_4 \times 5H_2O$). Incubation of the sample in the induction broth for 2 hours at room temperature is sufficient to induce resistance genes. The differential media is an MGY media amended with 200 mg/L of $CuSO_4 \times 5H_2O$. Once the bacteria have been incubated in the induction broth, the cultures are then streaked on the differential media and incubated at 37 degrees Celsius for 48 hours. If the bacteria are resistant to copper there will be plentiful colonies; if the bacteria is sensitive there will be no growth. The second part of identifying copper resistant strains of bacteria

involves molecular identification of the plasmid responsible for conferring resistance. This is done with a polymerase chain reaction (PCR) and Sanger Sequencing. The PCR reaction uses specific primers that only amplify the plasmid that confers resistance to copper.

130. A SIMPLE EPIDEMIOLOGICAL MODEL FOR HIV BASED ON ERLANG'S METHOD OF STAGES

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This research is to select an epidemiological model for HIV that uses few parameters while fitting the world prevalence and death data well. Here we consider a set of models based on Erlang's method of stages, including some with and some without social distancing. The use of stages is supported by biological studies which suggest that HIV passes through stages in each individual, although the exact number is not known. This set of models represents such stages by using a successive number of classes. To perform model selection, we compute R0 and use it to estimate initial values of the parameters in this model. We run thousands of iterations of a Nelder-Mead simplex search algorithm to determine the optimal values of parameters for each model and the error associated with each model. These errors are used to compute AICc values and then the AICc values are compared to select the most likely model. The selected model from this experiment contains the social distancing term as well as four infected classes/stages. We then perform identiability analysis and determine that the "true values" of the parameters for this model are uniquely determinable based on the data points.

131. ANALYZING THE FUNCTION OF NONCODING RNAS ON VIRAL LATENCY IN MURINE GAMMAHERPESVIRUS 68

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Gammaherpesviruses include Epstein-Barr Virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) and are associated with various malignancies including Burkitt's lymphoma, Hodgkin's disease, and Kaposi's sarcoma. Gammaherpesviruses have the ability to evade the immune response and establish lifelong latency in its host leading to pathogenesis. However, the biological mechanisms by which these viruses establish latency in vivo remains unclear due to the strict species specificity of these viruses. Murine gammaherpesvirus 68 (MHV68) infection in mice shares genetic and pathogenic features with human gammaherpesviruses presenting us with a robust animal model to depict the mechanistic in vivo studies between the virus and its host. MHV68 encodes 8 tRNA-miRNA-encoded RNAs (TMERs) each consisting of a viral tRNA-like structure (vtRNA) followed by two downstream pre-miRNA stem loops that are highly expressed during latency. In particular, TMER4 has been previously demonstrated in our lab to play an essential role in in vivo MHV68 dissemination, latency, and pathogenesis but its function remains unknown. To determine the mechanism for TMER4 function we compared the hypotheses of TMER4 acting via a trans function, binding to host proteins, versus TMER4 phenotype being related to genomic position by inserting a rescue of TMER4 into a different region of the virus to see if it restores wild-type activity. Using a two-step red-mediated recombination method alongside the MHV68 bacterial artificial chromosome (BAC) system, we are generating a recombinant virus that restores wild-type TMER4 into a new site within the MHV68.Δ5.6 TMER4 mutant virus (lacking both stem loops). We will generate recombinant virus

MHV68.Δ5.6.TMER4toTMER5, in which another viral noncoding RNA, TMER5, is replaced by the wild-type TMER4. We selected TMER5 since its deletion has shown no effect on MHV68 viral latency. Thus far we have been successful in generating the first recombination MHV68 BAC mutants containing insertion of the TMER4 in place of TMER5 sequence and deletion of the TMER5 sequence. Our recombinant virus is being compared across wild-type MHV68 TMER4 and MHV68.Δ5.6 TMER4 mutant virus to determine the ability of these viruses to establish latency with our control virus being MHV68.Δ5.6ΔTMER5 expression (full TMER5 deletion). If TMER4 is independent of genomic position, then it will display results equivalent to wild-type TMER4 and determine the mechanism behind TMER4 lies within its trans function instead of its genomic position.

132. COMPLETE GENOME SEQUENCING OF TWO NOVEL PAPILLOMAVIRUS TYPES IN INDIAN RIVER LAGOON BOTTLENOSE DOLPHINS (TURSIOPS TRUNCATUS)

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A next-generation sequencing approach was used to characterize enteric viruses in free-ranging bottlenose dolphins (BDs). Fecal swabs were collected from 12 BDs. Following the creation of two six-sample pools, total nucleic acid was extracted using a QIAamp Viral RNA Mini Kit. Combined DNA and cDNA libraries were created using a sequence-independent, single-primer amplification protocol. Libraries were sequenced using a V2 chemistry 500-cycle kit on a MiSeq platform. Assembly of the paired-end reads was performed in CLC Genomics Workbench. The assembled contigs were subjected to

BLAST searches using NCBI databases. One 693 bp contig was identical to a 437 bp sequence encoding the partial L1 gene sequence recovered from a papillomatous penile lesion in a male killer whale (Orcinus orca) that stranded in the UK. Primers based on the L1 contig sequence were used to generate the full genome sequence by nested inverse PCR and Sanger sequencing. The full genome was 7,299 bp and found to represent a new type of Dyopipapillomavirus 1, a species previously sequenced from a penile papilloma in a harbor porpoise (Phocoena phocoena PV4; PphPV4). A second contig was recovered containing the full genome of a new type of Omikronpapillomavirus 1 (OmikronPV1), a species previously sequenced from a BD penile papilloma (Tursiops truncatus PV; TtPV5). Both BD papillomavirus (BDPV) genomes displayed typical PV genome organization, including four early genes, two late genes and a long control region. Phenetic and phylogenetic analyses supported these two BDPV as novel types of DyopiPV1 and OmikronPV1, to be referred to as DyiopiPV1 (Tursiops truncatus PV8; TtPV8) and OmikronPV1 (Tursiops truncatus PV9; TtPV9), respectively. Separate specific endpoint PCR assays targeting the L1 genes were used to determine the prevalence of the novel PV types in fecal samples of the 12 BDs. An adult female BD displaying a genital papilloma was positive by PCR for DyopiPV1 (TtPV8). Three adult male BDs, one of which displayed a genital papilloma, were positive by PCR for OmikronPV1 (TtPV9). The genome sequencing of TtPV8 and TtPV9 expands the genomic diversity of PVs infecting BDs. In addition, our report of the identical PV sequence found in a BD and a killer whale suggests the capability of certain cetacean papillomaviruses to infect multiple delphinid species. Clinically, these findings may have management implications for facilities that manage mixed cetacean populations. Further investigation of the prevalence and associated health impacts of PVs on free-ranging BD populations are warranted.

133. 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 every 24-hours 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.

134. 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. At the same time PWID form one of the least studied groups who are prone to high HIV prevalence. Study of this population is challenging due to social stigmatization and possible criminal penalties for illicit drug use. To overcome these difficulties, referral-based recruitment can increase participation in studies of PWID. Here, we describe a study that investigates the impact of a suite of interventions targeting HIV acquisition and transmission among PWID in multiple sites in India. Using baseline and longitudinal information on the uptake of a number of interventions utilized in a cluster randomized clinical trial. This clinical trial is conducted by collaborators at the Johns Hopkins Bloomberg School of Public Health and YRG Care and has completed a baseline evaluation and one year of follow-up in intervention and control clusters. Using these data, we quantify the relative importance of: 1) needle exchange 2) antiretroviral therapy 3) opioid replacement therapy 4) HIV counseling. We use a dynamic transmission model to understand the importance of the interventions in dictating 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 clusters. Because the interventions target slightly different aspects of HIV transmission and acquisition risk, we hypothesize that there are both synergistic and antagonistic relationships to each other, resulting in non-additive effects on HIV acquisition, transmission and prevalence. To build the model, we used regression to identify individual and community level

determinants of HIV prevalence and used this information to inform the structure of our transmission model. We propose multiple approaches to parameterize our model to baseline data and data describing from one year after the beginning of the intervention.

135. 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. Routine examination showed no significant parasite burdens and the bacteria present appeared to be the result of secondary infection. Histopathology revealed prominent single cell necrosis and inflammation of the mucosal epithelium within the branchial cavity, pharynx, esophagus, and 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 (28-30 nm), naked, icosahedral particles within the cytoplasm, occasionally arranged in paracrystalline arrays. The virus was concentrated by ultracentrifugation prior to RNA extraction, cDNA library generation, and sequencing using an Illumina MiSeq platform. 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 qRT-PCR) within external and internal tissues.

136. GENOMIC CHARACTERIZATION OF PERCID HERPESVIRUS 1 (PEHV1) 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, enveloped virus particles with hexagonal 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 run generated 13,099,218 paired-end reads for this sample that 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 127,290 bp encoding 86 putative proteins including those conserved in all alloherpesviruses. Maximum Likelihood phylogenetic analysis based on the concatenated partial DNA-dependent DNA polymerase and terminase (exon 2) gene sequences (249 amino acid characters including gaps) revealed PeHV1 forms a novel branch between the genera Ictalurivirus and Salmonivirus. The phenetic analysis of the partial PeHV1 DNA-dependent DNA polymerase (151 amino acid characters including gaps) and terminase (exon 2; 98 amino acid characters including gaps) amino acid 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. Challenge studies are planned to confirm PeHV1 is the causative agent of the observed cutaneous disease in adult walleye.

137. IDENTIFICATION OF MACROPHAGE RESERVOIRS THROUGH TROPISM OF HIV-1 ENVELOPES

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Background: Despite advances in antiretroviral treatment (ART), eradication of HIV-1 is still not possible due to viral persistence in cell reservoirs. Myeloid cells, such as peripheral monocytes and tissue macrophages, which express significantly low levels of the CD4 receptor but they can be productively infected, have been proposed as a significant viral reservoir. To evaluate the hypothesis that macrophages are indeed a source for HIV-1 rebound viremia in individuals undergoing analytical treatment interruption (ATI), we investigated the evolutionary relationships of viral strains, that were also characterized by an in vitro assay quantifying replication capacity in macrophages, sampled before and after therapy interruption in multiple patients.

Methods: HIV-1 full-length envelopes were isolated by single genome amplification from three individuals at rebound plasma viremia followed ATI. To generate infectious recombinant viruses, env sequences were cloned into an infectious HIV-1 backbone, followed by transfection of HEK 293T. Monocyte-derived macrophages were infected with Env-recombinant viruses, and fusogenicity was assessed by a FRET-mediated assay. Replication

capacity was monitored for 14 days by reverse transcriptase activity. Bioinformatic prediction of CXCR4- or CCR5- co-receptor usage was performed using the publicly available WETCAT and geno2pheno algorithm. Phylogenetic relationships among the env sequences were reconstructed by inferring a maximum likelihood (ML) tree, using the best-fitting evolutionary model chosen according to Bayesian Information Criteria (BIC), with the IQ-TREE software. Intra-clade and inter-clade average genetic distances were calculated using both ML-composite estimated distances and p-distances, with standard error obtained by bootstrapping (1000 replicates) in MEGA7. Average diversity time (AVEDT) – representing the average time required to generate the diversity observed within well supported (bootstrap > 95%) monophyletic clades in the ML tree – and average divergence time between two well supported clades, were calculated assuming an average intra-host evolutionary rate for HIV-1 env of 7.53 10-3 nt substitutions/site/year. Bayesian inference of time-scaled phylogenies were carried out with BEAST v1.8.4 by enforcing a relaxed molecular clock and the SDR06 substitution model with empirical base frequencies and gamma distribution of site-specific rate heterogeneity. Two demographic priors were tested: constant population size or non-parametric Bayesian Skyline Plot, and the best demographic model was chosen by comparing marginal likelihood estimates, obtained using path sampling and stepping-stone sampling methods, with Bayes Factor (BF). Bayesian Markov Chain Monte Carlo were run for 200 million generations, and proper mixing of the MCMC was assessed on the basis of the effective sampling size (ESS) of each parameter estimate, accepting only ESS values >200.

Results: Although the majority of amplified env sequences showed little infectivity in macrophages, a small population of envelopes was able to fuse efficiently with these cells. Replication in macrophages of Env-recombinant viruses showed ability to replicate at an intermediate level, and as efficiently as the M-tropic strains ADA and YU2. Prediction of CXCR4- or CCR5- co-receptor usage based on V3 loop did not agree with in vitro M- or T- tropism. Phylogenetic analysis showed the circulation of several distinct HIV-1

subpopulations, with macrophage tropic viral lineages clearly originating from distinct ancestors that predated therapy interruption. The relatively low diversity within each clade suggested recent diversification from each common ancestor, but the estimated time frame of such diversification was still longer than the time interval between therapy interruption and when the sequences were actually sampled.

Conclusions: Overall, our results support the hypothesis that HIV-1 subpopulations of macrophage tropic viruses persist in each patient within distinct reservoirs that can be re-activated during rebound. HIV-1 replicates in tissues that are protected from the effects of ART, including microglia cells in the central nervous system, facilitating the presence of a persistent viral reservoirs. Our findings demonstrate that recombinant viruses containing envelopes isolated at rebound ATI were able to fuse and spread infection to macrophages. Phylogenetic relationships and molecular clock analysis also indicated that from the beginning of rebound to sampling there was not enough time for macrophage tropic variants to evolve from T-cell tropic ones, suggesting that Macrophage tropic variants may indeed constitute part of an independent HIV-1 reservoir.

138. INCREASE IN PERINATAL HIV INFECTION IN NORTH FLORIDA - MISSED OPPORTUNITIES FOR PREVENTION OF MATERNAL TO CHILD TRANSMISSION

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The rate of mother-to child transmission of human immunodeficiency virus (HIV) in the United States has significantly declined due to routine opt-out HIV testing of pregnant women and implementation of effective prenatal, intrapartum and postnatal interventions to prevent vertical transmission. This includes routine HIV testing in the first trimester and a recent trend for retesting in the third trimester in areas with high prevalence of HIV or in high risk social situations. The University of Florida Pediatric Infectious Disease division serves a 31 county area for pediatric HIV care that includes Gainesville, Tallahassee and the entire Florida panhandle encompassing numerous rural counties. There is currently one perinatal coordinator for HIV pregnant women serving 15 of the counties. Between 2008 -2012 there was one HIV infected infant in the entire catchment area. From 2013-2017 there have been 10 HIV infected infants. There has already been one infected infant in 2018. From 2013-17 statewide in Florida there were 41 infants diagnosed with HIV infection. In the past two years the North Florida region had 31% of the total number of HIV infected infants in the state. Seven of the eleven mothers transmitting infection were known to be HIV infected and were prescribed antiretroviral (ARV) therapy. Non -compliance with ARV therapy was documented in all 7. Two were teenagers and 3 were presumed infected during the third trimester. Only 3 mothers received no prenatal care and problems with insurance were reported in 3 mothers as reasons for ARV non-compliance. Mental illness and/or substance abuse was documented

in 6 women. Of the three non -HIV infected women prior to pregnancy 2 were tested in the first trimester, one was tested in the third trimester and one was tested only at delivery due to lack of prenatal care. Improving access to quality prenatal care with good case management for women known to be HIV positive as well as access to mental health and substance abuse services are seriously needed in rural areas. Improving compliance with ARV treatment during pregnancy is crucial in preventing maternal to child transmission. The number of perinatal coordinators needs to be significantly increased to support compliance and provide services. HIV testing in the first and third trimesters should become routine. HIV testing of all (but especially high risk women such as teenagers and those with mental illness or substance abuse) should be strongly considered at the time of delivery.

139. INTERLEUKIN 28B AND ABCC4 SINGLE NUCLEOTIDE POLYMORPHISMS AND RESPONSE TO HEPATITIS B VIRUS TREATMENT IN GHANAIAN HIV CO-INFECTED PATIENTS.

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Background: Sustained Hepatitis B virus (HBV) suppression in persons with and without human immunodeficiency virus (HIV) coinfection is important to reduce the progression of HBV infection to cirrhosis and hepatocellular cancer. While antiretroviral therapy (ART) using drugs with activity against HBV such as lamivudine (3TC) and/or tenofovir disoproxil fumarate (TDF) is effective treatment for HBV, failure to suppress HBV viremia is common. The factors associated with incomplete HBV DNA suppression are not well established. We hypothesized that single nucleotide polymorphisms (SNPs) of immunoregulatory cytokines genes and/or SNPs that influence the pharmacokinetics of nucleoside reverse transcriptase inhibitors with activity against HBV will be associated HBV treatment response.

Methods: ART-experienced HIV/HBV co-infected patients who had received at least 1 year of lamivudine-containing ART were enrolled at the Korle-bu Teaching Hospital (KBTH), Accra, Ghana. Genotyping for IL28B rs8099917, rs12979860, and rs12980275, as well as ABCC4 rs1751034, rs11568695, and rs3742106 SNPs were performed using validated TaqMan® genotyping assays. Association between each SNP and HBV DNA suppression (defined as HBV DNA < 20 IU/mL) was examined.

Results: Of the 152 participants in the study, the median age was 41.5 years, median CD4 count was 521 cell/μL and 58% were female. Of the 152 patients, 64 (34%) failed to achieved HBV DNA < 20IU/mL. Males and patients with HB e antigen positive were less likely to have HBV DNA suppression. Of the genetic factors examined, ABCC4 3463C>T (rs1751034) and 3724G>A (rs11568695) variants were associated with suppression of HBV DNA. Among the 89 with rs1751034 TT genotype, 30 (33.7%) had HBV viremia compared with 32 (52.5%) of 61 with TC/CC genotype (P = 0.034). Similarly, among the 100 with rs11568695 GG genotype, 36 (36.0%) had HBV viremia compared with 27 (54.0%) of 61 with GA/AA genotype (P = 0.054). None of the studied IL28B SNPs were associated with HBV viremia on ART.

Conclusions: Our study identified novel associations between ABCC4 SNPs rs1751034 and rs11568695 variants and unsuppressed HBV viremia on 3TC or 3TC/TDF-containing ART. The implicated SNPs may have a role in strategies to optimize HBV therapy and warrants further study with larger sample size.

140. ISOLATION AND MOLECULAR CHARACTERIZATION OF A NOVEL MAMMALIAN ORTHOREOVIRUS TYPE 2 FROM FLORIDA WHITE-TAILED DEER

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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.

141. ISOLATION AND PHYLOGENOMIC CHARACTERIZATION OF A NOVEL RHABDOVIRUS FROM AN ALASKAN HARBOR PORPOISE (PHOCOENA PHOCOENA)

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Rhabdoviruses possess bullet-shaped nucleocapsids enclosing the negative-sense single-stranded RNA genome that ranges in size from 10-16 kilobases. The family Rhabdoviridae within the order Mononegavirales includes 18 genera and 131 known species that infect plants, arthropods, and vertebrates including fish, reptiles, birds, and mammals. They are responsible for noteworthy diseases in human and veterinary medicine including rabies. To date, only a single report characterizing a rhabdovirus from a cetacean has

appeared involving a white-beaked dolphin (Lagenorhynchus albirostris) that stranded along the coast of the Netherlands in 1992.1 In 2017, an adult male harbor porpoise (Phocoena phocoena) stranded off the coast of Alaska and displayed scattered, mild ulcerative dermatitis, and necrotizing balanoposthitis. Body condition was poor and the lung, liver, subcutaneous tissues, and gastrointestinal tract were heavily parasitized. Histopathologic findings from the skin lesions included pustular epidermitis and dermatitis, with epithelial cell hydropic and ballooning degeneration. Occasional intracytoplasmic eosinophilic inclusion bodies were present. There was a necroulcerative subacute balanoposthitis. Skin and penile swabs were processed for virus isolation resulting in cytopathic effect on both primary beluga whale kidney (BWK) and Vero cells. Transmission electron microscopy revealed abundant bullet-shaped virions budding from the cell surface of BWK cells consistent with a rhabdovirus. A cDNA library was prepared using RNA extracted from the infected BWK supernatant and sequenced on an Illumina MiSeq sequencer. The de novo assembly of 2,607,928 paired-end reads using SPAdes genome assembler recovered the near complete genome of a novel rhabdovirus, the harbor porpoise rhabdovirus (PRV), that was completed by PCR and Sanger sequencing. Maximum Likelihood phylogenetic analysis based on the L gene revealed the PRV branches as the sister group to the white-beaked dolphin rhabdovirus (DRV). The cetacean rhabdovirus clade (PRV and DRV) was found to be nested within the genus Perhabdovirus which includes finfish rhabdoviruses. These data are consistent with a previous hypothesis1 that cetacean rhabdoviruses arose following a jump from a finfish host.

142. PESTE DES PETITS RUMINANT: USING A MIXED METHODS APPROACH TO PROTECT PASTORALIST LIVELIHOODS

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Peste des petits ruminants (PPR) is a highly infectious viral disease of small ruminants, with a primary emphasis on domestic sheep and goats. Worldwide, there are 76 countries which have current infections or are at-risk for PPR, affecting an estimated 1.7 billion sheep and goats, resulting in annual losses between $1.45-2.10 billion (USD) to approximately 330 million of the world's poorest. This Feed the Future Innovation Lab for Livestock Systems (UF/IFAS) project, in conjunction with partners Tufts University, Mercy Corps, and Makerere University, serves to assess innovative approaches to control PPR through the creation of self-sustaining vaccination delivery models and to scale vaccination in select regions of Kenya and Uganda. A mixed methods approach of qualitative data collection and analysis, community based participatory research, participatory epidemiology (PE), and disease modeling is being used to leverage qualitative data for optimum vaccine uptake in order to reach the necessary vaccination level to attain herd immunity. Peste des petits ruminants is a severe drain on pastoralist livelihoods in the region, which dramatically impacts human health and nutritional outcomes within the human population.

143. PHYLOGENOMIC CHARACTERIZATION OF A NOVEL MEGALOCYTIVIRUS LINEAGE FROM ARCHIVED ORNAMENTAL FISH SAMPLES

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The genus Megalocytivirus is the most recently described member of the family Iridoviridae; as such, little is known about the genetic diversity of this genus of globally emerging viral fish pathogens. We sequenced the genomes of two megalocytiviruses (MCVs) isolated from epizootics involving South American cichlids and three spot gourami sourced through the ornamental fish trade during the early 1990s. Phylogenomic analyses revealed South American cichlid iridovirus (SACIV) and three spot gourami iridovirus (TSGIV) possess nearly identical genomes (>99.9% nucleotide identity) and form a novel clade within the turbot reddish body iridovirus genotype (TRBIV Clade 2). Both genomes displayed a unique truncated paralog

of the major capsid protein (MCP) gene located immediately upstream of the full-length parent gene. Histopathological examination of archived oscar tissue sections that were PCR positive for SACIV revealed numerous cytomegalic cells characterized by basophilic cytoplasmic inclusions primarily within the intravascular and hematopoietic cells of various organs, particularly the anterior kidney, spleen, intestinal lamina propria, and submucosa. TSGIV-infected grunt fin (GF) cells grown in vitro displayed cytopathic effect (CPE; e.g., cytomegaly, rounding, and refractility) as early as 96 h post infection. Ultrastructural examination of infected GF cells revealed unenveloped viral particles possessing hexagonal nucleocapsids (120-144 nm in diameter) and electron-dense cores within the cytoplasm, consistent with the ultrastructural morphology of a MCV. Sequencing of SACIV and TSGIV provides the first complete TRBIV Clade 2 genome sequences and expands the known host and geographic range of the TRBIV genotype to include freshwater ornamental fishes traded in North America.

144. 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 sturgeons (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 total genomic DNA extracted from biopsied skin lesions and sequenced using a v3 chemistry 600 cycle kit on an Illumina MiSeq. 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 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 amino acid sequences of the partial pol and term amino acid sequences were 93.1 and 100% identical, respectively. This study provides the first complete AciHV1 genome sequence and expands the host range of this virus to include lake sturgeon.

145. PHYLOGENOMIC CHARACTERIZATION OF RANAVIRUSES DETECTED IN FISH AND AMPHIBIANS IN THAILAND

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Ranaviruses are emerging pathogens associated with epizootics in farmed and wild ectothermic vertebrates including fish, amphibians, and reptiles worldwide. In this study, we described the full genomes of seven ranaviruses isolated from cultured marbled sleeper goby (Oxyeleotris marmorata), goldfish (Carassius auratus), guppy (Poecilia reticulata), tiger frog (Hoplobatrachus tigerinus), Asian grass frog (Fejervarya limnocharis), and two East Asian bullfrogs (H. rugulosus) in Thailand. The full genomes of the fish and amphibian isolates were sequenced using an Illumina MiSeq sequencer. The nucleotide (nt) sequences of the major capsid protein (MCP) from the Thailand isolates compared to a Chinese isolate from tiger frog (H. tigerinus) were highly similar (99.9% nt identity). Comparison of the seven Thailand isolate MCP sequences to other 22 fully sequenced ranaviruses displayed a lower nt sequence identity ranging from 93.1-98.9%. Phylogenomic analyses based on the concatenated locally collinear blocks alignments obtained from Mauve 2.4 for 29 fully sequenced ranaviruses revealed that these eight Asian isolates formed a well-supported monophyletic group referred to as tiger frog virus (TFV) clade. Our findings confirm the transboundary movement of TFVs among Asian cultured fish and amphibians.

146. A SURVEY OF PESTICIDE RESISTANCE AND RESISTANCE MECHANISMS IN AMBLYOMMA AMERICANUM

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The lone star tick, Amblyomma americanum is a pestiferous species due to its ability to vector multiple human pathogens, its aggressive biting behavior, and high reproductive capacity. The application of pesticides, including permethrin, is a widely used management tactic to exclude A. americanum from specific areas or animals, and in some cases it is the only practical method available. Little is known about resistance development in A. americanum, despite the reliance on chemical management tactics. As part of the Centers for Disease Control and Prevention (CDC) Southeastern Regional Center of Excellence in Vector-borne Diseases, to understand more about the potential for resistance development in this species, we will complete the following objectives: 1) establish the discriminating concentration (DC) in permethrin-susceptible colony ticks, 2) survey for resistance in field-collected ticks and compare ticks collected from deer on farms and in wild populations, 3) determine the amount of pesticide to which these ticks and deer were exposed, and 4) characterize the resistance mechanisms. Research efforts to date have focused on the first two objectives. The discriminating concentration has been determined and this has been used to screen field populations. Engorged, adult female A. americanum were collected from deer on farms and in wild populations and stored in an incubator to lay eggs. After hatching, larval A. americanum were

placed in packets, following the Food and Agriculture Organization larval packet test protocol and treated with permethrin at the lethal concentration to 90% and 99% lethality (LC90 and LC99, respectively), the DC, and 2x the DC. The number of surviving and dead larvae were counted and stored in the two groups in ethanol at −80 °C for objective 4. Mortality at the LC99 and LC90 was 2.2% and 19.6% lower for ticks collected from farmed deer exposed to permethrin versus hunted wild deer from Florida wildlife management areas. As these wild deer are unlikely to have been exposed to pesticides, these results may suggest that A. americanum on deer farms are becoming permethrin tolerant. This project is funded by the CDC Southeastern Regional Center of Excellence in Vector-borne Diseases.

147. ANIMAL BITE AND SCRATCH INJURIES RELATED TO HURRICANE IRMA, FLORIDA 2017

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Background: Hurricane Irma made landfall in Florida on September 10, 2017, impacting most of the state. In preparation for the storm, approximately 6 million individuals evacuated. Domestic and wild animals are often displaced during these events and the resultant fear and stress can manifest in aggressive behavior. For this reason and because of increased instances of individuals initiating contact with unknown animals, these events are related to higher risk of injuries due to bites and scratches and potential rabies exposure. The goal of this study is to characterize animal bites and scratches that occurred in the month of Hurricane Irma landfall compared to previous years.

Methods: The Florida Department of Health captures emergency department (ED) chief complaint and discharge diagnosis data from 98% of Florida's hospitals in ESSENCE-FL, the Department's syndromic surveillance system. A query for rabies, bites, and scratches was used to extract all possible observations of interest

collected from EDs between September 1 and September 30 of the years 2011-2017. Reports of animal bites were summarized for 2017 versus a six-year baseline (September 2011-2016).

Results: There were 3,920 reports of animal exposures from EDs in September 2017; 43.5% of those reports were dated between 9/8/2017-9/16/2017. Most individuals were female (55%), between 35-54 years old (25%), and were evaluated for animal bites (93.8%). Injuries occurred predominantly to arms (11.2%), hands (22.2%), and legs (12.3%). The animals involved in these exposures were mostly dogs (60%) and cats (17.2%). Compared to baseline years, the number of ED visits related to animal exposures was 13.8% higher in September 2017 and 252.8% higher on September 11, 2017, adjusted for the number of EDs reporting into ESSENCE-FL. Adults were over-represented, especially those between 55-65 years old and those reporting scratch injury, injury to arms and hands, or exposure to cats was elevated in 2017. There was no difference in the proportion of individuals reporting leg injuries. In all years, Broward, Miami-Dade, Hillsborough, Orange, Palm Beach, Pinellas, and Volusia counties had the most cases.

Conclusions: Animal bite and scratch reports increased during Hurricane Irma in September 2017, with the distribution of burden among counties not changing from previous years. Education should be targeted to adults and focused on using caution when interacting with animals during evacuation events. Health departments should emphasize animal bite reporting particularly in the days immediately before and after these evacuation events to enhance appropriate follow-up and prevent rabies infection.

148. ANIMAL BREED COMPOSITION SHAPES GUT MICROBIOTA, AND ITS EFFECTS ON THE HOST METABOLIC AND IMMUNOLOGICAL STATUS

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Background: Great efforts have been made in breeding and genetic selection in the agricultural industry to generate animals with desirable traits. Host genetics can also influence the gut microbiota, which is considered a "second genome" that mediates host physiology. However, it is unclear how the gut microbiota is altered by the gradual changes of genetic composition, and its relationship with animal growth and health.

Methods: To evaluate effects of genetic variation on gut microbiota, growth rate, and physiological status, we collected fecal and blood samples from Angus-Brahman multi-breed cattle (n=240), with breed composition ranged from 100% Angus to 100% Brahman. Gut microbiota was detected by 16S rRNA gene sequencing, and analyzed by QIIME and PICRUSt. Metabolic and immunological status was evaluated by measurement of plasma glucose, triglyceride, and IgG1 levels. Associations among breed composition, gut microbial community and host phenotypes were assessed by Pearson’s correlation coefficient.

Results: The gut microbial community structure linearly changed with a shift in genetic composition in the multibreed herd of Angus and Brahman cattle. Groups of bacterial families and genera with their presence and proportion linearly correlated with breed composition were identified. Growth rate was positively correlated with Angus proportion, and obese-associated Rikenecellace was enriched in fast-growing Angus cattle. Plasma IgG1 level decreased with Brahman proportion. The relative abundances of butyrate-producing bacteria Faecalibacterium, Blautia and Coprococcus, which are biomarkers of a healthy gut, increased in infection-tolerant Brahman cattle. Plasma glucose level increased with Brahman proportion. Bacteria in cattle with more Brahman proportion were predicted to contain more genes involved in carbohydrate metabolism but less related to bacterial infections, while those with more Angus proportion were better involved in lipid biosynthesis. Associations between host phenotypes and microbial communities were also detected. Relative abundance of Faecalibacterium was positively correlated with growth rate. The plasma glucose level was positively correlated with relative abundances of Oscillospira, Bacteroides, [Prevotella], and Odoribacter.

Conclusions: Our findings indicate that animal breeds modulate the structure and function of the gut microbiota, and thus the altered microbiota are closely associated with animal's physiological and immunological status.

149. CHARACTERIZATION OF NEW FLUORINATED METHYLPHENYLAMIDE DERIVATIVES AS INSECTICIDES IN AEDES AEGYPTI, ANOPHELES GAMBIAE, AND DROSOPHILA MELANOGASTER

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Preventing biting to control the population of disease-vectoring mosquitoes remains the most efficient way to reduce the propagation of pathogens. Towards this end, repellents and insecticides are widely used, from which the systematic use of the same chemicals promotes resistance in the field. Accordingly, it is necessary to develop new compounds with new modes of action to bypass the newly acquired resistance. In this work, 4 compounds (A, B, C & D) with various halogenated moieties were synthesized from a new lead compound. Topical application assays showed toxicity (lethal dose effect 50%) as low as 30 ng and 8 ng per mg of body weight on Aedes aegypti (Ae) and Anopheles gambiae (Ag) respectively, comparable with commercial insecticides such as propoxur (1.3 ng and 1.08 ng for Ae and Ag, respectively). Their mode of action was further studied on the central nervous system (CNS) (isolated ventral ganglion, VG) from Drosophila melanogaster (Dm) Oregon-R wild type strain. This model relies on the recording of the spontaneous synchronized bursting activity of the motor neuron

fibers located in the abdominal nerves exiting the VG. These motor neurons are responsible for the locomotor activity of the larvae. In this model, the different compounds tested showed inhibiting effects, significantly reducing the bursting rate (e.g., compound B at 10 µM and C, D at 1 µM). They also reversed the inhibition induced by the application of 1 mM GABA to the VG, suggesting a mode of action targeting the GABA receptors in the CNS. Moreover, we showed that the CNS from another Dm strain (RDL 1675), being resistant to the dieldrin insecticide (a GABA receptor antagonist), were equally sensitive to compounds B, C and D. The promising preliminary results suggest these compounds are targeting both wild type and mutated GABA receptors and are toxic by topical application. Selective toxicity will be assessed on rodent models. Using this knowledge, additional derivatives will then be synthetized to improve selectivity and efficacy of the lead compound. Further characterization of those molecules will be conducted, with the use of more specific electrophysiological tools such as GABA receptor expression in Xenopus laevis oocytes.

150. DBD SURFACE PLASMA DEACTIVATION OF MULTI-ORGANISM BIOFILMS AND LEGIONELLA

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Legionella pneumophila (Lp), the major agent causing 90% of the registered cases of Legionellosis, is aquatic, inhabits diverse water sources and is highly capable of surviving in biofilms. Biofilms are complex heterogeneous colonies of microorganisms attached to surfaces as communities which provide the microorganisms living within them 5 to 1000 times more resistance towards disinfectants as well as adverse environmental conditions. Thus, Legionellae as well as other microorganisms residing in biofilms are difficult to eradicate with conventional disinfection methods. Although some literature can be found in disinfection of biofilms with gas discharge, research on disinfection of resistant sessile legionella with the help of surface plasma discharge has not been reported yet. Dielectric barrier discharge (DBD) is one way to create surface plasma where the electric discharge forms between two electrodes which are separated by a dielectric barrier. In this project, we have addressed two key issues, namely: (1) whether DBD surface plasma in direct contact with biofilms can inactivate the microorganisms residing in them and (2) whether surface plasma is capable of killing L. pneumophila. 5 to 7 day old biofilms were grown on plasma actuators using B. subtilis added to tap water containing Gram-negative organisms. Biofilms infected with Legionellae were produced by growing standard multi-organism biofilm for 5 days and then adding Lp to the biofilm culture which was incubated for three additional days. The biofilms grown on the actuators were exposed to direct DBD surface plasma for different exposure periods. The

control actuators grew 106 CFU/actuator of a mixed bacterial population and 103 CFU Legionella. Plasma exposure at 30V for 2 minutes resulted in a 3-4 log drop of all organisms and no detectable Legionella. Longer exposure (5 mimutes at 30 or 35V, 30V for 10 min) resulted in no surviving bacteria detected. These results indicate that DBD plasma actuators can be a potential inactivation agent for biofilms in general as well as legionella infected biofilms. The project was funded by SurfPlasma, Inc.

151. EFFECT OF RADIATION ON FEMALE AE. AEGYPTI PUPAE FOR SIT

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Aedes aegypti is a vector mosquito that can transmit pathogens that can cause disease in humans and animals. However, it is difficult to control them through larvicides and/or source reduction because they can develop in a wide range of water holding containers. One method that has been explored is the sterile insect technique using irradiation. Prior irradiation studies have primarily focused on the irradiation of male pupae, while ignoring the effect that radiation has upon female pupae. In this study we examine the effect that radiation has upon female adults after being irradiated as pupae, and we discuss the potential impact on feeding and oviposition behavior that these treated females may have if they were to be incidentally released with their irradiated male counterparts.

152. EFFECTS OF TOMATO ROOTSTOCK GENOTYPES AND GRAFTING ON FUNGAL NETWORKS AND DIVERSITY

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Root-associated fungal (RAF) communities are critical for supporting nutrient exchange between plants and the bulk soil environment and determining plant performance and health. We explore how rootstock genotype affects RAF communities, with the hypothesis that specific rootstock genotypes support distinct RAF communities. We grafted scion BHN589 on four tomato rootstock genotypes and profiled the associated endosphere and rhizosphere fungal communities by sequencing fungal Internal Transcribed Spacer (ITS2) regions. Only a small percentage of total OTUs were shared across all the rootstock genotypes, and taxa unique to each genotype were identified. PERMANOVA analysis based on the Bray-Curtis dissimilarity distance indicated that plant genotype explains a small percentage (2.07%, p < 0.01) of variation in RAF communities. Study site (8.34%, p < 0.001) and endosphere-rhizosphere compartment (8.92%, p < 0.001) explained the majority of the observed variation across all the rootstock genotypes. A significantly higher number of species (richness) was observed in the higher yielding rootstock (Maxifort) compared to the non-graft control. In addition, our analysis of fungal-association networks suggested higher interactions among fungal taxa in Maxifort compared to other rootstocks. Network analysis also showed an increase in the number of positive and negative associations with shared and unique taxa in Maxifort compared to other rootstocks. These results indicate a potential role of RAF communities in mediating the effects of grafting in vegetable production. Future approaches to modification of the RAF communities may enhance disease management in this

and related systems, when rootstocks are used to enhance disease resistance.

153. FECAL BACTERIAL COMMUNITIES OF WILD-CAPTURED AND STRANDED GREEN TURTLES (CHELONIA MYDAS) ON THE GREAT BARRIER REEF, AUSTRALIA

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Chelonia mydas are primarily herbivorous long-distance migratory sea turtles that contribute to marine ecosystems. Extensive research has been conducted to restore the populations of green turtles. Little is known about their gut microbiota which plays a vital role in their health and disease. In this study, we investigated and compared the fecal bacterial communities of wild-captured green turtles to stranded turtles by PCR amplification of a hypervariable region (V1-V3) of the bacterial 16S rRNA gene for each sample followed by sequencing on an Illumina MiSeq platform. At a phylum level, Firmicutes predominated among wild-captured green turtles, followed by Bacteroidetes and Proteobacteria. In contrast, Proteobacteria (Gammaproteobacteria) was the most significantly dominant phylum among all stranded turtles, followed by Bacteroidetes and Firmicutes. In addition, Fusobacteria was also significantly abundant in stranded turtles. No significant differences were found between the wild-captured turtles from two different locations. At a family level, 25 of the 53 families were identified in both the wild-captured and stranded green turtles, while 14 families were found only in stranded turtles. At the OTU level, 256 (48.7%) of the total OTUs (>1% abundance) were shared between the wild-captured groups of turtles, while absent in stranded turtles. The predominance of Bacteroides in all groups indicates the importance

of this bacteria in turtle gut health. In terms of microbial diversity and richness, wild-captured green turtles showed the highest microbial diversity and richness compared to stranded turtles. The marked differences in the bacterial communities between wild-captured and stranded turtles suggest the possible dysbiosis in stranded turtles in addition to potential causal agents.

154. FLORIDA MASTER GARDENERS' FOOD SAFETY KNOWLEDGE AND PRACTICE

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The Florida Master Gardener Volunteer (MG) program is a statewide program that has been providing scientific training on the subject of horticulture to individuals interested in gardening and planting since 1979. Previous studies have mainly focused on demographic characteristics and motivation for participation in a MG program. Little is known about MG volunteers' food safety knowledge and practices. Without sufficient attention to food safety, there could be foodborne illness risk among the targeted servicing population. The present study aimed to evaluate Florida MG volunteers' food safety knowledge and practice. A 40-item instrument was developed and distributed to all active Florida MG volunteers (n = 4005) via Qualtrics. One week later, 1012 completed responses were recorded and included for data analysis. The results showed that the majority of the respondents were female (80%), aged ≥ 60y (80%), and held college or postgraduate degrees (73%). The respondents correctly identified the five most common risk factors for foodborne illness, and 80% of them knew about the time/temperature controls necessary for food safety. Meanwhile, 87% of respondents correctly reported the safe minimum internal temperature for whole chicken or poultry. However, percentages knowing the safe minimum temperature decreased to 60%, 43%, and 42%, respectively, when asked about ground beef, leftovers and/or casseroles, and fresh whole cut beef. Moreover, the respondents were not knowledgeable about the specific vulnerabilities of different populations to foodborne illness. Regarding safe food handling behaviors, the

majority of participants reported safe handwashing practices (68%), safe fresh produce washing (77%), safe cross-contamination prevention methods (86%), and safe thawing methods (78%). However, significantly fewer respondents reported safe leftover storage methods (51%) and adhered to safe food thermometer monitoring of the internal temperature of meat products during cooking (<30%). In conclusion, the survey revealed certain gaps in food safety knowledge and practice that need to be addressed along with Florida MG volunteers' horticulture training.

155. GLOBAL CROPLAND CONNECTIVITY: A RISK FACTOR FOR INVASION AND SATURATION BY EMERGING PATHOGENS AND PESTS

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The geographic pattern of croplands is an important risk factor for the invasion of crop-specific pathogens and arthropods, and saturation by endemic pests. Understanding the structure of cropland networks supports sampling and mitigation strategies. We evaluated global networks of key vegetatively-propagated crops (banana, cassava, potato, sweetpotato, and yam) of particular importance to food security in the tropics. The risk of damage from diseases transmitted through vegetative propagation is a particular concern. We analyzed the structure of cropland networks for each crop, where the existence of a link between geographic location

pairs was determined using a gravity model, as a function of the distance between the pair of locations and the product of the harvested crop area in the two locations. Networks were evaluated using a novel index of pathogen or arthropod invasion and saturation risk, based on the role of locations in bridging cropland areas and the degree of connectedness of a location and its neighbors. For example, in addition to locations with high risk due to high cropping density, locations with high risk because of their role as bridges for cassava include South-Central Nigeria, Central Ghana, and Southwestern Democratic Republic of Congo. For potato, bridges include Central and Southern Poland and Northern Ukraine. The highly-linked hub and bridge locations we identified are likely priorities for surveillance and management, and for tracing intra-region movement of pathogens and pests. Integrated analyses of invasion and saturation risk can simultaneously evaluate risk due to cropland connectivity along with other risk factors such as climate and trade routes.

156. HEALTHY BEHAVIORAL CHOICES AND CANCER SCREENING IN INDIVIDUALS LIVING WITH HIV/AIDS ARE DIFFERENT BY BIOLOGICAL GENDER AND YEARS SINCE HIV DIAGNOSIS

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Background: Individuals with HIV have an increased risk of developing non-AIDS related malignancies. We hypothesize that, among individuals with HIV, time since HIV diagnosis may influence

the development of non-AIDS related malignancies partly through correlations with health compromising behaviors, biology, and treatment. Therefore, we investigated the prevalence of healthy behaviors and gender-specific cancer screening in a cohort of HIV infected individuals in Florida, by biological gender and time since HIV diagnosis.

Methods: We included a total of 517 individuals with HIV from the Florida Cohort Study which recruits individuals through county health departments and community clinics. Data were obtained from the cohort baseline and follow up questionnaires, electronic medical records, and Enhanced HIV/AIDS Reporting System. The prevalence of cancer screening for individuals at the recommended age of screening (anal cancer, colorectal cancer, prostate cancer, breast cancer and cervical cancer) and healthy behaviors (sustaining healthy body mass index, smoking, alcohol use and physical activity) was described overall as well as by gender and years since HIV diagnosis (≤ 13 years vs. >13 years). Prevalence across strata was compared using chi-square test.

Results: In the analysis by gender, females were more likely to be obese than males (57% vs. 22%, p <0.0001). Among males, the prevalence of overweight/obesity was significantly higher in those who had been diagnosed with HIV for >13 years 67% vs. 48%, p=0.02). Among males, 65% reported never having an anal pap-smear, 36% reported never having colonoscopy, and 39% reported never having prostate cancer screening. Among females, 50% reported never having an anal pap-smear, 46% reported never having colonoscopy, 8% reported never having cervical pap smear and 13% reported never having mammograms. The difference in anal pap-smear by gender was statistically significant (p<0.0001). Among males, the prevalence of never having a colonoscopy was higher in those who had HIV for ≤13 years (49% vs. 28%, p=0.02).

Conclusion: Prevalence of selected healthy behaviors and cancer screening differed by gender and/or years since HIV diagnosis suggesting a need for tailored cancer prevention efforts among HIV-infected individuals via, long-term gender-specific interventions.

157. HIGH-THROUGHPUT CELL BEHAVIOR EVALUATION USING LASER PRINTED CONSTRUCTS

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Conventional 2D and 3D cell culture models are limited in fully mimicking complex structures and functions of living organs and may fail to recapitulate crucial subtle organ-specific variations. To address this limitation, a microfluidic organ-on-a-chip device, capable of culturing printed cell-laden structures under pulsatile flow conditions is developed to simulate in vivo conditions as seen in typical tissues and organs. Adoption of laser printing during the fabrication of the organ-on-a-chip device enables direct creation of spatial heterogeneity of cells and biomaterials to mimic organ-specific variations for cells. The effect of extracellular matrix (ECM) on cell behavior is investigated using the designed organ-on-a-chip device. Laser printing is utilized to print combinations of living cells mixed with collagen and a varying content of alginate to mimic cells living in different ECM environments with varying mechanical stiffness. These cells are cultured under the same conditions of static as well as pulsatile flow of cell culture medium and then tested simultaneously after 24 and 72 hours, respectively. It is found that cell behavior is influenced by the mechanical stiffness of hydrogels since cells exhibit more elongated and less circular morphologies when cultured within less stiff hydrogels under both static and pulsatile flow conditions. It is also found that the pulsatile flow promotes the cell spreading

within ECM compared to static culture. The proposed organ-on-a-chip device provides a high-throughput platform for the simultaneous testing of different biological constructs as well as microbes under the same external stimuli.

158. HIV VIROLOGICAL FAILURE MODIFIES THE ASSOCIATIONS BETWEEN BMI AND HYPERTENSION AMONG PERSONS LIVING WITH HIV: A CROSS-SECTIONAL ANALYSIS OF THE FLORIDA COHORT DATA

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Objective: Studies have shown that overweight/obesity and HIV are each associated with hypertension. This study aims to test the interaction between overweight/obesity and virological failure on hypertension among persons living with HIV (PLWH).

Methods: We did a cross-sectional analysis using baseline data from 569 participants of the Florida Cohort Study. The participants were categorized into four subgroups based on the BMI (<25: normal vs. ≥25: overweight/obese) and viral load (<200 copies/mL: HIV viral suppressed vs. ≥200 copies/mL: virological failure). The multiplicative interactions were assessed using logistic regression models adjusting for the covariates and the potential confounders. The addictive interactions were assessed using three measures: Relative Excess Risk due to Interaction (RERI), Attributable Proportion (AP) and Synergy index (S).

Results: A total of 266 (40.36%) participants were recorded with hypertension as defined by objective medical record, self-reported diagnosis of hypertension, or self-reported use of antihypertensive

drugs through questionnaire. In the final model, compared to the participants with normal BMI and viral suppression, overweight/obesity was independently associated with hypertension (OR=1.66, 95%CI: 1.01, 2.76) while there was no independent association between virological failure and hypertension (OR=0.68, 95%CI: 0.29, 1.75). The odds ratio for hypertension was significantly higher for those with both overweight/obesity and virological failure (OR=3.37, 95%CI: 1.68, 6.79). The multiplicative interactive effect was statistically significant (p = 0.043). Further, the RERI for hypertension in overweight/obese individuals with virological failure was 1.34 (95%CI: 0.31, 3.47) more than the combination of individuals with either overweight/obesity or virological failure respectively. The AP due to the interaction between overweight/obesity and virological failure was 64% (95%CI: 36%, 92%).

Conclusion: Findings of this study suggest a significant association between BMI and hypertension among PLWH. HIV virological failure accentuated the correlation between increased BMI and hypertension. Individuals with both overweight/obesity and virological failure should be intensively managed for hypertension prevention and treatment.

159. OBJECTS OF REVERENCE AS CARRIERS OF DISEASES: HEALTH IMPLICATIONS OF PIGEON EXPOSURE IN MUMBAI

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Mumbai is home to a unique culture of people-feeding pigeons at designated pigeon-feeding stations, colloquially termed kabutarkhanas, for religious reasons. Mumbai has more than 50 such unregulated pigeon-feeding stations. Every kabutarkhana houses roughly 5000 pigeons, each of them potentially carrying over 60 human pathogens. The health hazards posed by these kabutarkhanas are manifold – not only are they located within communities, and frequently close to hospitals, but Mumbai also has a high prevalence of People Living with HIV/AIDS (PLWHA), with many of them dropping out of treatment every year, thereby exposing them to an increased risk of acquiring these infections. Common pathogens carried by pigeons are known, but several attributes such as the ecological niches for pigeon pathogens in an urban landscape, and the risk of pathogen transmission to vulnerable populations such as PLWHA are unknown. Pigeons pose a spatio-temporal threat to the health of not only the general population, but an increased risk to the HIV/AIDS-infected population of the urban environments they inhabit. This project involves a qualitative analysis of the reasons why people feed these pigeons, studying pigeon fecal samples for pathogens and comparing them to the risk perceptions associated with pigeon exposure among the city's physicians, and using risk-modeling techniques to estimate the risk of pathogen transmission to PLWHA in Mumbai. We aim to determine pathogens carried, and risk of pathogen transmission to PLWHA, providing data on spatio-temporal distribution of pigeon pathogen carriage and risk models for intervention efforts, and for efforts to increase the public health awareness surrounding pigeon exposure. The results will help determine the pathogen threat levels associated with these pigeon-feeding stations, providing data to local authorities for making

decisions on the future of these kabutarkhanas, besides the broader impacts in other cities having large pigeon populations, to additionally estimate the risk of the transmission of these pathogens to the vulnerable population of PLWHA.

160. ONE HEALTH @ UF

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The One Health Center of Excellence at the University of Florida (UF), housed at the Emerging Pathogens Institute, was created in 2010. With the arrival of a new team in mid-2016, we have redesigned the goals and activities of the Center to bring One Health, which is the interdisciplinary care of animal, environmental and human health, to a novel dimension: the One Health 2.0 vision. Previously, the center was focalized on infectious diseases; now we aim to embrace the broad array of different lenses of focus by expanding the disciplines and topics engaging with One Health. This new approach differs from many One Health initiatives around the globe which are mostly focused on specific interfaces within the biomedical realm. In order to propel this endeavor, we became an independent Center at the end of 2017. The Center's mission is to address complex problems related to the health of the biosphere by seeking novel solutions

originating from interdisciplinary and lateral thinking. The Center's vision is to capitalize on and exploit existing fields of excellence within and outside UF by creating novel areas of intersection, leading to an expansion of research and intervention spheres within the One Health paradigm. The Center aims at placing One Health as a perfectly suited philosophy and tool for the community to reach the United Nations Sustainable Development Goals. The Center is engaged on two fronts to accomplish this: training and interdisciplinary activities. The One Health Graduate Certificate, a collaborative effort with the College of Public Health and Health Professions, represents the training component. This certificate will gather students from high and low-middle income countries and with diverse professional backgrounds around complex topics pertaining to health. It will be composed of four courses and will have four suggested tracks: communication and behavior; ecosystems; urban development; and, pathogens and contaminants. The interdisciplinary activities component is represented by a series of seminars: monthly seminars, One Health talks and Ladies4Ladies, and, a yearly seminar, One Health International. The activities also involve bridging with different departments through research project development and dialogue. Current projects and interests span topics from influenza and diabetes type I, Zika epidemics prediction, human-animal bonding, to plant health. As one of the pillars of the Center, in collaboration with the National Science Foundation Center for Big Learning at UF, the center is currently creating frameworks and pathways to use Artificial Intelligence for analyzing current and future wicked One Health problems.

161. PARTICLE SIZE DISTRIBUTION OF INFECTIOUS VIRUSES USING WATER-BASED CONDENSATIONAL GROWTH TECHNOLOGY

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Inhalation of virus aerosols can cause a wide range of adverse health effects and even severe casualties depending on the species and size distribution of the viruses. The objective of this study is to investigate the size distribution of the infectious viruses in the size range of 30 nm-300 nm, using bacteriophage MS2 as surrogate for human viruses, with the newly introduced virus sampler- water-based condensation growth tube collector (GTC). Fine aerosols containing MS2 bacteriophage were generated from a Collison nebulizer, conditioned by a dilution dryer, size selected by a differential mobility analyzer (DMA), and then collected by the GTC. Particle size distributions of the virus-containing particles were measured by a scanning mobility particle sizer (SMPS), while the number of infectious viruses and the total viruses collected were quantified by the culture and quantitative reverse transcription-polymerase chain reaction (RT-qPCR). Particle size distributions of infectious viruses were also found to be dependent on the nebulization suspensions. For deionized (DI) water, size distributions of infectious MS2-containing particles approximate a cube-power scaling, suggesting a volume distribution instead of the number size distribution. In comparison, artificial saliva (AS) was found to be more protective than DI water and beef extract (BE), which approximate a four-power scaling particle size distribution. Results of this study will be of great importance for the accurate assessment of the infectivity and transmissibility of airborne virus.

162. PINEAPPLE HEART ROT ISOLATES FROM ECUADOR REVEAL A NEW GENOTYPE OF PHYTOPHTHORA NICOTIANAE

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Phytophthora nicotianae is an Oomycete pathogen that causes diseases in ~225 plant genera, thus standing out among other Phytophthora species in its geographic distribution, host range and economic impact. Pineapple heart rot disease, caused by P. nicotianae, is responsible for significant annual reductions in crop yield due to plant mortality. In Ecuador, new infections arise during the rainy season and increases production costs due to the need for biocontrol and fungicide applications. Studies of P. nicotianae population structure suggest that certain genetic groups are associated with host genera; however, it is not clear how many host-specific lineages of the pathogen exist or how they are related. The objectives of this study were to determine the level of genetic variation of P. nicotianae causing heart rot disease of pineapple in Ecuador and compare the genotypes found on pineapple to those previously reported from citrus, tobacco and ornamentals. Thirty P. nicotianae colonies were collected from infected leaves of four farms in two provinces of Ecuador: Santo Domingo De Los Tsáchilas and Los Ríos. We genotyped these isolates using nine simple sequence repeat (SSR) loci. In addition, we analyzed the DNA sequence of mitochondrial loci cox2+spacer and trnG-rns. Together, these loci supported a single clonal lineage with two MLGs differing in a single allele and low mitochondrial diversity. This lineage was distinct but closely related to isolates collected from vegetables and ornamentals in Italy. Our results support the hypothesis of host specialization of P.

nicotianae in intensive cropping systems and contribute to the understanding of population structure of this important pathogen.

163. PUBLIC HEALTH RESEARCH IN HAITI: CHALLENGES AND OPPORTUNITIES

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The Emerging Pathogens Institute, the School of Medicine, the College of Public Health and Health Professions at the University of Florida work in partnership in Haiti with local institutions to implement research projects at the University of Haiti's School of Medicine, the National Laboratory for Public Health and two laboratories in Gressier and Baradères. These activities include training and capacity building in new technology to assess presence of infectious diseases and high-risk areas, field assessments, laboratory, clinical and insectarium work. The team in Haiti is composed of dedicated 18 young scientists and technicians assisted by about twenty faculty and their graduate students. Among others, cholera, zika, other arbovirus, sexually transmitted infections are studied in partnership with GHESKIO (Groupe Haïtien d'Etude du Sarcome de Kaposi et des Infections Opportunistes), the Haiti's Ministry of Health and Population and its outposts in Jacmel, Gressier, Léogane and Baradères. Our facilities include a BSL2 and BSL3 laboratories in Christianville and a simple one at hospital in Baradères where specimens related to the studied pathogens are processed, tested and stored. A significant aspect of the research management model seems to be the partnership with local institutions since opportunities are constantly developing at better understanding the multiple challenges Haïti faces related to her natural history and socio-economic problems.

164. SEAFOOD WORKER HEALTH AND SAFETY SURVEILLANCE IN GULF COAST COMMUNITIES

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Commercial seafood harvesting provides fresh, high quality protein for our nation's tables, and is one of our nation's most dangerous professions. The fatal injury rate for fishers and related fishing workers is among the highest of any civilian occupation: 203.6 per 100,000 FTE workers, more than 50 times the all-worker rate of 3.5. The self-employed and uninsured nature of most of these jobs making the common non-fatal occupational injuries and health outcomes challenging to estimate. As part of the new NIOSH Southeastern Coastal Center for Agricultural Health and Safety at UF, 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. While initial focus in Florida Gulf coast fishing communities, the project and the Center's reach incorporates the southeastern US region including Florida, Alabama, Mississippi, Georgia, North and South Carolina, and the US Virgin Islands. Community partnerships are highlighted to reveal the importance of engaging with seafood workers with the study 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. Musculoskeletal injuries, cuts and lacerations, bites, spine punctures, 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, coupled with workplace

observations, will guide the development of interventions and outreach tools to support the health and safety of our coastal seafood harvesting workforce. This project is supported by NIOSH, the National Institute for Occupational Health and Safety (U54OH011230), the UF College of Public Health and Health Professions, and the Emerging Pathogens Institute.

165. SOUTHEASTERN COASTAL CENTER FOR AGRICULTURE HEALTH AND SAFETY

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The Southeastern Coastal Center for Agricultural Health and Safety (SCCAHS) is the newest center of a Centers for Disease Control and Prevention (CDC)/National Institute for Occupational Safety and Health (NIOSH) Agricultural Health and Safety Initiative. The Center focuses on occupational safety and health needs of people working in agriculture, fishing and forestry in Alabama, Florida, Georgia, Mississippi, North Carolina, South Carolina, and the US Virgin Islands. The University of Florida serves as the lead institution partnering with University of South Florida, Florida State University, Florida A & M University, Emory University, and the University of the Virgin Islands. The goals of the SCCAHS include conducting research-to-practice projects and utilizing research to develop target-audience specific education training and outreach. Research projects within the SCCAHS include occupational health and safety surveillance of Gulf Coast seafood workers; extent of agricultural pesticide applications in FL using best practices; pesticide and heat stress education for Latino farmworkers that is culturally appropriate; heat stress and biomarker of Renal Disease. Current pilot projects within

the Center include heat and pesticide stress in kidney; chronic low back pain in seafood workers; acute psychological and health impacts of Hurricane Irma in UF/IFAS Extension workers; mental, physical and occupational health among Haitian and Mexican Farm Workers in Immokalee, FL.

166. THE BURDEN OF TRAVEL-ASSOCIATED MORBIDITY IN FLORIDA, 2016

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Background: Florida is a large and diverse state with over 20 million residents and 113 million tourists in 2016. Travelers are a heterogeneous group with distinct behaviors and other factors that increase their potential for exposure to diseases outside their home state or country. Areas of endemicity contribute to travel-associated disease (TAD) patterns and vary by disease. However, TAD patterns also reflect travel patterns among people. The potential for travelers to carry non-endemic diseases between states or countries highlights the need to collect and analyze travel history data to increase awareness of imported diseases throughout the state.

Methods: Travel history data captured in Merlin, the state's reportable disease surveillance system, from 2007-2016 were reviewed. TADs were defined as confirmed or probable cases of reportable diseases where the person was infected or exposed in other U.S. states, U.S. territories, or outside of the U.S. Diseases excluded from this analysis included AIDS, HIV, tuberculosis, sexually transmitted diseases, chronic hepatitis, cancer, and congenital anomalies.

Results: Generally, 6-8% of all reported cases each year were TADs. Peaks occurred in 2010 and 2016, when 10% and 13% of cases were TADs, respectively. Peaks were related to an influx of Haitian residents entering Florida after a severe earthquake in 2010, a case definition change to giardiasis in 2010, and the large number of imported Zika virus infections in 2016. Of 2,756 TADs in 2016, 70%

were acquired outside the U.S., 20% in other U.S. states, and 9% in U.S. territories. Compared to previous years, a greater proportion of cases were acquired in U.S. territories due to the large number of Zika infections acquired in Puerto Rico. TADs identified in 2016 included 22 different diseases that accounted for 98% of all TAD cases. Additional characterization of TADs by disease, region of exposure, and county of residence will be presented.

Conclusions: This comprehensive review quantified and described the burden of imported reportable diseases. Identification of regions more likely to introduce and support increased transmission of a particular disease can direct activities to raise awareness and reduce TADs. This analysis demonstrated that both travel patterns and disease endemicity are important when strategizing to prevent TADs. More informed clinical networks and travel partners will increase preventative information provided to residents before they travel. Surveillance summaries provided to health care providers, travel clinics, and travel partners with the geographic distribution of diseases, seasonality, clinical presentation, and control measures may reduce TADs.

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