

The Dinglasan Laboratory is bioengineering mosquito midguts in vitro to characterize molecular interactions between the malaria parasite ookinete stage and the mosquito midgut epithelium. The overarching goal is to use the knowledge gained from this research to develop the next generation of mosquito-based malaria transmission-blocking vaccines. The same approach will be used to engineer an *Aedes aegypti* midgut in vitro to study Zika and dengue virus entry into gut cells. The image depicts a whole-mount female *Anopheles gambiae* mosquito midgut that is stained with anti-actin antibody (green) to identify striated gut muscles and DAPI (blue) for the nucleus.

For more information, visit the virtual home of the Dinglasan Lab at <http://www.dinglasanlab.org>

EPI RESEARCH DAY BOOK OF ABSTRACTS

TURNING THE TIDE AGAINST
DISEASE TRANSMISSION

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Welcome to the tenth annual EPI Research Day! As you look through the abstracts in this book, and view the correlating posters, you should get a feel for the wide range of emerging pathogens-related research conducted by EPI members and collaborators. We are particularly pleased to welcome investigators from outside of UF, 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.

Dr. Jean Pape is the Howard and Carol Holtzmann Professor of Clinical Medicine at Weill Cornell Medical College, and the director of Centres GHESKIO in Port-au-Prince, Haiti. He founded GHESKIO in 1982 as the first institution in the world dedicated to the fight against AIDS. Dr. Pape's work has had significant public health impact, contributing to a 50% decrease in the national infantile mortality rate and a similar decrease in national HIV seroprevalence in Haiti. He is a member of the U.S. Institute of Medicine, and has received numerous international awards, including the French "Legion d'Honneur; in 2014 he was awarded Haiti's highest recognition: "Honneur et Mérite, Grade Commandeur." He is joined by Dr. Mark Eloit, leader of the Pathogen Discovery Laboratory at Institut Pasteur (Paris), and professor of virology at the Veterinary School of Maisons-Alfort. His group has been a leader internationally in work with the viral causes of encephalitis, with a particular focus on agents found in Southeast Asia; his talk will explore approaches to viral identification by untargeted next generation sequencing.

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

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

9:00 AM - 10:00 AM	Registration, Breakfast, and Poster Setup <i>CGRC 1st and 4th floors</i>
10:00 AM - 1:00 PM	Poster Session <i>Presenters, please stand by your posters</i>
12:00 PM - 12:45 PM	Lunch <i>CGRC 1st floor Lobby</i>
12:45 PM - 1:00 PM	Keynote Assembly <i>CGRC Auditorium 101</i>
1:00 PM - 1:10 PM	Welcome <i>Dr. Thomas A. Pearson, Executive VP for Research and Education</i> Introductions <i>Dr. J. Glenn Morris, Director, EPI</i>
1:10 PM - 3:15 PM	Keynote Speeches
3:15 PM - 4:00 PM	Poster Removal

(1:10-2:10)

Dr. Jean William Pape

Professor of Clinical Medicine at Cornell Medical College
Director of GHESKIO Centres in Port-au-Prince, Haiti

**“From Infantile Diarrhea to AIDS
and Global Health in 30 Years”**

(2:10-3:10)

Dr. Marc Eliot

Professor of Virology at the Veterinary School of Maison-
Alfort
Head of the Pathogen Discovery Lab at Institut Pasteur

**“Virus Identification at
Homeostasis and Disease in
Clinical Samples by Untargeted
Next Generation Sequencing”**

01. CHARACTERIZATION OF A SHIGA TOXIN AND THE NON-TOXIN ASSOCIATED CONTRIBUTIONS OF ITS HOST BACTERIOPHAGE ON SHIGELLA FLEXNERI LYSOGENS

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The emergence of Shiga toxin (Stx) production in traditionally non-toxin producing *Shigella flexneri* isolates is cause for concern because Shiga toxin is associated with bloody diarrhea and hemolytic uremic syndrome (HUS), a severe and life-threatening kidney damaging sequela. These symptoms are more commonly associated with infections by bacteria that produce Stx2 toxin, an RNA N-glycosidase that inhibits protein synthesis and ultimately results in host cell death. Our lab has reported the presence and expression of Stx1 in *S. flexneri* clinical isolates. This toxin is antigenically distinct but enzymatically similar to Stx2 and is encoded by genes carried by a bacteriophage, ϕ POC-J13. Here, we demonstrate that Stx1 from ϕ POC-J13 is (i) localized in the periplasm of *S. flexneri*, (ii) localized independently of bacteriophage-encoded products, and (iii) unlike other Shiga toxins, is not secreted or released into the extracellular environment under standard laboratory conditions. Other studies have suggested a role for Stx in survival within host environments. Thus, we hypothesize that Stx1 has an alternate role in *Shigella* virulence aside from its well-described RNA N-glycosidase activity. Additionally, we demonstrate that *S. flexneri* lysogens have a significant increase in acid resistance, an essential feature of *Shigella* pathogenesis within its host and its ability to survive in low-pH environments outside of the host. These results show the importance of considering other consequences of ϕ POC-J13 lysogeny independent of Shiga toxin production.

02. COMPARATIVE GENOME ANALYSIS REVEALS THAT PO157 CONFER PERSISTENCE AND PREDOMINANCE TO ESCHERICHIA COLI O157:H7 IN HOSTS

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Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 is a particular type of *E. coli* causes life-threatening disease such as hemolytic uremic syndrome (HUS) and hemorrhagic colitis. Since STEC O157 primarily colonize in the terminal recto-anal junction (RAJ) of cattle without detectable symptoms, the spread of this pathogen has emerged as a serious problem in food industry. Importantly, persistent strains of STEC O157 found in animals and on farms have been well adapted in their environment and would be responsible for a large portion of O157 outbreaks. In the previous studies, an *E. coli* O157:H7 subtype strain (FRIK2455) was predominantly found on a farm during several years while the other clonal variant (FRIK2533) was not. In this study, we conducted whole genome sequencing using PacBio sequencing and Illumina Miseq technique to identify bacterial genetic factors that may explain the predominance of FRIK2455. Through the comparative analysis of these two genomes, we found that these strains share almost identical whole genome architecture and genetic composition, with a few SNPs on chromosomal DNA. However, FRIK2533 lost a pO157 plasmid, which was previously shown to play critical roles in bacterial adhesion and

colonization. Therefore, our study confirms that pO157 is essential for full pathogenicity of E. coli O157 strains.

03. DNA PHOTO-LABELING WITH PCR-BASED METHOD FOR DIFFERENTIATION OF LIVE AND DEAD E. COLI O157:H7

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The CDC estimates Shiga Toxin-Producing E. coli (STEC) causes over 30 deaths and 265,000 infections each year in the U.S. The most common STEC serotype, E. coli O157:H7, accounts for approximately 36% of those illnesses. In many detection methods, dead cells can result in false-positive results while only viable E. coli O157:H7 cells are responsible for causing illness. DNA photo-labeling is able to neutralize DNA from dead cells while live cells are selectively amplified during PCR. The purpose of this study was to differentiate live and dead E. coli O157:H7 through the development and optimization of a rapid, PCR-based detection method combined with DNA photo-labeling. In this study, the photo-labeling method was optimized to fully neutralize dead cells while allowing amplification of live cells. Both live and dead E. coli O157:H7 (K1266) culture were either treated with or without DNA photo-labeling dye ethidium monoazide (EMA) under the LED light, and analyzed using multiplex PCR (mPCR) and quantitative PCR (qPCR). Results showed that incubating E. coli O157:H7 for 5 min with 25 μ M EMA followed by 5-min high-intensity LED light exposure is optimal to achieve DNA neutralization of dead cells and PCR amplification of live cells. Live and dead cells were successfully differentiated with detection limit of 10^4 CFU/ml in mPCR. However, for qPCR analysis an additional DNA extraction step was needed to remove unbound EMA, which caused significant sensitivity loss with a detection limit of 10^8 CFU/ml. This data suggests that live and dead E. coli O157:H7 cells can be differentiated using DNA photo-labeling combined with PCR.

Despite the success of this method, it does require a relatively high number of cells, therefore requiring an appropriate cell concentration or enrichment for application in food testing.

04. ESCHERICHIA COLI O157 TRANSMISSION AMONG CATTLE IN A CLOSED BEEF FEEDLOT FACILITY: RISK FACTORS AND DYNAMICS

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Background: Cattle are considered the main reservoir for *Escherichia coli* O157, and understanding how *E. coli* O157 is transmitted among cattle is important for the prevention and control of the diseases to ensure food safety. Super shedding events were defined as cattle shedding high concentrations of *E. coli* O157 that can be detected in cattle's fecal samples. Using statistical models, we aim (1) to evaluate the carriage dynamics of *E. coli* O157 among cattle, (2) to assess the association of potential risk factors, such as exposure history and super shedding events, with infection of and recovery from *E. coli* O157, and (3) to detect potential heterogeneity of transmission dynamics among different subtypes of *E. coli* O157 in the setting of closed cattle feedlot.

Methods: A longitudinal study was conducted by following 168 cattle steers for 22 months on a feedlot facility at biweekly intervals in Ohio, U.S. Samples were collected on 12 different days at biweekly intervals, analyzed by the microbial techniques, and were

subsequently analyzed by Multiple Locus Variable-number Tandem Repeat Analysis (MLVA) using standard protocol. Subtypes of A, B and C were derived from the MLVA. Logistic regressions were used to explore determinants for carriage. An individual-based discrete-time Markov-state model was used to analyze the transmission dynamics, which was fitted with the maximum Likelihood methods.

Results: The transmission dynamics models demonstrated that super shedders significantly increased the rate of infections comparing to non-super shedders. The odds of infection by late-stage shedders was 2.89 fold as compared to early-stage shedders. Exposure to super shedders increased the odds of infection by 4.7 times as compared to exposure to non-super shedders. Recovery rates of cattle that were super shedders were 5.36 times higher as compared to the non-super shedders. Cattle that had historical infections were less likely to have super-shedding as compared to naive cattle.

Conclusion: These models showed that super shedding events increased the risk of transmission of *E. coli* O157 but also shortened the carriage period among cattle steers. Our results cast light on future research about super-shedding events of similar pathogens in the human population.

05. EVOLUTIONARY ORIGIN OF SHIGELLA FLEXNERI 2A CARRYING SHIGA TOXIN-CONVERTING TEMPERATE PHAGE IN HISPANIOLA

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The emergence of new pathogens due to horizontal phage transfer has been studied in *Shigella* and other bacterial species. However, the mechanisms of phage transmission in *S. flexneri* have not yet been described. *Shigella* is the causative agent of shigellosis, a severe diarrheal disease. Bacteria of the *Shigella* spp. can acquire virulence genes, such as genes encoding Shiga toxins (*stx*), which contribute to disease. It has been previously reported that patients travelling to the island of Hispaniola (Haiti and the Dominican Republic), were infected with *stx1*-encoding strains of *S. dysenteriae* 4. In 2014 we identified isolates of *S. flexneri* 2 carrying *stx1a* encoded by a lambdoid prophage and each patient who reported recent foreign travel, had visited Hispaniola. Here, we present a study of the evolutionary and epidemiological history of *S. flexneri* 2a strains in patients returning from the Dominican Republic, Haiti, French Guiana, Peru, Mexico and India between 1999 and 2014. We report a comparison of a set of clinical samples of *Shigella* spp. collected within the USA, focusing mainly on samples of *S. flexneri* 2a. Samples were cultured in order to isolate individual bacterial strains. Bacterial genomic DNA was extracted and sequenced using Illumina next generation sequencing technology. Presence of *stx1*-encoding phage

was assessed by PCR. The presence of the phage was confirmed in stx1-positive strains, using the PHASTER algorithm. Two populations of bacterial strains were identified as stx1-positive or stx1-negative, and the latter used as negative controls. Maximum-Likelihood (ML) and neighbor joining (NJ) phylogenies were inferred from genome-wide alignments of homologous genes among *Shigella* species, using bootstrapping to assess classification accuracy and confidence. Phylogenetic analyses revealed that earlier lineages in each cluster were originally stx1-negative, suggesting that at a certain time point stx1-negative strains acquired the phage ϕ POC-J13. Moreover, after the phage introduction, all bacterial strains maintained the ϕ POC-J13 phage, thus indicating a possible advantage from phage acquisition. Both ML and NJ trees indicate bidirectional dissemination of *Shigella* spp. between Haiti and the Dominican Republic, and suggest Hispañiola as one of the possible epicenters of *Shigella* dissemination in the Caribbean area. A full phylodynamic and phylogeography characterization of *Shigella* spp. in Hispañiola may, therefore, prove extremely useful to understand epidemic outbreaks in the area and develop proper intervention strategies.

06. FLRA AND MSHA ACT INVERSELY ON VPS INDEPENDENT BIOFILM PRODUCTION BY VIBRIO CHOLERAЕ IN NUTRIENT POOR LAKE WATER MICROCOSM

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Biofilms, produced by a variety of pathogenic bacteria, can promote the microorganisms' ability to confer resistance against numerous stressful survival conditions. *Vibrio cholerae* O1, the causative agent for epidemic cholera, is known to form vibrio polysaccharide (VPS) mediated biofilm. Using quantitative biofilm assay and confocal microscopic analysis, we report here that *V. cholerae* can also produce VPS independent (VPS-Ind) biofilm. Interestingly, biofilm production increases when the *flrA* gene, responsible for the motility of the bacteria, is deleted. In contrast, it is positively regulated by *mshA*. The VPS-Ind biofilm is specifically formed in nutrient-poor lake water microcosms and not in nutrient rich L-broth. Furthermore, unlike VPS dependent biofilm (rugose biofilm), VPS-Ind biofilm is required for the active uptake of nutrients, including chitin, phosphate, ammonium nitrate and sucrose. In addition to VPS-Ind biofilm, we also observed that major chemotaxis genes, including *cheA2* and *cheY3* are required for uptake of these nutrients when supplemented in filtered sterilized lake water microcosms. We hypothesize that VPS-Ind biofilm plays a critical role in the persistence and acquisition of nutrients when *V. cholerae* persists in nutrient-poor aquatic reservoirs.

07. FROM CHICKENS TO CAMPYLOBACTER, HOW ENVIRONMENTAL ENTERIC DYSFUNCTION IS AFFECTING A GENERATION IN ETHIOPIA

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Although stunting rates have decreased substantially over the past 25 years in Ethiopia, they are still amongst the highest in the world at 40% in children under the age of 2. With stunting representing a more chronic form of undernutrition, it represents a general failure of the individual to reach full genetic potential for optimal health, both in physical health and cognitive ability. Until recently, the dominating theory has been that diarrheal disease and poor WASH conditions (specifically human feces) were general risk factors for undernutrition. However, recent reports out of Ethiopia have contradicted this by stating that ingestion of chicken excreta may not only be a risk factor for diarrheal disease but also for environmental enteric dysfunction (EED), which is damage to the intestine in the form of a “sub-clinical” inflammatory response that occurs in the small intestine. This impairs absorption of nutrients which leads to undernutrition and stunting. The MAL-ED study is a large cohort study performed in eight sites in South America, sub-Saharan Africa and Asia which looked to better understand the complex inter-relationship between enteric infections and malnutrition. They found high rates of stunting in several sites with *Campylobacter* (associated with chicken excreta) having the most impact on growth of children. *Campylobacter* was also the most frequently isolated pathogen. Ethiopia has developed a Livestock Master Plan that includes increasing poultry production at the household level by 250% by 2020. Therefore, this project includes a scoping trip to Ethiopia including visits to key stakeholders and an overall assessment of current poultry production systems and the food safety systems in place in Ethiopia. Plans for future interventions in Ethiopia are developed, including the introduction of a complete package to convert the traditional scavenging poultry system to an improved

family poultry system which provides training and education to the smallholder farmer, provisions for poultry housing to minimize contact with poultry excreta, and livestock/food safety training. Stool samples from children under the age of 2, chicken excreta and environmental samples will be cultured for *Campylobacter* spp., which will then undergo metagenomic analysis to obtain a better understanding of the relationship between the microbiomes of both the children and the animals. The ultimate goal of this project is to reduce exposure to *Campylobacter* spp., and thus improve stunting rates in Ethiopia.

08. HAZARD ASSESSMENT OF GLOBAL PRODUCE CHAINS 2010 – 2015: US, EU, AND OTHER REGIONS

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Understanding biological hazards associated with produce commodities is of greatest concern from a global perspective and can inform risk management activities to protect public health and enhance international trade. This study aimed to identify biological hazards and associated products in the global fresh produce chain. Produce-related outbreaks, illnesses, and recall data were collected from currently available annual reports and databases of foodborne outbreak surveillance systems in different regions and food recall programs from 2010 to 2015. The global pattern and regional differences of documented foodborne outbreaks and recalls were analyzed for different fresh produce groups, pathogens, and food-pathogen pairs. There was a total of 956 outbreaks and 42,924 cases in all regions, including the US, EU, Japan, Australia, and New

Zealand. The US and EU together contributed to over 80% of outbreaks and over 90% of cases. Many produce-related outbreaks were attributed to unspecified or multiple food groups. The top three food categories contributing to most produce-related outbreaks were “vegetables & non-fruits” (221 outbreaks, 23.1%), “unspecified vegetables” (154 outbreaks, 16.1%), and “mixed foods” (144 outbreaks, 15.1%). The top three pathogens causing the most produce-related outbreaks were norovirus (396 outbreaks, 41.4%), Salmonella (186 outbreaks, 19.4%), and Staphylococcus aureus (79 outbreaks, 8.2%). Some produce outbreaks were dominated by certain pathogens, such as Shiga toxin-producing Escherichia coli in “vegetable row crops” outbreaks and norovirus in “small” fruits outbreaks; and some produce categories were commonly associated with certain pathogen outbreaks, such as “seeded vegetables” and “sprouts” for Salmonella outbreaks. There were 466 recalls/notifications in the US, Canada, Europe, Australia, and New Zealand between 2010 and 2015. The top pathogens leading to recalls were Salmonella (49%), Listeria monocytogenes (20%), and generic E. coli (11%), and the top food categories were herbs (35%), vegetable row crops (14%), sprouts (10%), and small fruits (7%). The complexity of foods consumed across the globe and the difficulty in tracing illnesses back to specific food ingredients are highlighted, as are important food-pathogen pairs that need further efforts to prevent outbreaks and recalls. This study can be used to inform future risk assessment studies on important food-pathogen pairs and support risk management activities for fresh produce.

09. IMPACT OF WANING ACQUIRED IMMUNITY AND ASYMPTOMATIC INFECTIONS ON CASE-CONTROL STUDIES FOR ENTERIC PATHOGENS

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Case-control studies of outbreaks and of sporadic cases of infectious diseases may provide a biased estimate of the infection rate ratio, due to selecting controls that are not at risk of disease. We use a dynamic mathematical model to explore biases introduced in results drawn from case-control studies of enteric pathogens by waning and boosting of immunity, and by asymptomatic infections, using *Campylobacter jejuni* as an example. Individuals in the population are either susceptible (at risk of infection and disease), fully protected (not at risk of either) or partially protected (at risk of infection but not of disease). The force of infection is a function of the exposure frequency and the exposure dose. We show that the observed disease odds ratios are indeed strongly biased towards the null, i.e. much lower than the infection rate ratio, and furthermore even not proportional to it. The bias could theoretically be controlled by sampling controls only from the reservoir of susceptible individuals. The population at risk is in a dynamic equilibrium, and cannot be identified as those who are not and have never experienced disease. Individual-level samples to measure protective immunity would be required, complicating the design, cost and execution of case-control studies.

10. ISOLATION AND CHARACTERIZATION OF EXTENDED-SPECTRUM BETA-LACTAMASE PRODUCING ESCHERICHIA COLI FROM BEEF CATTLE FARMS

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The rapid dissemination of Extended-spectrum β -lactamase (ESBL)-producing *E. coli* (ESBL-*E. coli*) has threatened public health all over the world. The emergence of ESBL-*E. coli* from food-producing animals is especially a critical concern because the animals could contribute to the burden of ESBLs and ESBL-*E. coli* limits antibiotic usage as the treatment of bacterial infection. Understanding of ESBL-*E. coli* occurrence from farm animals is essential to mitigate the global transmission of ESBL-*E. coli*. However, sufficient information is not available regarding the prevalence of ESBLs on beef cow/calf operations. In this study, we examined the prevalence of ESBL-producing Enterobacteriaceae (ESBL-E) and ESBL-*E. coli* on 17 commercial beef farms located in Florida to understand the spread of ESBLs among food animals in the farms. Furthermore, the characteristics of ESBLs isolated from animals were analyzed using polymerase chain reaction (PCR) to screen ESBL genes, minimum inhibitory concentration (MIC), and antibiotic susceptibility test. 1,096 samples were collected from the 17 farms including feces of calves and cows, soil, water, and forage. ESBL-E and ESBL-*E. coli* were isolated by plating on MacConkey agar containing cefotaxime (4 $\mu\text{g/mL}$) and ChromAgar *E. coli* media. All of the farms had ESBL-E indicating the prevalence between 13.15% and 63.63%. In case of ESBL-*E. coli*, 65% (11/17) of beef farms had ESBL-*E. coli* and the overall prevalence was 7.42%. Total 61 ESBL-*E. coli* were isolated and all of the isolated ESBL-*E. coli* harbored CMY, TEM, or CTX-M genes. The most predominant ESBL gene was CTX-M. Moreover, they

showed a MIC of cefotaxime ≥ 16 $\mu\text{g/mL}$ and multidrug resistance, showing the highly virulent characteristics. Our results suggest that commercial beef farms have ESBL-E. coli even though antibiotics were not extensively used for prophylactic purpose and the isolates from food-producing animals could be a potential life-threatening factor. Therefore, our data can provide critical knowledge to better understand the prevalence and characteristics of ESBLs on beef operations.

11. MAJOR SHIFT OF TOXIGENIC V. CHOLERAЕ O1 FROM OGAWA TO INABA SEROTYPE ISOLATED FROM CLINICAL AND ENVIRONMENTAL SAMPLES IN HAITI

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In October of 2010, an outbreak of cholera was confirmed in Haiti for the first time in more than a century. A single clone of toxigenic *Vibrio cholerae* O1 biotype El Tor serotype Ogawa strain was implicated as the cause. Five years after the onset of cholera, in October, 2015, we have discovered a major switch (ranging from 7 to 100%) from Ogawa serotype to Inaba serotype. Furthermore, using wbeT gene sequencing and comparative sequence analysis, we now demonstrate that, among 2013 and 2015 Inaba isolates, the wbeT gene, responsible for switching Ogawa to Inaba serotype, sustained a unique nucleotide mutation not found in isolates obtained from Haiti

in 2012. Moreover, we show that, environmental Inaba isolates collected in 2015 have the identical mutations found in the 2015 clinical isolates. Our data indicate that toxigenic *V. cholerae* O1 serotype Ogawa can rapidly change its serotype to Inaba, and has the potential to cause disease in individuals who have acquired immunity against Ogawa serotype. Our findings highlight the importance of monitoring of toxigenic *V. cholerae* O1 and cholera in countries with established endemic disease.

12. MICROBIOLOGICAL SURVEY OF INDICATOR AND PATHOGENIC BACTERIA ON FRESH PRODUCE SOLD AT FLORIDA FARMERS' MARKETS

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The number of farmers' markets in the U.S. has gone up with the consumer demand for locally grown fresh produce. The increase in farmers' markets highlights the importance of food safety at these markets. The purpose of this study was to assess the microbial safety of fresh produce from Florida farmers' markets. One hundred and ninety-nine produce samples were collected from 9 farmers' markets in North and Central Florida from July 2016 to January 2017. Of the commodities analyzed, there were 72 tomatoes, 61 leafy greens, 36 berries, and 30 spinach samples. Samples were tested for total coliforms and generic *E. coli* using 3M Petrifilm EC plates. The presence of *L. monocytogenes*, *Salmonella*, and *E. coli* O157:H7 were tested for with real time PCR assay kits. Coliforms were present in lettuce, spinach, tomatoes, and berries at a rate of 84.6%, 85%, 65.7%, and 20%, respectively. Spinach had the highest coliform presence, with a median concentration of 3.90 log CFU/g. One tomato and one spinach sample were positive for generic *E. coli*, with a prevalence of 1.15%. Of 199 samples, 1% (n=2) were positive for *L. monocytogenes*. No *Salmonella* and *E. coli* O157:H7 were detected. The data from this study provides useful information on

the safety and quality of produce from Florida farmers' markets and may aid in the development of food safety programs.

13. PREVALENCE AND CHARACTERIZATION OF EXTENDED-SPECTRUM B-LACTAMASE-PRODUCING ESCHERICHIA COLI ISOLATED FROM MECONIUM OF NEWBORN CALVES

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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*. In this study, meconium samples were collected from the rectal anal junction of 322 newborn calves within 24 hours after their births. ESBL-producing *E. coli* were identified from the samples by plating on MacConkey agar supplemented with Cefotaxime (4 μ g/mL). Isolates were further characterized with ChromAgar *E. coli* and CTX-M gene amplification using PCR. ESBL-producing *E. coli* was detected in 7.5% (24/322) of meconium samples of newborn calves. Illumina MiSeq was employed for Whole Genome Sequencing (WGS) of 37 strains from 24 calves. After assembly using SPAdes 2.0, 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 groups that coincided with their STs. All the isolates carried a variety of virulence genes and were resistant to multiple antibiotics, suggesting that these strains may threaten public health if they contaminate food products. In particular, we identified hyper-virulent strains of ST117 that harbored Shiga toxin-encoding genes (stxAB), which may cause severe human diseases. This was the first study that accessed the prevalence and characterization of ESBL-producing *E. coli* in meconium of newborn calves, indicating animals are even start to be exposed to AMR bacteria in the uterus.

14. SECRETOMIC ANALYSIS OF MACROPHAGES INFECTED WITH SALMONELLA ENTERICA REVEALS PRESENCE OF EXTRACELLULAR VESICLES DURING THE EARLY STAGES OF INFECTION

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The extracellular proteins of cells play significant roles in many physiological processes. This study focuses on canonically and non-canonically secreted proteins of macrophages as well as specific role of extracellular vesicles (EVs) in infection. EVs are nano-sized vesicles and include exosomes, microvesicles and apoptotic bodies. These EVs are secreted by cells to transport various components including proteins, which aid as messengers in response to changes in the environment allowing intracellular communication and stimulation of an immunological response in the presence of a pathogen. *Salmonella enterica* serovar Typhimurium is a Gram-negative intracellular pathogen which invades the human macrophages and has been well known to promote the secretion of pro-inflammatory

cytokines along with disrupting the host secretory pathway. We provide a proteomic analysis of the extracellular proteome of human macrophages infected with Salmonella. We also provide evidence of CD63- and CD9- positive exosome-like vesicles, which are produced at an early stage of infection. These vesicles contain specific protein cargo and have an ability to induce pro-inflammatory cytokines in uninfected cells.

15. SENSITIVITY STUDY OF LIQUID CRYSTAL-BASED DETECTION ASSAY FOR ESCHERICHIA COLI O157:H7 IN ALFALFA SPROUT AND COOKIE DOUGH

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Escherichia coli O157:H7 is the predominant serotype of Shiga toxin-producing E. coli (STEC), causing human infection and death in the United States. To identify E. coli O157:H7 in foods, rapid detection assays have been developed as alternatives to traditional culture method. Liquid crystal-based immunoassay (LCIA) is a novel assay using immunomagnetic separation and liquid crystal-based biosensor to detect foodborne pathogens. Without enzyme- or dye-labeled secondary antibody involved, LCIA was able to detect E. coli O157:H7 with reduced assay time (total assay time of 1.5 hrs) than traditional sandwich ELISA. The goal of this study is to evaluate the sensitivities of LCIA in detecting E. coli O157:H7 in various foods. When E. coli O157:H7 is present, E. coli O157:H7-immunomagnetic bead (IMB) forms aggregates and distorts liquid crystal matrix, which was detected using Crystal Diagnostics Xpress system to confirm the presence of pathogen. In this study, alfalfa sprouts and commercial cookie dough were inoculated with 100 to 10⁵ CFU/100g and 100 to 10⁵ CFU/20g of E. coli O157:H7, respectively. Samples were collected for analysis after being enriched at 37°C for 0 h, 3 h, 6 h, 12 h, and 18

h. Non-O157 STEC and common foodborne pathogens were also tested for specificity of the LCIA.

In this study, LCIA was able to detect *E. coli* O157:H7 in alfalfa sprout and cookie dough samples with detection limits of 105 CFU/ sample without enrichment. With 6-hr enrichment, LCIA was able to detect *E. coli* O157:H7 as low as 1 CFU/100 g for alfalfa sprouts. LCIA detected 1 CFU/20g of *E. coli* O157:H7 in cookie dough with 9-hr enrichment. The developed assay was highly specific to *E. coli* O157:H7 and did not show any cross-reactivity with non-O157 STEC or other common foodborne pathogens. This study is the first to compare the sensitivity of LCIA in detecting *E. coli* O157:H7 in food samples. This novel immunoassay shows a great potential as a rapid and sensitive detection method for *E. coli* O157:H7 in various foods including complex matrices with high fat and high sugar.

16. SPATIAL ANALYSIS OF CHOLERA DATA IN FAR NORTH CAMEROON

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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 2010-2011 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 elevation came from WorldClim and ArcGIS Online databases respectively. Railways, water bodies, waterways, and road data came from the USGS database. 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. Data were spatialized, and buffer regions of 0.8 km were created for water bodies, waterways, roads, and railways in relation to village-level data. Service area network analyses were then performed.

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. A visual inspection of the graph of cases by eco-zone showed that spikes in case counts occurred during or near times of increased rainfall. Water body and waterway buffers of 0.8 km did not appear in the mountain eco-zone, though bodies of water existed nearby. We will further examine the association between cases and precipitation within each eco-zone by adjusting for transportation, population density, and human mobility, and discuss research needs for the next step.

17. SPATIAL ANALYSIS OF CHOLERA DATA IN FAR NORTH CAMEROON

Sonal Sathe - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Mingjin Liu** - Department of Biostatistics, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Yang Yang** - Department of Biostatistics, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Song Liang** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

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18. SURVIVAL STUDY OF SALMONELLA AND E. COLI O157:H7 ISOLATES FROM DIFFERENT ORIGINS IN COOKIE DOUGH DURING STORAGE

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Since the multistate *Escherichia coli* O157:H7 outbreak in 2009 associated with commercial pre-packaged cookie dough, cookie dough product is recognized as a novel vehicle for transmission of pathogens including *E. coli* O157:H7 and *Salmonella*. Considering that cookie dough has been associated with pathogens and consumers' risky behavior of consuming raw cookie dough have been frequently reported, it would be beneficial to understand pathogens' survival in cookie dough during storage, which has not been fully studied. In this study, we compared the survival of *Salmonella* and *E. coli* O157:H7 isolates from different origins in commercial cookie dough. Cookie dough samples were separately inoculated with approximately $6.0 \log_{10}$ CFU/g of *Salmonella*

isolated from peanut outbreak, egg container, and clinical samples, and E. coli O157:H7 isolated from cookie dough outbreak, salami, and environmental samples. Viable cell population of each isolate was obtained during 8 weeks of storage at 4°C. During 8 weeks of storage, reductions of Salmonella and E. coli O157:H7 in cookie dough were achieved ranged from 0.84 to 1.30 log and 0.48 to 0.87 log CFU/g, respectively. Notably, Salmonella Tennessee isolate from peanut outbreak had much lower viable cell count than S. Enteritidis isolates from eggs and clinical samples (P-value <0.05). Also, E. coli O157:H7 isolate from the cookie dough outbreak had a significantly lower number of viable cells than other E. coli O157:H7 isolates from salami and environmental samples (P-value <0.05). Our data suggests that Salmonella and E. coli O157:H7, once introduced, can remain viable in cookie dough for at least two months. The survival of pathogens is affected by the origins of the isolates. Understanding the factors contributing to such phenomenon will be essential for the control of these pathogens in cookie dough.

19. THE ROLE OF PGE2 IN MACROPHAGE SURVIVAL UPON SALMONELLA INFECTION

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Cellular metabolites are crucial in regulation of the host immune responses. As an example, prostaglandins are lipid mediators produced by cyclooxygenases with various functions in inflammatory processes. One such prostaglandin, PGE2, can promote or suppress inflammation depending on the type of receptor it binds and stimulates. We found that PGE2 secretion was enhanced upon infection of human macrophages with Salmonella in what appears to be a COX-2 dependent manner based on gene expression analyses

and targeted metabolomic analysis. Pre-treatment of human macrophages with PGE2 significantly increased IL-1B secretion and cell survival upon infection, which also led to changes in cell conformation as determined by confocal microscopy. Trials with combinations of EP2/EP4 agonists and antagonists revealed that this signaling works primarily through the EP4 receptor as inhibition of EP4 reduces secretion of IL-1B to the level comparable with the uninfected control. In summary, Salmonella activates COX-2 pathway upstream to the PGE2 mediated IL-1B production, thereby enhancing inflammation and leading to enhanced survival of human macrophages.

20. ADDRESSING BARRIERS TO INFLUENZA IMMUNIZATION AMONG COLLEGE STUDENTS - A CROSS SECTIONAL SURVEY

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Background: Influenza vaccination rates for college age students are known to be very low. Increasing vaccination rates will require an understanding of the barriers to immunization and motivations in relation to influenza vaccination in this age group.

Objective: Identify barriers to immunization among college age students using an on-line anonymous cross sectional survey tool.

Design/Methods: A cross sectional survey was designed and approved by the University of Florida (UF) Institutional review Board and was distributed via University colleges to their undergraduate student listserve via REDcap (Research Electronic Data Capture) - a secure online research tool from January 2016-March 2016.

Results: Surveys were distributed to a diverse range of colleges across UF. The demographics of the respondents matched the overall demographics at UF except the majority of respondents were

female. Respondents were primarily from the colleges of Agricultural and Life Sciences, Public Health or Engineering. 1105 responses were received. 85% of students had received a flu vaccine in the past. For the 2015/16 influenza season, 59% reported receiving flu shot, 4.4% FluMist, 7% were not vaccinated but intended to and 30% did not plan to be vaccinated. The top 4 barriers to immunization were 1) it's never been a priority for me 2) I do not believe flu vaccines are effective 3) I am concerned about adverse reactions 4) I do not believe flu is severe enough for me to need a vaccine. A majority of students surveyed had a preference for IIV with prior use of IIV and lack of knowledge regarding LAIV noted for the preference. Lack of routine vaccination growing up predicted lower influenza vaccine uptake in college. Students primarily preferred to learn more about influenza vaccines from their healthcare providers as compared to online resources or on-campus health education campaigns.

Conclusion(s): Barriers to immunization in the college age student are similar to those that have been identified in the adult population and likely reflect the attitudes and beliefs of their parents. Lack of routine vaccination growing up predicted lower influenza vaccine uptake in college. The majority of students in this survey had received IIV and had a preference for IIV over LAIV. Lack of knowledge about LAIV and having received the flu shot in the past influenced this decision. Educational efforts to increase college student vaccination rates will need to primarily utilize medical providers as resources for information.

21. ASSESSING THE ROLE OF BENZATHINE PENICILLIN G PROPHYLAXIS IN PREVENTING ALL-CAUSE ACUTE RESPIRATORY DISEASE IN US MILITARY RECRUITS: A MACHINE LEARNING APPROACH

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Background: Acute respiratory disease (ARD) is a national security threat as it is responsible for approximately 27,000 missed basic combat training (BCT) days annually among US military recruits. Since the 1950s, the military has used benzathine penicillin G (BPG) prophylaxis to prevent streptococcal infections in recruits at BCT, which costs over \$6M per year. After a 12-year manufacturing freeze, the adenovirus vaccine was re-introduced in 2011, resulting in drastic decreases of ARD. Here, in light of the shift in ARD dynamics resulting from the adenovirus vaccine, we evaluated the effectiveness of BPG prophylaxis at preventing ARD in military recruits.

Methods: We fit a random forest model to 25 years of weekly ARD case counts from recruits in BCT, controlling for availability of the adenovirus vaccine, BPG availability, BCT site, population size at the BCT site, month and year. Percent increased mean squared error (MSE) was used to rank variables in terms of how important they are at predicting ARD. The MSE of a variable is a measure of how much worse the model would perform if the variable were removed. K-fold cross validation was used to ensure that the model was not over-fit.

Results: Our model explained 85.4% of the variance in the data. Adenovirus vaccine availability, the months of January and February, and population size at the BCT site were the four most important variables, and removing them from the model would have resulted

in 90%, 68%, 65% and 59% increase in MSE, respectively. BPG was among the least important variables with a <1% increase in MSE.

Conclusions: BPG is not an important predictor of ARD, suggesting that it has a minor role in ARD control. We recommend a clinical trial to empirically estimate the effect of BPG on ARD before eliminating BPG from recruits' prophylaxis regimen.

22. COLLECTION OF AIRBORNE INFLUENZA VIRUS IN A STUDENT HEALTH CARE CENTER THROUGH WATER-BASED CONDENSATION GROWTH

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Transmission of influenza virus between humans mainly occurs through three routes: direct or indirect contact, large droplet spray, and aerosol. The relative importance of the aerosol route remains contentious since there is limited direct evidence of infection mediated by virus-containing aerosols. One of the reasons for this lack of evidence is the challenge in collecting aerosols of infectious influenza viruses. In this study, ambient virus aerosol particles were collected by a newly developed Viable Virus Aerosol Sampler (VIVAS) and by an SKC BioSampler at a student health center during the 2015-2016 flu season. The VIVAS works by particle size-amplification

through water vapor condensation and gentle deposition of the enlarged particles onto liquid collection media. In contrast, the BioSampler is an impinger that deposits collected particles onto collection media in a more aggressive swirling motion. Our prior study with lab-generated influenza H1N1virus showed much higher efficiency for viable collection with the VIVAS. In the student health study reported here, both samplers were operated in parallel under the same parameters. Efforts were made to isolate viable human respiratory viruses by inoculation of the collected material onto a variety of indicator cell lines: A549, LLC-MK2, MDCK, MRC-5, Vero E6. Whereas viable viruses were collected by both samplers, virus-induced cytopathic effects were observed first in cells inoculated with material collected using the VIVAS before those collected by the Biosampler, suggesting a higher concentration of viable viruses therein compared to material collected using the BioSampler. The efficacy of the VIVAS at the collection of airborne viruses and maintaining the viability of the collected viruses, is superior to that of the BioSampler, as infectious influenza viruses and Human Respiratory syncytial virus (RSV) were present in all the collection media of the VIVAS, and some but not all of the BioSampler. Results of this study also showed that different human respiratory viruses could be in the breathing air of a health care facility; these viruses included influenza A H1N1, influenza A H3N2, influenza B virus, RSV, Adenovirus C (Type 5), HPIV type 3, HPIV-4a, HPIV-2, HCoV-229E, and HCoV-NL63. After genomic sequencing to confirm identities, the isolated influenza viruses are consistent with the known outbreak pattern of influenza viruses in Florida during the test periods. All these suggest that the VIVAS is a promising device for sampling viable influenza virus in healthcare facilities.

23. DETECTION OF ALPHACORONAVIRUS VRNA IN THE FECES OF BRAZILIAN FREE-TAILED BATS (TADARIDA BRASILIENSIS) FROM A COLONY IN FLORIDA, USA

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Background: Bats are natural reservoirs of corona viruses (CoV) and other viruses with zoonotic potential. After it was found that SARS-CoV probably originated in bats, a flurry of investigations uncovered many more novel bat CoVs. The recent description of a bat CoV related to MERS-CoV in Mexican bats emphasized the relevance of investigating neotropical bats for CoVs. Brazilian free-tailed bats (*Tadarida brasiliensis*), also known as the Mexican free-tailed bats, roost in colonies in natural and artificial structures in Florida. Unlike

their counterparts in Brazil and Mexico, the viruses harbored by the Florida bats are underexplored.

Objective: To determine whether CoV vRNA could be detected in the feces of Brazilian free-tailed bats in Florida.

Study: Nineteen (n=19) fecal samples were collected from capture/release bats in Florida in May, 2016. Filtered homogenates prepared from the fecal samples were inoculated onto a variety of cell cultures for virus isolation. Viral nucleic acids extracted from the filtered homogenates and cell culture spent media were screened for CoV RNA by RT-PCR for the amplification of a conserved 440-bp CoV RNA dependent RNA polymerase (RDRP) gene sequence. Amplicons were then sequenced for confirmation and phylogenetic analyses.

Results: Virus-specific cytopathic effects were not observed in inoculated cells during a four-week observation period. A CoV-specific 440-bp amplicon was generated from 2/19 bat fecal samples. The sequence for both amplicons was identical and submitted to GenBank (Accession: KX663833.1). Phylogenetic analyses suggest that the RDRP gene sequence from these bats clusters with bat CoV sequences from Brazil. Most of the clades show the RDRP sequence clustering by bat species, indicating that the virus evolves according to species. Yet the clade with our sequence of interest from Florida clusters with the Brazilian sequences, which contain multiple different species (*T. brasiliensis* and *Molossus molossus*) of bats.

Conclusions: We surmise that this and highly related alphacoronavirus are carried by Brazilian free-tailed bats living in a wide eco-spatial region. As various CoVs that affect humans emerged from bats, our study raises the question whether CoV such as the one detected in our work are yet to be detected pathogens of humans and animals other than bats. Although restricted in sample number, location and single bat species investigated, our study suggests that surveillance and identification of coronavirus in Florida bats is worthy.

24. EFFICACY OF OSELTAMIVIR AND ZANAMIVIR COMBINATION CHEMOTHERAPY FOR WILD-TYPE AND DRUG-RESISTANT MUTANT INFLUENZA A VIRUSES IN THE HOLLOW FIBER INFECTION MODEL (HFIM) SYSTEM.

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The global emergence of resistant influenza highlights the pressing need for alternative dosing strategies with clinically available antivirals. One potential dosing strategy is to use two or more antiviral agents to treat influenza infections to prevent viral replication and inhibit the selection of drug-resistant viruses.

Our aims were to evaluate the efficacy of two neuraminidase inhibitors, oseltamivir and zanamivir, in mono- and combination therapy against wild-type and drug-resistant influenza A viruses. We performed pharmacodynamic studies in the HFIM system with oseltamivir (75 mg Q12h, $t_{1/2}$: 8 h) and zanamivir (600 mg Q12h, $t_{1/2}$: 2.5 h), given as mono- or combination therapy, against wild-type pandemic 2009 H1N1 A/Mexico, oseltamivir-resistant pandemic 2009 H1N1 A/Hong Kong, and zanamivir-resistant seasonal 2008 H1N1 A/Brazil influenza viruses. Additionally, dosage regimens were evaluated against two different mixed virus infections. The first mixed virus infection consisted of 99% wild-type A/Mexico and 1% OS-resistant A/Hong Kong. The second mixed infection represented the worst-case infection scenario and was comprised of 50% of OS-resistant A/Hong Kong and 50% of ZAN-resistant A/Brazil strains. Combination therapy was effective against all influenza viruses tested, including mixed infection models, suppressing viral growth to levels similar to those reported for the most effective monotherapy regimen. Moreover, our results showed that the combination therapy prevented the amplification of drug-resistant mutants in

both the 99/1% Mexico and Hong Kong mixture and the worst-case infection scenario (50/50% mixed virus infection with OS- and ZAN-resistant viruses). Our findings indicate that oseltamivir-zanamivir combination therapy does not provide enhanced viral suppression relative to the most effective treatment arm, but is effective at preventing the emergence of resistant viruses. Therefore, we conclude that oseltamivir-zanamivir combination therapy is a potential therapeutic option for complicated influenza A virus infections.

25. IMPACTS OF CARBON NANOTUBES ON LUNG CELL LIPIDOME AND HOST IMMUNE RESPONSES FOLLOWING INFECTION WITH PANDEMIC INFLUENZA A VIRUS

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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 (> \$132 billion in 2005 in the United States). 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 consumer and industrial products and in biomedical applications, 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, with a particular focus on lipid composition and associated gene expression, including IFITs (interferon-induced proteins with tetratricopeptide repeats) and IFITMs (interferon-induced transmembrane proteins). We hypothesized that SWCNTs are changing normal cellular lipid profiles, which contribute to a repressed immune response and increased viral titer. For our approach, we exposed SAECs to 20 µg/mL SWCNTs for 24 h. Following exposure, we extracted cell lipids and performed global lipidomics using a mass spectrometry approach and analysis using LipidView and MarkerView platforms. We additionally quantified the mRNA expression of IFIT and IFITM genes via quantitative realtime PCR (qRT-PCR). Data from the lipidomic analysis revealed that SWCNTs did not result in large shifts in overall lipid classes, but did cause changes in specific lipid species. Gene expression analysis showed that SWCNTs did not alter the expression of IFITs and IFITMs alone, but did repress IAV-induced expression of several IFIT and IFITM family members.

These results suggest that SWCNTs impact lipid composition of SAEC and impair innate immune mechanisms that normally protect against viral infections. These studies highlight the importance of assessing health endpoints that include pathogen susceptibility when evaluating the environmental health and safety of nanomaterials.

26. ISOLATION AND IDENTIFICATION OF HUMAN CORONAVIRUS 229E (HCOV-229E) FROM FREQUENTLY TOUCHED ENVIRONMENTAL SURFACES IN A CLASSROOM

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Background: Environmental surfaces can contain pathogens from different sources, including respiratory secretions. One route of infection by respiratory viruses is contact transmission, which occurs when virus-containing fomites are indirectly transferred to mucous membranes of the upper respiratory tract.

Objective: To determine whether viable respiratory viruses could be isolated from high contact surfaces of a classroom over several days during the start of “influenza season”, when students were observed coughing and sneezing during classroom sessions.

Study: Swab samples from frequently touched surfaces in a classroom were collected in November 2016 using a commercial collection and transport system. Replicate sets of subconfluent A549, HeLa, LLC-MK2, MDCK (NBL-2), MRC-5, LLC-MK2, and Vero E6 cells were inoculated with aliquots of the collected material and one set incubated at 33°C, the other at 37°C. The inoculated cells were then observed daily for virus-specific cytopathic effects (CPE). Viral nucleic acids were extracted from the cell lysate and virions in spent culture media of cells displaying CPE, and virus identity attempted using a GenMark multiplex PCR eSensor XT-8 Respiratory Viral Panel.

Results: Some inoculated cultures from samples collected over a 5-day period displayed cell rounding, followed by clumping and detachment of the cells within 3-11 days post infection. The CPE first appeared in cells incubated at 33°C, then later at 37°C. Viral nucleic acids extracted from cell lysates and virions in spent culture media

from the infected cells were identified as Human coronavirus 229E by the GenMark eSensor system.

Conclusion: Human coronavirus 229E, which causes upper respiratory tract infections and more serious disease in patients with comorbidities, is thought to be spread by respiratory droplets and fomites. Our study shows that this virus remains infectious on environmental surfaces in a classroom setting, supporting the notion that contact transmission may be an important route of infection for the virus.

27. NO FLUMIST - THE IMPACT ON CONTROL FLU - ALACHUA COUNTY'S SCHOOL-LOCATED INFLUENZA VACCINATION PROGRAM

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Background: School- located influenza vaccination (SLIV) programs are known to improve influenza vaccination rates and lessen the impact of seasonal influenza. Control Flu, Alachua County, Florida's SLIV program has offered live attenuated influenza vaccine (LAIV, Flumist*) at no cost to all pre K – 12 grade students in public, private and charter schools since 2009.

Objective: Assess the impact of the change from administering only FluMist to only Flu shot on the Control Flu Program

Design/Methods: Vaccination data was collected for the 2106/17 season and directly compared to preceding seasons.

Results: In 2015-2016, 13,727 doses of Flumist were administered through the program resulting in a 41% overall student vaccination rate (49% elementary, 44% middle and 31% high). Without Flumist

for the 2016-2017 season, inactivated influenza vaccine (IIV, Flu shot) was offered. This resulted in a 41% decrease in overall vaccination rate in public schools. The biggest decline (51%) was seen in elementary schools due to the local requirement for a parent to be present for the child to be vaccinated. Seven private/charter schools chose not to participate due to shots. Of those participating there was a 24% decline in vaccination rate. There was no requirement for parents to be present at these schools. There was a 51% decline in vaccination rate for publicly insured children as compared to a 29.75% decline in privately insured children. Students accepted the shot better than expected. They were more anxious entering the vaccination clinic but relieved and happy it was not as bad as anticipated exiting the clinic. Distraction techniques used by vaccinators were crucial to the success of shots at all ages. Student behavior and vaccine acceptance was not improved with parents present. Cost per dose was higher due to the need for purchasing needles, alcohol wipes, sharps containers and gloves.

Conclusions: The change from LAIV to IIV resulted in a significant decline in the Control Flu vaccination rate. The decline was greatest for elementary students partly due to a local requirement for parents to be present for younger students. There was a greater negative impact on vaccination rate for publicly insured children than for privately insured children. Although school administrators and nurses were anxious about administering flu shots in schools, the vaccine clinics went more smoothly than expected. We would anticipate an improved uptake of flu shots and vaccination rates if only flu shots will be available for next season.

28. PAPER-BASED MICROFLUIDIC DEVICES FOR DETECTING RNA FROM FLU VIRUS

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Introduction: Point-of-care (POC) tests can deliver quick results directly at the site of analysis. Also, they can be carried out by personnel without laboratory medical training. These advantages make POC tests appropriate for preventing and controlling the epidemic outbreak of infectious diseases. Conventional methods to detect pathogenic bacteria and viruses, including plaque culture, enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR), are generally not practical for POC test. A miniaturized analytical device, however, offers many advantages over the conventional methods. Tests performed by micro-devices include fewer and simpler manual steps, thus are accessible to unexperienced medical personnel and even patients themselves. Paper-based analytical devices (PADs) do not need a pump to operate, require small sample volume and are intrinsically cheap. Our research groups have developed laminated paper-based analytical device (LPAD) for detection of proteins, glucose and cotinine in synthetic urine samples. We have been working on a POC device for the detection of low concentration of nucleic acid from viruses.

Materials and Methods: The LPAD was fabricated by cutting glass fiber paper/nucleic acid extracting FTA cards, followed by laminating with films. The LPAD was then attached to a polycarbonate (PC) holder with double-sided tapes. The reverse transcriptase loop-

mediated isothermal amplification (RT-LAMP) was developed for detecting RNA from H1N1 virus with colorimetric signal.

Results and Discussion: The LPAD contains the RNA sensing pad sealed with films and a PC holder to hold the amplification buffer during incubation step. Gel electrophoresis confirms the RTLAMP system for detecting RNA from down to 10 TCID₅₀ in a single piece of device.

Conclusion: Our experiments have shown the successfully functioning of LPAD to detect flu virus with colorimetric method. Further automation to make the platform more desirable for filed use is under development.

29. RSV-ASSOCIATED PEDIATRIC MORTALITIES IN FLORIDA, JANUARY 2010-SEPTEMBER 2015

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Background: Respiratory syncytial virus (RSV) is a common virus which typically causes upper respiratory tract infections, though in immunocompromised individuals, the very young, and the elderly, it may lead to lower respiratory tract infections and severe illness requiring hospitalization, and which may be fatal. Palivizumab is an immunoprophylactic therapy available for children who are considered high risk for a severe infection, though it is administered according to national RSV seasonality rather than Florida's RSV seasonality. The purpose of this review was to pilot the RSV-associated pediatric mortality case report form and to describe Florida cases medically and demographically in order to determine high risk populations and immunoprophylaxis uptake among cases.

Methods: Cases of RSV-associated pediatric mortalities in the state of Florida were identified by querying initial literal causes of death on death certificates accessible via Florida's Electronic Surveillance System for the Early Notification of Community-based Epidemics

(ESSENCE-FL). After these cases were identified, death certificates were obtained for those individuals through the Florida Department of Health (FDOH). County health department staff and infection control practitioners were asked to obtain cases' medical records, after which the investigator abstracted data from those records using a case report form (CRF) created by merging Centers for Disease Control and Prevention (CDC) and FDOH CRFs.

Results: Nearly half of identified cases were under one year of age, and minorities were disproportionately represented. Most cases expired in the months October through March, with the Central Region experiencing the most overall RSV-associated pediatric mortalities. All cases with medical history reported at least one pre-existing medical condition. None of the cases' medical records indicated that they had received palivizumab immunoprophylaxis prior to presenting at the hospital.

Conclusions: The case report form piloted in this review captured all relevant information, though the age field had to be refined throughout the abstraction process. Consistent with previous studies, most cases were under one year of age and most had pre-existing conditions which likely contributed to the severity of their illness. Many of those cases would have met the criteria to receive palivizumab prophylaxis and, had it been administered according to RSV seasonality in Florida, it is possible that these cases would not have experienced mortality as a final outcome. It is important to ensure that pediatricians in communities and hospitals are aware of the benefits of administering palivizumab quickly and consistently for high risk patients, especially during RSV season.

30. SINGLE-WALLED CARBON NANOTUBES INCREASES INFLUENZA A VIRUS INFECTIVITY THROUGH OXIDATIVE STRESS IN VITRO

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Background: Extensive application of nanomaterials has raised concerns regarding their potential health impacts. Our previous research shows that pre-exposure of lung epithelial cells to single-walled carbon nanotubes (SWCNTs) modulates expression of several inflammatory and anti-viral genes in concert with increased viral titers following subsequent exposure to influenza virus H1N1 (IAV). Evidence indicates that SWCNTs induces oxidative stress which can impair cell function and impact on innate immune signaling pathways. To investigate possible mechanism of increased IAV infectivity by SWCNTs, we assessed the effect of oxidative stress induced by SWCNTs on innate antiviral responses in small airway epithelial cells (SAEC).

Methods: Reactive oxygen species (ROS) were measured using a DCFDA method in SAEC exposed to SWCNTs (0.2-30 $\mu\text{g}/\text{mL}$) or IAV (MOI=0.5) singly and in combination for 6 hours for 6 hours. Before the sequential exposures to SWCNTs (20 $\mu\text{g}/\text{mL}$) and IAV (MOI=0.5) for 24 hours respectively, SAEC were treated with the antioxidant (N-acetyl-L-cysteine (NAC)) and measured with ROS production, RNA expression of inflammatory and antiviral genes, and virus titers (TCID₅₀).

Results: A dose-dependent increase of ROS production was observed in the dose range of 0.2-30 $\mu\text{g/mL}$ of SWCNTs with the lowest observed adverse effect level (LOAEL) determined to be 2.0 $\mu\text{g/mL}$. SWCNTs (20 $\mu\text{g/mL}$) synergistically produce ROS with IAV in SAEC and significantly inhibited expression of inflammatory and antiviral genes (RIG-I, MDA5, TLR3, IFN β 1, CCL5, IL8, IFIT2, IFIT3) while increasing IAV virus titers. With pre-treatment of NAC, the gene expression levels and virus titers in cells treated with SWCNTs+IAV showed no significant changes compared with those treated with IAV only.

Conclusion: SWCNTs inhibited pulmonary immune responses and increased IAV infectivity in part through oxidative stress mechanisms.

31. BABESIA BOVIS RAD51 INFLUENCES EPIGENETIC REGULATION OF THE VES MULTIGENE FAMILY

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Babesia bovis employs cytoadhesion via the immunologically and structurally polymorphic ligand, VESA1, to sequester in the deep microvasculature. VESA1 is a heterodimeric protein comprised of subunits encoded by the 1(alpha) and 1(beta) branches of the ves multigene family. Both are highly diverse antigenically, with variation arising mainly via segmental gene conversion (SGC), a presumed subclass of homologous recombination (HR). Rad51 proteins are considered pivotal to HR in eukaryotes. To assess the contributions of BbRad51 to SGC we knocked out the Bbrad51 gene encoding the orthologous protein, and characterized the parasite phenotype. No phenotype was observed in Bbrad51 null parasites with regard to parasite morphology, replication, sensitivity to (gamma)-irradiation or methylmethane sulfonate, or rate of gross DNA repair following (gamma)-irradiation. To look at effects on

antigenic variation, ves1(alpha) transcripts were analyzed by deep sequencing of RT-PCR libraries made using universal ves1(alpha) primers. SGC persisted, but the cumulative SGC tract length distribution midpoint was reduced from 90 bp in wt to 79 bp in knockouts. Whereas the frequency of unique SGC tract formation was unchanged when normalized by the number of active ves loci, total SGC formation was significantly reduced in knockouts. In situ transcriptional switching among ves loci occurred significantly more frequently in wt parasites, and showed evidence of a locus switching hierarchy. These results indicate that BbRad51 is not essential to SGC (although another paralog may be), but suggest that it influences both the rate and overall diversity of antigenic variation primarily through epigenetic transcriptional regulation of the ves multigene family. Supported by R01AI055864 and departmental funds.

32. FUNCTION AND MECHANISMS OF O-FUCOSYLATION OF MALARIA PARASITE TSR-DOMAIN PROTEINS

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The thrombospondin type I repeat (TSR) domains of several key proteins of the malaria parasite facilitate attachment to human and mosquito host cells. We hypothesize that the Plasmodium biosynthetic machinery modifies these domains with the sugar fucose, using a protein-O-fucosyltransferase 2 (PoFUT2) enzyme. Throughout the life cycle of Plasmodium, multiple proteins are

expressed on the surface of the parasite and contain TSR domains. Our aim is to describe this modification and evaluate its biological significance during the parasite transmission from humans to mosquitoes and back. To test our hypothesis, we have generated a PoFUT2 null mutant of Plasmodium and compared the grow and survival of parasites throughout multiple stages of the life cycle. Furthermore, we have characterized by mass spectrometry one of the most abundant proteins on the sporozoite stage of the parasite, showing that TSR domain of circumsporozoite protein (CSP) is indeed fucosylated by human PoFUT2.

33. INFECTION INCREASES VULNERABILITY TO CLIMATE CHANGE VIA EFFECTS ON HOST THERMAL TOLERANCE

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We currently face increases in rates of infectious disease emergence and unprecedented changes in the global climate. Host factors, such as infection with emerging pathogens, may alter species' sensitivity to temperature to an extent that threatens survival in a warming world. However, few studies have addressed interactions between infection and host thermal physiology. We investigated interactions between infectious disease and upper thermal tolerance by focusing on frogs as a model system. The critical thermal limits of frogs have been well-studied, and frogs are currently experiencing the fastest and largest extinction event ever recorded, largely due to global emergence of the fungal pathogen *Batrachochytrium dendrobatidis* (Bd). We experimentally infected frogs with Bd and acclimated them to constant cool temperatures or daily heat pulses mimicking the body temperature regimes of frogs in nature. We then examined the effects of Bd infection and acclimation on their critical thermal maxima (CT_{max}). We found that Bd infection reduced the CT_{max} of hosts by up to ~4°C. Acclimation to realistic daily heat pulses enhanced thermal tolerance among infected individuals, but the effect of infection was at times greater than the effect of acclimation. Our findings suggest that infection may increase host

vulnerability to projected climate changes such as increases in temperature extremes and that the benefits of thermal acclimation may not be sufficient to protect animals from the thermal consequences of infection. Moreover, in disease systems in which the thermal tolerance of hosts exceeds that of the infectious organism, infection risk or the intensity of existing infections may be moderated when hosts elevate their body temperatures. A recently proposed conceptual model that expands on the relationship between CT_{max} and infection risk predicts that infection risk will decrease as the difference in the CT_{max} of the host and pathogen increases (tolerance mismatch hypothesis). Our study demonstrates that in ecological systems in which tolerance mismatch is very small, high parasite burdens can shrink the gap between host and pathogen thermal tolerances even further. This effect could increase risk of reinfections that may build to lethal levels as individuals are increasingly compelled to occupy cool refuges at the expense of behaviors that facilitate heat- and immune-mediated parasite clearance. We conclude that the effects of infectious disease are an underappreciated factor in estimates of species' vulnerability to climate change.

34. SCHISTOSOMA MANSONI INFECTION AMONG NONHUMAN PRIMATES IN AFRICA: A REVIEW

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Nonhuman primates have been recognized as zoonotic hosts of *Schistosoma mansoni* in Africa, but their epidemiological roles in the parasite transmission, in particular implications in human infections, remain largely unknown, mainly due to dearth of information on the molecular epidemiology of the schistosome parasite in human and non-human primates. As the first attempt to gain some insights into this, here we conduct a comprehensive review on *S. mansoni* infections among nonhuman primates in Africa. A comprehensive literature search was conducted using databases including PubMed, Google Scholar, Web of Science, WHO library database, World Cat, and Science Direct. Keywords “*Schistosoma mansoni*”, “*S. mansoni*”, “schistosomiasis”, “zoonosis”, “primate”, and “Africa” were used in the search. A total of 17 studies reported in nine countries, including Cameroon, the Democratic republic of Congo, Ethiopia, Kenya, Nigeria, Senegal, Tanzania, Uganda, and Zimbabwe were identified. The non-human primate species reported in these studies include 12 species, namely, *Cercocebus albigena*, *Cercocebus torquatus*, *Cercopithecus aethiops*, *Cercopithecus mitis*, *Colobus abyssinicus*, *Eythrocebus patas*, *Pan troglodytes*, *Papio anubis*, *Papio cynocephalus*, *Papio cynocephalus anubis*, *Papio hamadryas papio*, and *Papio ursinus*. The studies employed a variety of different methods to test infections. The most frequently used diagnostic technique was faecal sample test, followed by serological and urine tests, and in a few cases, necropsy. Regardless of testing methods and combining the results, these surveys reported primate infections by *S. mansoni* in the range of 0 to close to 24%, with highest percent

of infection reported in Uganda (19.55%, n = 179), followed by Tanzania (18.37%, n = 245), and then Nigeria (15.46%, n = 97). Regarding infections with respect to species of primates, the highest percent of infection was found in *Pan troglodytes* (23.62%, n = 199), followed by *Papio hamadryas papio* (23.53%, n = 17) and *Papio anubis* (16.92%, n = 402). We review the types of human and primate interface in each study setting and discuss the likely human-primate interactions in the context of *S. mansoni* transmission.

35. SPATIAL DISTRIBUTION OF SCHISTOSOMA MANSONI ENDEMIC AREA OF ETHIOPIA

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Background: Schistosomiasis is an important public health problem in tropical and subtropical areas. According to World Health Organization, 78 countries reported the schistosomiasis transmission in 2015 with 218 million infected people and 800 million people are at risk. In Ethiopia, the intestinal form of schistosomiasis caused by *Schistosoma mansoni* is the most widely distributed in the country. Although many school- and community-based surveys were conducted in the past decades and schistosomiasis was estimated to have infected 4 million people with more than 35 million at risk of infection, the actual national distribution of schistosomiasis endemic areas and associated environmental determinants remain less well understood. This study aims to identify environmental and ecological factors associated with the endemic areas, and to develop a niche model to predicting the potential distribution of schistosomiasis

endemic areas based on our initial data for the two lake areas (Lake Tana and Lake Langano) in Ethiopia.

Methods: In this study, we use Maximum Entropy (MaxEnt), one of the most popular tool for species distribution and environmental niche modeling, to assess a set of environmental factors and to estimate the probability of *S. mansoni* distribution across the landscape. As the initial exploration, we use *S.mansoni* survey data (1984-2013) from previous studies in the lake area in Ethiopia as the known sites of presence. Environmental factors including climate data from Worldclim global dataset, altitude, slope, and Normalized Difference Vegetation Index (NDVI) are used as the background data in MaxEnt.

Results/concluding remark: Our initial exploration suggests that the MaxEnt model provides a reasonably good discrimination of endemic and non-endemic areas (the area under the curve, AUC, 0.834). The precipitation of wettest month/quarter, and altitude are the most important factors identified by the model. The MaxEnt modeling approach seems a promising tool for the identification of environmental and ecological conditions sustaining the transmission of *S. mansoni* in Ethiopia. As a next step, we propose to refine and validate the model, and extend the prediction at the national scale.

36. SUGAR CANE PROCESSING RESIDUE, BAGASSE, ENHANCES DECOMPOSITION OF CITRUS LEAVES AND COULD CONTRIBUTE TO CITRUS BLACK SPOT MANAGEMENT

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Citrus black spot (CBS) is a quarantine disease, reported in Florida in 2010. The pathogen, *Phyllosticta citricarpa* (Pc), survives in twigs and leaf litter during the off-season. Speeding up leaf litter decomposition could contribute to reducing disease pressure and potential spread. Objectives were to investigate (a) effects of bagasse, cellulolytic microbes and urea on leaf litter decomposition; (b) relations between litter composition, moisture content and decomposition rate; and (c) effects of the treatments on Pc survival. Two laboratory and two field experiments were conducted in Northern Florida, where the pathogen is absent. In microcosms, the effects of bagasse +/- cellulolytic microbes, just microbes, urea, and a control treatment were compared with respect to decomposition of Pc-inoculated mature sweet orange leaves on soil, Pc reisolation and detection by qPCR. In two disease-free sweet orange groves, effects of bagasse and urea on decomposition of nylon-bagged sweet orange leaves on soil were compared to a control at various distances from micro-sprinklers. Bagasse resulted in a leaf dry weight reduction of 93% in microcosms and 67% in groves compared to 36% in both controls after two months. Dry weight reductions declined with distance from sprinklers, except in the bagasse treatment which maintained leaf moisture content. Pc was not reisolated, but its DNA was detected in all inoculated but not in non-inoculated samples after one month. Bagasse enhanced decomposition of sweet orange leaves on soil. Additional experiments are needed in CBS affected groves to investigate effects of bagasse on Pc survival in leaf litter.

37. GENOMIC EPIDEMIOLOGY OF MAJOR M. TUBERCULOSIS OUTBREAK IN COPENHAGEN: A RETROSPECTIVE WHOLE GENOME SEQUENCING COHORT STUDY COVERING 23 YEARS

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Despite its status as a tuberculosis low-burden country, Denmark has experienced sustained, active transmission of the disease. The Danish Mycobacterium tuberculosis (Mtb) strain collection at the International Reference Laboratory of Mycobacteriology includes specific outbreak strains spreading in the population. The most prominent example is the so-called “C2/1112-15” cluster, which is a collection of isolates displaying identical genotypes based on their IS6110 RFLP/MIRU-VNTR patterns. The cluster was first identified in the beginning of the 1990’ies in only a few patients. Since then, it has caused disease in more than 1000 individuals, making it the predominant lineage in Scandinavia. In 2001, the cluster was found in Greenland. In this preliminary study, we have conducted WGS on 114 representative isolates from the C2/1112-15 cluster. These isolates were mainly collected in the Greater Copenhagen area and span the years 1992-2014. Using phylogenetic analysis, we found that all isolates are confined to the same Mtb sub-lineage, commonly known as lineage 4.8. By comparing the 114 genomes to publically available genomes belonging to the same lineage, we observe that C2/1112-15 constitutes a monophyletic clade clearly distinct from other outbreaks publicly available. We observe a major and a minor lineage within C2/1112-15 with a most common recent ancestor dating back to 1959. Using molecular clock analysis, we calculated an overall mutation rate of the cluster to be 0.24 SNPs/genome/year. Using a median-joining network approach we also determined the existence of seven discrete transmission chains within the major lineage that all originate from a clonal group of isolates, the earliest of which was collected in 1993.

38. IDENTIFICATION OF OPTIMAL LINEZOLID REGIMENS FOR THE TREATMENT OF MYCOBACTERIUM TUBERCULOSIS USING THE HOLLOW FIBER INFECTION MODEL (HFIM) SYSTEM

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Background: Linezolid (LZD) is an oxazolidinone with potent activity against Mycobacterium tuberculosis (TB), including multi-drug resistant (MDR) and extensively-drug resistant (XDR) strains. However, prolonged courses of LZD therapy are required to treat TB infections which often results in several adverse side effects such as thrombocytopenia, neutropenia, and/or anemia. These drug-related toxicities are attributed to the inhibition of mammalian mitochondrial protein synthesis. We have previously shown that trough (Cmin) is the pharmacodynamic index most closely linked with toxicity, indicating that less frequent dosing intervals are required to minimize toxicity. The aim of this study was to determine whether every other day dosing (Q48h) of LZD could limit drug-related toxicities without compromising antibacterial activity.

Methods: Six hollow fiber (HF) cartridges were inoculated with 5x10⁶ K562 cells, a human myeloid leukemia cell line with platelet-specific properties. LZD was administered into five cartridges to simulate clinically relevant regimens either as the total daily dose once daily (Q24h) or two-times the total daily dose Q48h. One cartridge served as a no-treatment control. Cells were harvested from HF cartridges at various times throughout the 16 day study and mitochondrial toxicity was evaluated by measuring oxidative phosphorylation (OXPHOS) complex 4 proteins via ELISA. Moreover, the same LZD dosage regimens were evaluated for effectiveness

against TB in the HFIM system when administered in combination with moxifloxacin (MOX) either as Q24h or Q48h dosing intervals. Exposures were linked to response with a sigmoid-Emax effect model using ADAPT V software.

Results: LZD inhibited OXPHOS complex 4 proteins in an exposure dependent manner. However, complex 4 protein levels were higher in the treatment arms receiving Q48h dosing when compared to Q24h dosing. These findings suggest that Q48h dosing minimizes drug-related toxicities with prolonged LZD therapy. Combination regimens with LZD and MOX Q48h regimens were as effective as the corresponding Q24h regimens in killing TB and preventing the emergence of LZD- and MOX-resistance.

Conclusions: These findings indicate that Q48h dosing of LZD has great potential to minimize toxicity without compromising antibacterial effectiveness. Together, these data provide valuable insight for designing optimal LZD regimens that are part of prolonged combination treatment courses for the treatment of MDR- and XDR-TB.

39. LUNG TISSUE CONCENTRATIONS OF MOXIFLOXACIN AMONG PATIENTS WITH MULTIDRUG-RESISTANT TUBERCULOSIS

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Background: The global emergence of multidrug-resistant tuberculosis (MDR-TB) is an enormous public health threat and major barrier to effective TB control. To exert its pharmacological effect a drug has to get to the target site at sufficiently high concentrations. Lower drug concentrations in cavitary lesions, the ultimate site of action, may lead to development and amplification of resistance and ultimately to treatment failure. Little is known about target site penetration of moxifloxacin. Improved knowledge regarding tissue penetration of anti-TB drugs will help guide drug development and optimize drug dosing and management.

Material/methods: Patients with culture-confirmed pulmonary tuberculosis receiving moxifloxacin and being scheduled to undergo adjunctive surgical resection were enrolled in Tbilisi, Georgia. Serum samples were collected immediately before and 1, 4, and 8 hours after receiving moxifloxacin. Another serum sample was collected at the time of surgical cavity removal (approximately at t_{max}). Microdialysis (μD) was performed in the ex vivo tissue immediately after surgical removal of the cavitary lesion. Drug concentrations in collected microdialysates (free drug) and serum samples were measured using validated LC-MS-MS assays. Noncompartmental

analysis was performed and a tissue-to-serum-concentration-ratio was calculated. Free serum concentrations were calculated by multiplying total serum concentrations times expected fraction unbound (0.49).

Results: Seven patients undergoing surgical resection for drug-resistant TB were enrolled (median age: 25 years; 57% males; 57% with no history of prior TB treatment; 43% co-infected with either hepatitis B or C virus). One patient had isoniazid resistant and rifampin susceptible TB, while 3 had multidrug-resistant TB and another 3 had extensively drug-resistant TB. The minority of patients (29%) had C_{max} concentrations within the recommended range of 3-5 µg/ml. The median t_{1/2} (7.0 h) and t_{max} (2.0 h) were similar to those reported in the literature. The median free serum concentration at time of surgical resection was 1.23 µg/ml; median free lung tissue concentration was 3.37 µg/ml (range 0.81-5.76). The median free-tissue/free-serum-concentration-ratio was 3.2 (range 0.66-28.08).

Conclusions: Moxifloxacin showed excellent penetration into TB diseased pulmonary tissue among patients with a variety of radiological lesion types. With the exception of one subject all patients had higher tissue concentrations when compared to serum. The majority of patients (71%) had suboptimal serum C_{max} and AUC values which can be explained, in part, by the high inter-patient variability in moxifloxacin pharmacokinetics. The excellent tissue penetration of moxifloxacin highlights its importance in the treatment of MDR-TB and may even make it a candidate for susceptible TB treatment.

40. OPTIMIZING THE DOSE OF RIFAMPIN: PHARMACOKINETIC RESULTS FROM THE HIRIF TRIAL

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Background: Rifampin displays profound concentration-dependent killing of *M. tuberculosis*, which is not fully exploited by the standard 600 mg dose. This blinded, randomized trial compared 10, 15 and 20 mg/kg of rifampin delivered orally, 7 days per week for 8 weeks, at participating centers in Peru. Patients also received standard oral doses of isoniazid, pyrazinamide, and ethambutol, and a continuation phase of isoniazid 10 mg/kg/day and rifampin 10 mg/kg/day 3 days/week for 18 weeks.

Materials & Methods: The study protocol and informed consent documents were reviewed and approved by Institutional Review Boards at all participating institutions. Rifampin and placebo were provided by the Sanofi-Aventis Groupe. The study population included adults with newly diagnosed, previously untreated, smear positive (>2+) pulmonary tuberculosis. All study treatment doses were directly observed. After no fewer than 13 and no more than 56 (including 3 consecutive daily) doses of RIF, plasma samples were collected at 0, 2 and 6 hours post dose (sparse, 67%) or 0 hours plus sampling windows centered on 0.5, 1, 1.5, 2, 6 and 14 hours post dose (intensive, 33%). Samples were stored at - 80°C until assayed at the University of Florida using a validated LC MS MS assay. The plasma standard curve for rifampin ranged from 0.05 to 50 mcg/mL. Phoenix v6.2 software was used for the non-compartmental analysis. Statistical tests were performed using JMP v10 (SAS Institute, Cary, NC).

Results: Results are presented in order for the 10, 15 and 20 mg/kg rifampin groups. There were 58, 57, and 53 patients evaluable for pharmacokinetics. Median (range) values for C_{max} were 6.20 (0.62-12.55), 10.18 (0.58-27.00) and 13.33 (3.88-39.22), and for AUC₀₋₆ 24.94 (2.63-57.94), 43.13 (3.49-111.58) and 55.47 (13.63-132.88).

Median ratios for the 15/10 and 20/10 mg/kg doses were C_{max} 1.64 and 2.15; AUC₀₋₆ 1.73 and 2.22. Median T_{max} was 2 hours across all groups, and median half-lives were 2.29, 2.46, and 2.31 hours. There were no statistically significant differences among the recorded adverse events across the 3 dosing groups.

Conclusions: Rifampin doses up to 20 mg per kg were well tolerated and produced slightly more than proportional increases in C_{max} and AUC₀₋₆. Peak concentrations, including in the 10 mg/kg/day arm, were higher and achieved more consistently at 2 hours than previously reported among TB patients in Peru treated with locally sourced rifampin. The concentrations in the control group in HIRIF were similar to those achieved in a recent dose-ranging study of rifampin in South Africa; C_{max} in the 20 mg/kg arm in Peru was lower and more variable than that reported in the South Africa study (21.6 [16.0-31.9]). The present results, and those of other studies, support continued investigation of higher doses of rifampin for potential efficacy improvements and treatment shortening benefits.

41. PREVALENCE OF MULTI-DRUG RESISTANT 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 nonconsecutive 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 at least 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. 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 especially for the MDR TB are greatly needed.

42. STUDY OF CD4+CD25+ REGULATORY T CELLS FREQUENCY AND IFN-GAMMA SECRETION IN PATIENTS SUFFERING FROM PULMONARY TUBERCULOSIS DURING 6 MONTHS TREATMENT

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Tuberculosis (TB) remains a major cause of morbidity and mortality around the world. CD4+CD25+ regulatory T cells (Treg) play a central role in the prevention of autoimmunity and in the control of immune responses by down-regulating the function of effector CD4+ or CD8+ T cells. In infectious diseases Tregs may inhibit protective responses facilitating pathogen multiplication and dissemination, but they may also limit the inflammatory response diminishing tissue damage. Although there is experimental and clinical evidence that Tregs are induced during Mycobacterium tuberculosis infection, their role in the immunopathogenesis of tuberculosis is still not completely understood. So, we studied the frequency of Treg cells and secretion of IFN-gamma in PBMC during treatment of patient suffering from pulmonary tuberculosis.

Material and Method: Peripheral blood mononuclear cells (PBMC) were obtained before treatment and after 2, 8 and 24 weeks of treatment from 30 patients with active pulmonary TB and positive smear Referred Tuberculosis Center University of Medical Sciences. The frequency of Treg cells were obtained by Flow cytometry and secreted IFN-gamma was measured in supernatant using ELISA after re-stimulation of PBMC by PPD.

Result and Conclusion: During a successful treatment, the number of Treg cells started significant statistically to increase in compared to the day before the treatment beginning. Inversely, the amount of IFN-gamma secreted after re-stimulation of PBMC started significant statistically decreasing. These observations support further investigations into the possible utility of these parameters as markers to evaluate the active disease or successful treatment and

also the obtained data emphasize important roles of Treg cells and IFN-Gamma in the development or healing of immunopathogenesis caused by Mycobacterium tuberculosis infection.

43. WHOLE GENOME SEQUENCING OF MYCOBACTERIUM TUBERCULOSIS FOR THE INVESTIGATION OF A TUBERCULOSIS OUTBREAK INVOLVING PRISON AND COMMUNITY CASES IN FLORIDA, USA

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Introduction: An extensive tuberculosis (TB) outbreak involving U.S-born persons in the prison system and foreign-born persons in the community occurred in Florida, U.S. over a 10-year period (2004-2015). Genotyping by spoligotyping and 24-locus MIRU-VNTR showed the outbreak was clonal but contact investigation could not identify a source. We used whole-genome sequencing (WGS) and phylogenetic analyses to delineate the outbreak.

Hypothesis: In this investigation, we tested the hypothesis that the outbreak bacterial population was more diverse than observed when using traditional genotyping methods.

Methods: We sequenced the complete genomes of 21 of the 74 Mycobacterium tuberculosis isolates, which constituted a representative spatial and temporal sample of the outbreak cases.

Contigs were ordered and aligned using the laboratory strain CDC1551 as a reference genome and SNPs called de Novo. We compared the mean genetic distances between and within community and prison strains; foreign and US-born cases. A phylogenetic relationship between the strains was investigated using distance-based and maximum likelihood methods. We applied a Bayesian coalescence-based phylogenetic framework implemented in Beast v2.4.1 to infer the timescale of the outbreak and estimate the date of the most recent common ancestor, using a strong prior for the clock rate. A posterior distribution of trees and model parameters were generated by running 5×10⁷ Markov Chain Monte Carlo (MCMC) generations, sampling every 5,000 steps.

Results: The genomic data revealed two *M. tuberculosis* lineages with identical spoligotyping and 24-locus MIRU-VNTR profile, suggesting two concomitant TB outbreaks. The lineages descended from a common source of foreign origin. Almost thirty percent of the outbreak cases were HIV co-infected, 37% were incarcerated at the time of diagnosis, 16% had a history of drug use and 4% were homeless in the year prior to diagnosis. These clinical and social factors likely contributed to the outbreak. The Bayesian phylogenetic analyses suggest that the source strain circulated in the community for some time before the clustered cases in the prison system triggered the outbreak.

Conclusions: These data highlight the increasing need for more discriminatory methods such as WGS in the fight against TB in low incidence settings.

44. AN INTERACTIVE TOOL TO DETERMINE POTENTIAL COSTS AND CONSEQUENCES OF SCREENING AND TREATING STRATEGIES FOR MALARIA IN WEST AFRICA

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Several malaria prevention and control strategies consist of presumptively treating the entire population (e.g., mass drug administration or seasonal chemotherapy) or a subset of it (e.g., intermittent preventive treatment in pregnancy, treating neighbors of index cases). A common alternative to several of these strategies consists of first screening individuals (using either microscopy or, more commonly, rapid diagnostic tests [RDTs]) to then treating those detected to be positive. The potential benefit of first screening and then treating is a potential reduction of cost and decreased risk of emergence of drug resistance. However, baseline malaria prevalence and RDT performance can vary substantially across region and, as a result, screening and treating might not be equally effective across the entire country. To facilitate policy decision making, we aim to create an interactive tool that informs managers about the potential costs and consequences of screening and treating vs. presumptive treatment in each subnational region in seven West African countries. We fit generalized linear mixed effects model to data from children under five years old using data from Demographic and Health Survey or Malaria Indicator Survey from seven countries. By implementing the models within Bayesian framework, we estimate the probabilities of malaria infection, the “field” RDT sensitivities and specificities. Based on these estimates, and the user’s input on the cost of treatments and tests, the tool will then calculate and display the potential costs and number of untreated infected patients of each strategy in each region. This information can aid managers in determining which strategies are likely to be more effective in each region.

45. CHEMICAL COMPOSITION OF ZINGIBER ZERUMBET (L.) SMITH: ESSENTIAL OIL AND ITS REPELLENT ACTIVITY AGAINST THE YELLOW FEVER MOSQUITO, Aedes Aegypti L.

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Mosquitoes are vectors for many pathogens that cause severe human morbidity and mortality. There is continued research and development of natural products to discover repellents which protect humans from mosquito-borne illnesses. The present study was an attempt to assess repellency of the essential oil of *Zingiber zerumbet* (L.) Smith. against *Aedes aegypti* (L.) mosquitoes. *Z. zerumbet* is commonly known as the pinecone or shampoo ginger because the inflorescence is a pinecone shape and the mucilaginous substance present in the inflorescence is used as a natural hair conditioner by the Hawaiians. They also apply the compressed rhizomes to sore spots, bruises, and cuts, as well as to treat headaches, toothache, skin disease, joint pains, sprains and stomach-ache. Rhizomes were subjected to hydrodistillation and analyzed by GC-FID and GC-MS. The main constituents of *Z. zerumbet* oil were characterized as zerumbone (87.1%), humulene epoxide-I (2.0%) and camphor (1.4%). The major compound zerumbone was identified and its structure was characterized by ID-NMR spectroscopic data analyses. The repellent data will be discussed in the presentation.

46. COMMON PATHOGENS AND CAUSES OF DEATH IN CAPTIVE WHITE TAILED DEER

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The state of Florida has approximately 400 deer farms and hunting preserves that provide both meat and hunting opportunities to residents and visitors alike. This viable industry generates about 12.8 million dollars in revenue each year in a combination of licensure and hunting. These farms and preserves have created 1700 jobs in rural counties, giving small towns a thriving industry. To keep this business viable, it is essential to keep these animals as healthy as possible. White tailed deer are susceptible to several bacterial and viral pathogens, which infect both adult and fawn at different times throughout the year. CHeRI is currently working to understand the prevalence of these pathogens in order to prevent future outbreaks of disease and mortality.

CHeRI obtains organ, blood, fecal and nasal samples from deceased animals via necropsy. These samples are tested for an assortment of viral and bacterial pathogens, and infections can be detected through microbiological and molecular techniques. In 2016, more than 80 animals were submitted for post mortem diagnostic testing through CHeRI. The general findings for these animals yielded very useful and interesting data throughout the different seasons.

In the beginning of the summer, fawn are the most susceptible to infection. They are almost exclusively infected with bacterial pathogens from May through August. Additionally, most of these mortalities were caused by coinfections of multiple bacteria. The most common species found were E.coli, Streptococci and Trueperella. Later in the fall and early winter months, more viral pathogens were found, specifically Blue Tongue Virus and Hemorrhagic Disease Virus. Interestingly, the viral pathogens infected high numbers of both adults and young animals. Viral infections were commonly found in conjunction with a septic bacterial infection as well, suggesting that the combination of

multiple pathogens leads to a higher level of mortality due to a highly compromised immune system. CHeRI continues to look for solutions to help lower the incidence of these infections. Thus far, we can conclude that because a number of these pathogens are capable of persisting in the environment, changes in husbandry practices have the potential to decrease the incidence of these co-infections.

47. COMPARISON OF IXODID TICK COLLECTION METHODS: DRAGGING VS. SURVEYS OF FERAL SWINE IN SOUTH CENTRAL FLORIDA

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Surveillance of tick species is important as many ticks can transmit pathogens of livestock, wildlife, and humans. In the United States, feral swine are hosts for native and exotic Ixodid tick species, and serology results show feral swine are exposed to multiple tick-borne pathogens. Traditional active surveillance methods for tick collection such as dragging cloth over vegetation to collect questing ticks, attracting ticks with carbon dioxide, or trapping animals to survey them for ticks, can be time consuming, costly, and inefficient. Feral swine are large-bodied, habitat generalists that potentially attract diverse tick species. As they are often taken by land and wildlife managers or by hunters, feral swine could potentially provide a convenient and cost-effective host for tick surveillance. In our study, we determine potential benefits and drawbacks of feral swine as tick surveillance tools. We compare species diversity, number of ticks

collected, and life stage of ticks collected from feral swine versus collected by dragging on a cattle ranch in south central Florida. More than 200 feral swine and more than 40,000m of vegetation were surveyed for ticks from May 2015 – October 2016. Tick surveys of feral swine detected a greater number of adult ticks and a greater diversity of adult tick species than dragging. Dragging methods collected all three life stages, whereas feral swine surveys detected only adults. In conclusion, feral swine surveys were better at detecting adult species richness and for collecting adult ticks, whereas dragging was better at collecting larval and nymph life stages.

48. DENGUE AND CLIMATE IN THE MEKONG DELTA, VIETNAM

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Climate plays an important role in the geographic distribution and burden of disease due to dengue. Dengue is a significant cause of morbidity and mortality in Can Tho, a province in the Mekong Delta in Vietnam. In this region, average temperatures have increased by 0.5°C since 1980. To better understand the influence of climate on dengue, this study examines dengue hospitalizations over a ten-year span, as well as vector breeding behavior and human mosquito avoidance behaviors.

We used Seasonal Autoregressive Integrative Moving Average (SARIMA) modeling to assess the association between dengue cases and meteorological factors between 2001-2011. We then sampled 400 households prospectively every 1-2 months between June 2012 and June 2013 in Can Tho. Entomological indices of the immature forms of the dengue vector, *Aedes aegypti*, and the productivity of different types of household containers were determined. Human mosquito avoidance behaviors, such as the use of fans, mosquito

repellant, and larval elimination strategies were also recorded in monthly or bimonthly interviews. Correlations of these variables, dengue hospitalizations, and meteorological factors were established. Relative pupal productivity of containers and *Ae. aegypti* larval abundance risk factors were also determined.

The time series analysis demonstrated that the rate of dengue hospitalizations was significantly associated with maximum relative humidity with a lag of one month. Rainfall and temperature were not predictors of dengue hospitalization rate. In the prospective study, the house- (HI), container- (CI), and Breteau indices (BI), and the number of larvae per household (LH) were strongly correlated with relative humidity, moderately correlated with precipitation, and weakly correlated with temperature and hours of sun. All indices were significantly higher in the rainy vs. dry season. Dengue hospitalization rates were also higher during the rainy vs. dry season (2.2/10,000 popn per month vs. 1.4/10,000 popn per month), and moderately correlated with humidity. However, mosquito avoidance behaviors were more frequent in the dry season (92.5% vs. 86.0% of interviewees endorsed one or more forms of mosquito prevention). There was significantly less use of fans (67.6% vs. 80.0%), repellant coils (35.2% vs. 47.2%), and larval elimination strategies (39.2% vs. 50.5%) during the rainy versus the dry season. The most productive containers were large jars used ubiquitously for water collection (relative pupal productivity 87%). *Ae. aegypti* larval abundance was associated with not cleaning water storage jars (RR=2.5, 95% CI 1.6-3.7), not having access to municipal waste pick-up (RR=3.2, 95% CI 2.1-4.8), and season (RR[rainy season]=3.1, 95% CI 2.2-4.5).

Our study reveals moderate to strong correlations between relative humidity, entomological indices and dengue hospitalization rates. Further studies on the role of relative humidity on dengue may be warranted as climate changes. Our findings also demonstrate that there is a temporal disconnect between mosquito avoidance/larval elimination behaviors and peak periods of immature *Ae. Aegypti* abundance and hospitalization rates.

49. DETECTION OF ARBOVIRUS CO-INFECTIONS OF HUMANS DURING A CHIKUNGUNYA VIRUS OUTBREAK, HAITI, 2014

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Background: A large outbreak of Chikungunya Fever (CF) occurred from May to September 2014 in Haiti. During outbreaks such as this, standard diagnostic methods focus on detection of the expected causative agent only. Sparse information exists regarding co-infections during CF outbreaks, with a current estimate that 10% of individuals harbor a co-infection by Chikungunya virus (CHIKV) and another arthropod-borne virus (arbovirus) such as Dengue virus (DENV) or Zika virus (ZIKV).

Methods: Plasma samples collected between May and August from schoolchildren with CF symptoms were screened by real time (rt) RT-PCR for CHIKV genomic RNA (vRNA). One hundred (n=100) CHIKV-positive samples were then tested by rtRT-PCR for DENV types 1-4 and ZIKV vRNAs, and aliquots thereof used for virus isolation. The complete genomes of viruses in co-infected samples were subsequently determined by Sanger Sequencing.

Results: In total, 6/100 plasma specimens and their corresponding cultures were rtRT-PCR positive for both CHIKV and either DENV type

2 (n=1) or ZIKV (n=5) vRNAs. All CHIKV genomic sequences were highly similar (99%) to each other and to a CHIKV isolate from Haiti in May 2014 reported by the CDC. The earliest ZIKV detected in this study was from May 29. All 5 ZIKV genomes were similar to each other but from a different lineage than the ZIKVs from December that we previously isolated from Haitians. The DENV type 2 sequence is highly similar to strains that had circulated for at least 5 years in the Caribbean.

Conclusions: Based on discovery of five CHIKV/ZIKV and one CHIKV/DENV type 2 co-infections, a general estimate of 10% co-infection rate seems reasonable. This study reveals separate introductions of ZIKV into Haiti since the virus lineages in May and December are different. DENV type 2 appears to be in circulation throughout many Caribbean and Central American countries with little genetic variation.

50. DETECTION OF CHIKUNGUNYA-, DENGUE- AND ZIKA VIRUSES IN MOSQUITOES COLLECTED IN HAITI, 2016

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Background: The emergence of arboviruses, including Chikungunya virus (CHIKV), Dengue virus types 1-4 (DENV) and Zika virus (ZIKV) in the Americas and Caribbean has been of major public health concern for the past 4 years. The suspected primary vector of these viruses, *Aedes aegypti* is increasing global distribution, but other mosquito species may also play a role in virus transmission. This on-going surveillance study is designed to detect arboviruses present in

mosquitoes in Haiti, and to identify the mosquito species that carry them.

Methods: BG Senintel traps were set at several locations in our study site in Gressier, Haiti and checked weekly. Mosquitoes were identified and pooled by location, species (*Aedes aegypti*, *Ae. albopictus*, *Culex quinquefasciatus*, and other) and sex (male and female), then frozen at -80°C until processing. Pools of ≤ 15 mosquitoes (by location, species, and sex) were subsequently homogenized in PBS and an aliquot of homogenate used for RNA extraction, with the remaining homogenate stored at -80°C for future work. Following RNA extraction, screening for CHIKV, DENV, and ZIKV was done by real-time (rt)RT-PCR.

Results: Between May and September 2016, a total of 8293 mosquitoes were trapped. To date, 51 pools of 449 mosquitoes have been screened for arboviruses. Four pools (35 total mosquitoes) tested positive for: CHIKV (n=1), DENV type 3 (n=1) and ZIKV (n=2). The CHIKV-positive pool consisted of two female *Ae. albopictus* caught May 17, 2016. The DENV type 3-positive pool consisted of 11 female and male *Ae. aegypti* caught May 23, 2016. And the two ZIKV-positive pools consisted of 15 male *Ae. aegypti* caught May 17, 2016, and 7 female and male ‘other species’ caught July 4, 2016.

Conclusions: To our knowledge, this is the first detection of CHIKV in *Ae. albopictus* in Haiti, and warrants further study to determine whether the virus has adapted to this mosquito species. The presence of DENV type 3 in *Ae. aegypti* is consistent with well-established virus-vector correlations. Our detection of ZIKV in male *Ae. aegypti* is novel for Haiti and suggests germline transmission of the virus, while its presence in “other” mosquito species raises the question whether they too are vectors of ZIKV.

51. EFFECT OF EXPOSURE HISTORY ON DENGUE INFECTION AND DISEASE: A STATISTICAL APPROACH AND ITS APPLICATION TO THE DENGUE COHORT IN NICARAGUA

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A cohort of children has been established in Nicaragua since 2004 to the epidemiology, virology and immunology of the dengue virus, an mosquito-borne arbovirus with four co-circulating serotypes. Each subject was bled annually and followed up for dengue-fever-like symptoms. Serum samples were tested by ELISA, and subset of ELISA-positive samples were serotyped with other lab-methods. We proposed a statistical competing-risk framework to assess the infection risks of all four serotypes during the study years and the effects of exposure history on both infection and probability of disease given infection. The model couples the cohort data with surveillance data to infer the exposure history at the individual level. The model is fitted with a data-augmented MCMC sampling algorithm with an efficient sampling approach for individual exposure history.

We demonstrated by extensive simulation studies that the proposed algorithm can provide unbiased estimation of model parameters. By applying our method to the Nicaragua pediatric cohort, we found that there were three outbreaks triggered by three different serotypes during the study years. The probability of disease for DENV-4 was much lower than other three serotypes. Compared with those without prior infection, having 1 (2 or more) prior infections were associated with 2.15 [95% CI: 1.78, 2.60] (3.79 [95% CI: 2.49, 5.56]) fold higher risk of infection, likely due to exposure heterogeneity. With at least one symptomatic prior infections, the probability of disease given infection was lowered to 35% (95% CI: 11%, 92%) of that without prior infection. Children of 9 years or older had 1.53 (95% CIL 1.19, 1.97) fold higher probability of disease given infection, compared to younger children.

52. EPIDEMIOLOGY AND PATHOGENICITY OF MAYAROVIRUS

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Arthropod-borne viral (arbovirus) diseases are of great public health interest as they exist in a vast geographic range and have been responsible for several pandemics in recent years. These diseases include but are not limited to dengue, chikungunya, West Nile, and Zika. Despite the extent of disease and extensive range attributed to arboviruses, there are still gaps in our knowledge regarding epidemiology and pathogenicity. Mayaro virus (genus Alphavirus, family Togaviridae) is currently endemic in Brazil and could expand its geographic range in the future due to increasing climate change, deforestation, urbanization, and migration. Mayaro is a zoonotic disease that causes fever and joint pain. We report the epidemiology and immunology of Mayaro virus by performing serosurveys and by studying the humoral immune response. In addition, we are identifying potential human target cells by conducting immunofluorescence and flow cytometry on several target human cell types – Human Dermal Fibroblasts, HEK 293, and Macrophages. Arboviruses are also known to exhibit antibody cross-reactivity. In the future, we will use serum from Panamanians previously infected by alphaviruses to examine the potential cross protective immunity arising from antigenetically similar viruses and whether this heterologous immunity influences the emergence of a new pathogen.

53. EVALUATING THE EFFICACY OF ATTRACTIVE TOXIC SUGAR BAITS FOR MOSQUITO CONTROL IN HAITI

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Background: Many vector borne diseases continue to plague Haiti including malaria, lymphatic filariasis. Additionally, dengue, chikungunya and Zika are a threat to both residents and non-residents. Furthermore, the earthquake in 2010 also resulted in the displacement of people and changes in land use have led increase in mosquitoes. Typical outdoor treatment for adult mosquitoes involves thermal fogging or ultra-low volume (ULV) insecticide application. Both of these methods deliver a short term relief and require frequent reapplication for continued control of adult mosquitoes. Attractive toxic sugar bait (ATSB) applied to vegetation has been shown to effectively reduce mosquito populations from multiple genera over periods of up to one month from a single application.

Methods: The study was done at the UF's Haiti field site in Gressier. The compound has defined borders, within which there is sparse vegetation, and a barrier of dense vegetation along its perimeter and therefore closely mimics a military compound. Prior to spraying ATSB, control data was obtained through collecting mosquitoes at specified sites within and outside the for one week. Attractive Toxic Sugar Bait (Westham Innovations LTD, Tel Aviv, Israel) consisting of 0.4% beta-cyclodextrin microencapsulated garlic oil and 99.6% mixture of sugar water was applied on vegetation along the entire perimeter of the study site. Mosquito surveillance using CDC light traps, BG sentinels and CDC gravid traps was done at the aforementioned sites for six weeks following ATSB application. All chemicals were applied using a Stihl backpack sprayer. Pre- and post- treatment surveillance data was compared for the study site.

Results: A total of 11,043 mosquitoes were caught over a 2 month period in the sampling locations. A total of 3477 mosquitoes were caught in the ATSB collection sites (intervention sites) versus 7566 mosquitoes in the control sites. The pre ATSB application mosquito counts in the intervention sites were 2541 while post ATSB mosquito counts was 936 mosquitoes. Overall, there was a reduction in the density of mosquitoes caught after the application of the ATSB. Significant differences were observed in the density of *Ae. aegypti* and *Ae. albopictus* mosquitoes caught in the control versus the intervention areas.

Conclusions: The ATSB strategy might be useful in controlling mosquitoes in an enclosed environment.

54. EVIDENCE FOR TRANSMISSION OF ZIKA VIRUS FROM MOTHER TO BABY BY BREAST MILK

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We isolated infectious Zika virus (ZIKV) from the breast milk of a symptomatic patient and from the urine of her 5 month-old child. The child was breastfeeding and did not develop symptoms of ZIKV infection. Whole-genome sequencing of ZIKV isolates from breast milk and from the child's urine revealed a near-exact match of consensus sequences. These findings suggest that ZIKV can remain infectious in breast milk.

On March 25, 2016, a 32 year-old female patient from Barquisimeto, Venezuela presented a 1-day history of symptoms associated with acute ZIKV infection: malaise, arthralgias, conjunctival hyperemia, and maculopapular rash. At that time, she was breastfeeding her 5 month-old child, who was asymptomatic. Serologic analyses revealed that the mother had IgM and IgG antibodies against ZIKV but no detectable antibodies to Chikungunya virus. She was also IgG positive but IgM negative for Dengue virus (DENV) using a pan-serotype test, indicating past infection by one or more of the DENV serotypes. The mother remained symptomatic with arthralgias and malaise for 10 days, with the macular papular rash and conjunctival hyperemia resolving 4 days after the onset of symptoms. In contrast, the child remained asymptomatic throughout the observation period.

Breast milk, serum, and urine specimens were collected from the mother 4 days after onset of acute Zika Fever symptoms, and serum and urine from the child the same day. Breast milk and urine tested negative for ZIKV viral genome (vRNA) by conventional RT-PCR. To determine whether the virus was present below the detection limits of the RT-PCR tests, Vero E6 and LLC-MK2 cells were inoculated with the mother's and child's specimens. Cytopathic effects characteristic of ZIKV infection were observed 9 and 12 days post-inoculation of the mother's and child's specimens, respectively, onto Vero and LLC-MK2 cells, and the presence of ZIKV vRNA therein confirmed by RT-PCR. Full-genome sequencing of ZIKV isolated from breast milk and the baby's urine revealed an exact match; the virus sequences were nearly identical to ZIKV isolates from Colombia. The finding of live virus in the mother's breast milk and a matching isolate from her

breastfeeding infant may suggest post-natal transmission from mother to child.

55. IDENTIFYING MALARIA RISK FACTORS IN A HYPERENDEMIC SETTING USING BAYESIAN MODEL SELECTION

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The epidemiological dynamics of malaria, as with many other vector-borne diseases, have been linked to a wide variety of environmental, socio-economic, and demographic factors. Traditional statistical approaches for evaluating the contribution of each of these potential disease drivers present critical tradeoffs. Modeling all possible combinations can be computationally intensive and make it difficult to draw definitive conclusions when numerous disease factors are considered. Conversely, selecting a subset of potential drivers can fail to describe the relative importance of a particular covariate and can exclude important risk factors. To address these issues we propose a Bayesian probit regression model that contains a model selection procedure which proposes new candidate models through the random addition, subtraction, or swapping of covariates. A new model is proposed and evaluated at each step of the iterative Markov Chain Monte Carlo algorithm, generating parameter estimates and inclusion frequencies for each potential disease driver. We used this approach to simultaneously evaluate the relative importance of a wide range of environmental, socio-economic, and medical risk factors for malaria in the Bunkpurugu-Yunyoo district of northern Ghana, using existing data from six malaria surveys conducted in 2010-13. Our analysis identifies substantial protective socio- economic and medical factors related to the two modest “urban” centers in this small geographic area, indicating that the small towns in this hyper-endemic setting may buffer nearby rural

areas from environmental conditions that are traditionally linked to high malaria transmission. This Bayesian model selection technique offers a promising solution for dealing with the practical and computational constraints of evaluating numerous diverse risk factors for malaria and other diseases.

56. IMMUNOBIOLOGY OF THE KUPFFER CELL-SPOROZOITE INTERACTION

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Malaria is a devastating disease, causing approximately 600,000 deaths annually. The *Plasmodium* parasite responsible features an asymptomatic liver stage within the vertebrate host during which many *Plasmodium* sporozoites traverse Kupffer cells (KCs), liver-resident macrophages, before invading hepatocytes. The job of KCs during a typical infection is to detect, phagocytose, and present foreign antigens, while producing cytokines to activate an immune response. It has been shown that sporozoites are able to traverse KCs without being phagocytosed or killed, suggesting that sporozoites are altering the normal KC response to pathogens. However, the mechanism by which this occurs is not understood. It is well-documented that the parasite can evade the immune system during the blood stages through various mechanisms, including direct suppression of immune responses. To test whether similar phenomena occur with KCs in the liver stage, we analyzed the primary rat KC cytokine response to *P. berghei* sporozoites in vitro. We hypothesized that KC responses would be skewed towards a tolerant, or anti-inflammatory, phenotype. Here, we report that KCs up-regulate cytokines of both the M1 (pro-inflammatory or classically activated) and M2 (alternatively activated) variety in response to sporozoites. Interestingly, we noted that the cytokine response is rapid and short-lived, with cytokines being found in the supernatant just 10 minutes after exposure, and levels dropping

below the limit of detection after 2 hours, suggesting sporozoite silencing of KC cytokine secretions. Additional work focuses on the interaction of KCs with a sporozoite secreted protein, the *P. berghei* homolog of macrophage migration inhibitory factor, which is involved with the disruption of immunological memory during the blood stages and may play a similar immune evasion role during the liver stage. Taken together, our work sheds new light on the mechanism by which sporozoites modulate KC activity to remain hidden from the host immune system during traversal.

57. IMPACT NETWORK ANALYSIS OF AVOCADO LAUREL WILT DISEASE

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In 2004, Redbay trees in coastal Georgia began dying from a previously unidentified disease: Laurel Wilt. Researchers quickly identified the causal organism as the ascomycete fungus *Raffaelea lauricola*, which was vectored by the introduced Redbay Ambrosia Beetle, *Xyleborus glabratus*. The invasive pathogen and pest were likely co-introduced on packing material. Dead standing trees become a breeding ground for beetles, who can then move the pathogen to new hosts. In addition to movement by the beetle, there is strong evidence for human-mediated spread of infested firewood. As Laurel Wilt moved south, it began to affect the avocado production region of Florida, centered in Miami-Dade County. Understanding the factors involved with spread within and between

avocado groves is critical for future management of disease. Impact Network Analysis examines how new disease control strategies will impact both the biology of the system as well as the social structure of the managers and stakeholders. Work is underway exploring the efficacy and cost-efficiency of injecting protective fungicides to inhibit colonization and insect pheromones to prevent movement of beetles into groves. Drones equipped with hyperspectral cameras and disease-sniffing dogs can detect differences between diseased and healthy trees. Once detected, worker applying insecticides to prevent movement of beetles from culled trees and chip the remaining wood. Future long-term strategies for disease prevention and control include resistance-breeding efforts and grafting horticultural scions to resistant rootstocks. Managing laurel wilt is critical to sustainable Florida avocado production, as well as protecting production elsewhere in the US, Mexico and the Caribbean.

58. INDIVIDUAL-LEVEL PREDICTORS OF DENGUE VIREMIA: A META-ANALYSIS

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Dengue is an arthropod-borne virus endemic to regions of southeast Asia, Africa, the Americas, and the Mediterranean that infects up to 400 million people annually. The majority of these infections are unapparent, but a portion result in undifferentiated fever or mild illness, classified as dengue fever (DF), or more severe clinical manifestations, dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). There is also substantial individual variation in viral load across all levels of clinical outcome, which has important implications for infectivity and transmissibility. The risk factors

associated with severe clinical outcomes and higher viral load are only partially understood. While secondary heterologous dengue infections and high viral load are often associated independently with DHF or DSS, these relationships become more complex when various factors – including viremia, serological status, infecting serotype, day of infection, and other individual characteristics – are considered in combination, and different studies have produced conflicting reports. Here, we perform a meta-analysis of 18 studies with more than 5,000 individual-level measurements of dengue viral titer to assess predictors of higher viral load. Viral load varies from 0 to 1.3×10^{11} genome copies/mL ($n=4,719$) and 0.06 to 3.4×10^5 PFU/mL ($n=383$) across cases of all severities and all serotypes. We will use general additive models (GAMs) with disease severity (DF or DHF), serological status (primary or secondary infection), serotype (DENV1, 2, 3, or 4), and day of infection as initial predictors of viral load. Preliminary results indicate that viremia is higher in cases with more severe disease but lower in cases of secondary infection, and that viral load differs significantly between the four serotypes. However, work remains to refine these models. A better understanding of the risk factors for dengue viremia will help elucidate transmission dynamics and assist in clinical and public health decision making.

59. INSECTICIDAL ACTIVITY OF SOME NOVEL TRIFLUOROMETHYLPHENYL AMIDES

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Mosquito-transmitted diseases such as malaria, dengue, and yellow fever, produce several million human deaths yearly. Climate change and global warming can enhance the vectorial capacity and the temporal and spatial distribution of mosquito populations. To find more effective tools for mosquito control we focus on the development of new repellents and insecticides to prevent mosquito bites and so reduce disease risk to humans. In recent years, we had been designing trifluoromethylphenyl amides (TFMPAs) as potential insecticides. The 4th generation set consists of 27 compounds that have been designed based upon active structures from bioassays of the compounds from the previous three generations (currently a total of 52 compounds). The 27 compounds were evaluated for toxicity against larvae and adults of *Aedes aegypti*. At a concentration of 100 μM , 10 compounds produced 100% mortality after 24 h, while 15 compounds produced 100% mortality after 48 h against *Aedes aegypti* larvae. At a concentration of 10 μM , 7 compounds produced 100% toxicity after 24 h, and 8 compounds showed 100 % toxicity after 48 and 72 h. At a lower concentration of 1 μM , one compound was active with 60 ± 15 % toxicity after 24h; 5 compounds showed toxicity after 48h. The most active produced $73.3 \pm 23\%$ mortality. At 0.1 μM 2 compounds showed $1.7 \pm 2.9\%$ mortality after 72 h. The fipronil standard was active at 0.01 μM with 96.7 ± 5.8 to 100 % mortality after 24, 48 and 72 h. In this presentation we will show LC50s and bioassay results for the active

compounds against *Aedes aegypti* adults. These studies revealed novel structures that could lead to discovering new compounds with insecticidal and repellent activity.

60. ISOLATION OF DENGUE 4 IN CLINICAL SPECIMENS FROM VENEZUELA DURING THE OUTBREAK OF ZIKA VIRUS

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Zika, Dengue, Yellow Fever Virus (Flavivirus; Flaviviridae) are on the rise in Venezuela. In April 2016, we began collecting plasma, urine and other specimens from patients who exhibited symptoms of Zika febrile illness and checked into five participating hospitals in Barquisimeto, Venezuela. We present preliminary results based on laboratory cell culture work and molecular diagnostic work. More than 600 patients have been sampled; 142 specimens have been delivered to the University of Florida, and 44 have been grown in cell culture and tested for ZIKV and DENV. We have isolated ZIKV from two patients and DENV4 from two patients who later developed psoriasis. Remaining specimens exhibit cytopathic effects but are negative for ZIKV and DENV. We discuss the clinical symptoms observed and future directions for understanding the epidemic of febrile illness that is occurring in Venezuela.

61. MALARIA IN HAITI: A GENOMIC APPROACH TO ITS EPIDEMIOLOGY AND BIOLOGY

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Malaria in the Caribbean has been eliminated from all islands except Hispaniola. In Haiti, located in the western half of the island, malaria is endemic and becomes epidemic in the rainy seasons. The malaria parasite (*Plasmodium falciparum*) was introduced into Hispaniola through the slave trade, and during this 300 year period ending in early 1800s, ~11 million Africans were brought to the Caribbean. Hispaniola received a large fraction of this total, and a substantial percentage of these individuals carried the parasite, since malaria is holoendemic in West Africa. We hypothesize that in establishing transmission through its new vector (*Anopheles albimanus*), the parasite population underwent bottlenecks and was subjected to powerful selection pressures. Knowing historically when the parasite arrived on the island and the comparative isolation of this parasite population on Hispaniola, represents a unique opportunity to examine the evolution of *P. falciparum*. We have undertaken a detailed study of the genetics of this parasite population utilizing whole genome sequencing. Principal component and phylogenetic analyses based upon single nucleotide polymorphisms (SNPs) indicate that the Haitian parasites have an ancestral relationship with parasites from Africa, and are clearly distinguishable from those found in South America. Loci under selection represent mutations

allowing *P. falciparum* to adapt to the novel vector and environmental conditions on Hispaniola. Mutations of biomedical relevance, such those conferring drug resistance or potentially altering future vaccine efficacies, plus those suited to tracking the movement of the Haitian parasite to other countries, provide information useful for tailoring elimination plans.

62. MOVEMENT, POPULATION STRUCTURE, AND DISEASE PREVALENCE OF CATTLE EGRETS (*BUBULCUS IBIS*): A PROPOSAL

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The distribution of cattle egrets has dramatically expanded in the past two centuries. Native to central Africa, southern Portugal, Spain, and the Asian tropics, cattle egrets are now found in all continents, except Antarctica. Despite their rapid expansion, little is known about cattle egret movements including migration and dispersal, and the potential for cattle egrets to transport harmful pathogens. The objectives of this project are to survey the genetic population structure; to estimate the movement and migration rates of cattle egrets within Florida and along the Mississippi and Atlantic flyways; and to determine cattle egrets' role in disease transmission. Live cattle egrets are trapped and sampled for blood, ticks, feathers, and morphological measurements. Dead cattle egrets are collected from USDA Bird Air Strike Hazard (BASH) control efforts and sampled for blood, ticks, feather, liver, spleen, and morphological measurements. SNP microarrays will be used to survey genetic population structure and estimate movement. PCR and gel electrophoresis will be used to determine prevalence of

Plasmodium, Trypanosoma, Babesia, Ehrlichia, Toxoplasma, Samonella, Dermatophilus, and Anaplasma. This project will provide a better understanding of cattle egret movement and their role in disease transmission.

63. NOVEL APPROACHES TOWARDS QUANTIFYING THE CELLULAR RESPONSES OF THE HOST AND PATHOGEN IN MALARIA TRANSMISSION BIOLOGY

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As proteomic techniques provide a robust means to identify and quantify proteins globally, they are useful tools to unravel the complex host-pathogen interactions of Plasmodium parasites, the malarial pathogens, and in particular explore the developmental programming of the transmission stages of the parasite. In the human host, a small portion of the asexual parasite population undergoes sexual development to become gametocytes, a mosquito transmissible form. Using a Systematic Subtractive Bioinformatic analysis on the NF-54 and Dd2 gametocyte proteomic data, we have established a male- and a female-enriched gametocyte proteome. In addition, three female specific genes PF3D7_0906100, PF3D7_0309100, and PF3D7_1218800 were identified as potential female-enriched biomarkers. Even though characterizing the proteome of early sex-specific gametocyte would help to understand mature gametocytes, it has been a daunting analytical challenge to study the initiation of male-female divergence during the immature gametocyte stages, especially for the male gametocytes, due to their smaller size, reduced number (1 male for every 4 females) and a

lower overall protein abundance per cell. Clearly, new methods to characterize these earlier male gametocyte developmental stages are needed to overcome this obstacle. We postulate that MS1 intensity based label-free quantification is a suitable LC-MS/MS method to characterize the developing gametocyte proteome because: (1) there is no sample loss due to the chemical labeling process; and (2) this approach provides sufficient sensitivity to capture the potential biomarkers in very limited amount of samples. Combined with the subtraction strategy previously demonstrated, we aim to identify sex-specific biomarkers for the stage I-II gametocytes. In the preliminary experiment, 356 proteins and 1462 peptides were identified after a triplicate analysis for the asexual and gametocyte proteome. Based on the signal intensities, retention time consistency and the identification confidence of individual peptides in the LC-MS/MS analysis, 779 peptides were selected for MS1 intensity based quantification. Transmission-blocking vaccine targets like 6-cysteine protein (PF3D7_0209000), osmiophilic body protein G377 (PF3D7_1250100) and putative secreted ookinete protein (PF3D7_0513700) were identified among the gametocyte enriched proteins. In addition, PF3D7_1430800 and PF3D7_1431100, which were previously found in developing gametocytes (stage I/II), were also found enriched in the gametocyte proteome. These results demonstrate that the quantification approach could provide sufficient sensitivity to identify proteins enriched during gametocytogenesis.

64. NOVEL FIELD METHODS UTILIZING EXPERIMENTAL TRAP FOR STUDY OF ARBOVIRAL NATURAL TRANSMISSION CYCLES

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With a recent rise in incidence of diseases caused by arthropod-borne viruses, they have acquired global significance. Many arboviruses are zoonoses, requiring a vertebrate host to propagate the virus. Many gaps in knowledge exist with regards to the natural transmission cycles of arboviruses, including the identification of animal hosts and mosquito vectors. Traditional methods for establishing host and vector species require the experimental inoculation of putative hosts and vectors with virus in a laboratory setting. These methods are hampered by numerous logistical issues, such as the need for experienced personnel, high cost, and lack of suitable animal facilities to study lab-based infections. Therefore, in this proof-of-concept study, we propose a combination of innovative field methods that include the use of an experimental trap, housing a vertebrate host as bait to attract potential mosquito vectors allowing for efficient collection of multiple transmission parameters.

In this study, we examine the role of rodents as hosts of eastern equine encephalitis virus (EEEV; genus Alphavirus, family Togaviridae) using different iterations of the experimental trap. The most successful version trapped the largest amount of mosquitoes (over 50 mosquitoes/night). Eighty-six percent were of the species *Culex erraticus*, 7% *Mansonia titillans*, and 7% *Coquillettidia perturbans*, all species capable of transmitting EEEV. In addition, rodent blood samples were analyzed for the presence of EEEV by qRT-PCR. None had detectable levels of EEEV viral RNA. Key features of the final, successful experimental trap consist of the placement of

a fan to prevent mosquitoes from escaping, the wider diameter of the trap to prevent the rodent from biting through the net, and the addition of more pockets to allow for more efficient aspiration of mosquitoes. This novel field method has demonstrated potential to elucidate important elements of arboviral transmission that may prove especially useful in resource-scarce settings.

65. PREVALENCE AND DISTRIBUTION OF PATHOGEN INFECTION AND PERMETHRIN RESISTANCE IN TROPICAL AND TEMPERATE POPULATIONS OF RHIPICEPHALUS SANGUINEUS LATRIELLE

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The brown dog tick, *Rhipicephalus sanguineus* Latrielle, is a peridomestic, cosmopolitan parasite of dogs known to vector numerous pathogens of veterinary and medical importance. Recent phylogenetic analyses separate this tick into temperate and tropical lineages. Populations of *R. sanguineus* have been reported to exhibit sodium channel target site insensitivity to permethrin, which is likely to be due to the prolonged use of pyrethroids against many pests in and around the home. In this study, populations collected in North America, the Caribbean, Africa, Europe, and Asia were tested to elucidate the population relationship between latitude, resistance mechanisms, and pathogen-vector interactions. Using molecular assays, populations from 29 distinct locations were simultaneously screened for bacterial infection by *Rickettsia*, *Ehrlichia*, and *Babesia* and the presence of a sodium channel single nucleotide polymorphism known to confer permethrin resistance. Both permethrin resistant and susceptible phenotypes were screened. DNA sequencing was used to identify bacteria to species. Implications on *R. sanguineus* management in association with latitudinal distribution will be discussed.

66. RELEVANCE OF AVIAN MALARIA AND OTHER HAEMOSPORIDIA IN THE ARCTIC SHOREBIRD ASSEMBLAGE: INVESTIGATION INTO LIFE-HISTORY CORRELATES AND PHYLOGEOGRAPHIC PATTERNS OF TRANSMISSION

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Migration is accompanied by extreme physiological and energetic demands, hence evolution of such a complex and costly behavior must be highly beneficial. Although migrations has been postulated as a mechanism that reduces prevalence of infectious diseases in host populations (i.e., migratory escape and migratory culling), the broad geographic ranges and diverse habitats encountered throughout the annual cycle of migrants may actually increase infection rates and contribute to the emergence and spread of disease agents. Some migratory species experience a disproportionate infection risk due to underlying differences in life-history traits that alter the exposure and susceptibility to disease agents. We evaluate the transmission ecology of vector-borne diseases and their implication on migration facilitated movement using haemosporidian parasites of Arctic-breeding shorebirds as a model system. Furthermore, as current and future anthropogenic stressors threaten the long-term viability of shorebird populations, we evaluated how life-history traits determine patterns and dynamics of disease transmission. We found significant correlations between haemosporidia prevalence and life history traits that affect shorebirds' exposure to disease agents. Overall, species with large body size and preference for freshwater habitats exhibited a higher prevalence of haemosporidia. This lends support not only to the habitat selection hypothesis, which proposes differences in parasite pressure due to habitat use but also suggests that species already vulnerable to decline may be further decimated due to infection with vector-borne disease agents. Life-history traits not only shape

haemosporidia infections patterns of migratory shorebirds, but also appear to mitigate transmission dynamics throughout the Western Hemisphere. We found that Arctic transmission of haemosporidia significantly contributed to the observed infection prevalence as well as indication of migration mitigated spread of these disease agents. Taken together, our findings suggest that while migratory shorebirds contribute to the spread of vector-borne disease agents, the contribution is likely species-specific due to underlying differences in life-history traits. Lastly, the evidence of Arctic-transmission may be reflective of changes in climatic conditions that allowed for a northward expansion of vector-borne disease transmission and thus highlights the importance of continued surveillance of these disease agents in the Arctic.

67. SPATIAL HETEROGENEITY CAN UNDERMINE THE EFFECTIVENESS OF COUNTRY-WIDE TEST AND TREAT POLICY FOR MALARIA: A CASE STUDY FROM BURKINA FASO

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Background: Considerable debate has arisen regarding the appropriateness of the test and treat malaria policy broadly recommended by the World Health Organization. While presumptive treatment has important drawbacks, the effectiveness of the test and treat policy can vary considerably across regions, depending on several factors such as baseline malaria prevalence and rapid diagnostic test (RDT) performance.

Methods: To compare presumptive treatment with test and treat, generalized linear mixed effects models were fitted to data from 6510 children under five years of age from Burkina Faso's 2010 Demographic and Health Survey.

Results: The statistical model results revealed substantial regional variation in baseline malaria prevalence (i.e., pre-test prevalence) and RDT performance. As a result, a child with a positive RDT result in one region can have the same malaria infection probability as a demographically similar child with a negative RDT result in another region. These findings indicate that a test and treat policy might be reasonable in some settings, but may be undermined in others due to the high proportion of false negatives.

Conclusions: High spatial variability can substantially reduce the effectiveness of a national level test and treat malaria policy. In these cases, region-specific guidelines for malaria diagnosis and treatment may need to be formulated. Based on the statistical model results, proof-of-concept, web-based tools were created that can aid in the development of these region-specific guidelines and may improve current malaria-related policy in Burkina Faso.

68. SPATIOTEMPORAL DYNAMICS OF DENGUE VIRUS IN PUERTO RICO, 2009-2014

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Dengue virus is a prominent vector-borne disease that has established its global dominance since the mid-20th century, with current estimates suggesting that about 40% of the world's population is at risk of infection. Since the 1960s, Puerto Rico has experienced reoccurring outbreaks of dengue every 3-5 years, with the largest recorded outbreak occurring in 2010. While there have been numerous studies investigating the spatial patterns of dengue, few have actually focused on analyzing the microscale spatiotemporal transmission dynamics. Here, we analyze the point locations of 6149 dengue infections from 2009-2014 in the northeast region of Puerto

Rico, including the city of San Juan. DENV1 and DENV4 accounted for more than 99% of the cases in the data set. To analyze the spatial dependence of cases, we use the phi function to calculate the relative probability of a pair of cases occurring in the same window of space and time, versus if their clustering in space and in time were independent. This function accounts for heterogeneities in underlying population structure that could create clustering, such as seasonal factors or population density differences. Here, we assess clustering in cases of all serotypes and in homotypic cases of a single serotype. We explored the dependence of cases that were 100m apart, within 15 days of each other, and within a 30km² area. We observed specific spatiotemporal clustering ($\Phi > 1.0$) up to 10 kilometers among all serotypes. Similarly, DENV1 showed spatiotemporal clustering up to about 10km, which was expected since DENV1 cases account for more than 77% of all infections. DENV4 cases revealed a spatiotemporal signal up to about 2km. Preliminary results suggest that there is some spatiotemporal clustering in the San Juan region, which may be due to numerous factors. Work still remains to understand these clustering patterns in heterotypic cases and stratified by age.

69. SPATIOTEMPORAL EVOLUTION AND RECOMBINATION OF MAYARO VIRUS ISOLATES COLLECTED OVER 60 IN THE AMERICAS: THE DAWN OF A NEW EPIDEMIC?

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Mayaro virus (MAYV), the causative agent of Mayaro fever, is an arbovirus transmitted by the *Haemagogus* species of mosquito endemic to the Amazonian forest regions in South America. MAYV has received recent attention in the field of infectious diseases due to the identification of several cases in Brazil, Mexico and the Caribbean. Despite its role in a highly debilitating disease and recent evidence of spread areas outside of the Amazonian regions of Central and South America, limited information about the evolution and the epidemiology of MAYV represent an important barrier to prevention of further spread. We present a thorough study of the evolutionary and epidemiological history of MAYV strains collected within the Amazon basin, São Paulo State, and Haiti. A data set including all currently available full-genome viral sequences was

assembled to quantify codon usage and adaptability, which revealed specific adaptations to a broad host and vector range, including humans and the *Aedes* mosquito species, and assess potential recombination events. Bayesian phylogeography based on molecular clock calibrated genealogies was used to investigate the spatiotemporal spread of MAYV lineages in South American and the Caribbean areas, as well as to infer the origin of detected recombination events. The analysis showed that the first recombinant strain appeared between 2002 and 2012 in Pará State (Brazil), and moved to São Paulo State, giving rise to a second recombinant that was eventually isolated in Haiti in 2015. We hypothesize that human mobility and adaptability to a broad range of host and vector species played a central role in the emergence of recombinant strains, which are usually rare among arboviruses. Moreover, the potential urbanization of this virus might be the beginning of a much larger, more global epidemic and deserves, therefore, close monitoring in the immediate future.

70. SURVEYING FOR HEMORRHAGIC DISEASE IN FLORIDA WHITE-TAILS (*ODOCOILEUS VIRGINIANUS*): A COMPARISON OF ON- AND OFF-RANCH CERVIDS

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The growth of the cervid farming industry has led to many questions as to what constitutes a healthy deer, and in the event of illness, what pathogens are responsible. Following an outbreak of hemorrhagic disease in Florida cervids in 2012, the need for disease

recognition, rapid diagnosis, and an understanding of deer health became crucial to the industry. Hemorrhagic disease is caused by two orbiviruses: epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV). In order to understand how density affects hemorrhagic disease prevalence and exposure levels, we bled high-density farmed white-tails, on-ranch but unfarmed 'preserve' animals to represent a mid-range density, and low-density wild white-tails from an adjacent wildlife management area. These samples were collected during the spring of 2016 and were then assessed via qPCR and serum neutralization assays to determine overall prevalence and circulation of EHDV and BTV. We report no EHDV- or BTV-positive qPCR results during this time period. However, there was an overall 92% (76/83) seroprevalence for EHDV across sampling locations, indicating most animals are exposed to EHDV. Captured wild cervids displayed a 57% (8/14) seroprevalence for at least one EHDV serotype, while farmed deer kept at artificially high densities displayed a 98% (51/52) seroprevalence. This implies that deer kept at artificially high densities may be more prone to exposure with EHDV. Understanding how these viruses circulate and which factors influence this can lead to more informed management practices in the cervid farming industry.

71. SYNERGISTIC EFFECT OF PERMETHRIN WITH POTASSIUM CHANNEL BLOCKERS ON ANOPHELES GAMBIAE

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Chemical insecticides remain a major component of vector control, and resistance to some chemicals, especially pyrethroids, challenges our efforts to control vectore borne diseases. The purpose of this research was to explore the possibility of co-applying permethrin (an agonist of voltage-sensitive sodium channels) with the potassium channel blockers 2S-65465 and 4-aminopyridine (4-AP), in order to potentiate the neurological effect of this pyrethroid and reduce the amount of permethrin that is needed for effective control of *An. gambiae*. Voltage-sensitive sodium and potassium channels work coordinately for the proper initiation and maintenance of action potentials and electrical excitability in nerve. We hypothesize that the ability of pyrethroids to cause persistent sodium currents will be augmented by blockage of outward postassium current flow, which normally repolarizes the membrane potential during a nerve membrane action potential. Topical treatments were performed on the insecticide-susceptible *An. gambiae* G3 strain. Permethrin had a 24 hr LD50 value (lethal dose for 50% mortality) of 0.26 (0.19-0.43) ng/mg of mosquito weight; the LD50 values of the compounds 4-AP and 2S-65465 were 0.49 (0.39-0.60) µg/mg and 0.12 (0.10-0.16) µg/mg, respectively. Co-application of compound 2S-65465 at 125 ng/mosquito (a dose causing 10% mortality, the LD10) with increasing amounts of permethrin showed an LD50 value for permethrin of 0.03 (0.01-0.16) ng/mg, which was around 9-fold lower than permethrin alone. Another significant aspect of pyrethroid toxicity is rapid knockdown (mosquitoes are unable to stand or fly normally long before death ensues). Topical treatment with permethrin at 0.02 ng/mosquito (3% knockdown) and 0.04 ng/mosquito (3% knockdown), along with 2S-65465 at the LD10 dose

(12% knockdown) increased 1 h knockdown to $54 \pm 14\%$ and $63 \pm 9\%$, respectively. Poisoned mosquitoes often shed their legs, and in identical combination treatments, the number of legs shed per ten mosquitoes at both doses of permethrin were significantly ($p < 0.05$, t-test) increased by 2S-65465, 167% and 229%, respectively, compared to permethrin alone. Similarly, compound 4-AP at 25 ng/mosquito (3% mortality) combined with 0.04 ng/mosquito permethrin (16% mortality) significantly increased mortality of the G3 strain to $50 \pm 12\%$. These data suggest that co-application of either potassium channel blocker with permethrin can synergistically increase the mortality of an *An. gambiae* susceptible strain. Further experiments using this co-application method will be performed using other candidate compounds, as well as on a pyrethroid-resistant strain of *An. gambiae*.

72. THE EFFECTIVENESS OF ANTIVIRAL AGENTS WITH BROAD-SPECTRUM ACTIVITY AGAINST CHIKUNGUNYA VIRUS VARIES BETWEEN HOST CELL LINES.

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Introduction: Chikungunya virus (CHIKV) is a mosquito-borne virus that has recently emerged in the Western Hemisphere affecting many countries including the United States. Currently there are no approved vaccines or antiviral treatments for CHIKV-infected patients. This study aims to evaluate the therapeutic potential of commercially available broad-spectrum antivirals against CHIKV. Vero cells are typically the gold standard for evaluating the antiviral effectiveness of agents against arboviruses, including CHIKV. We hypothesized that antiviral activity of potential anti-CHIKV agents may differ depending on the host cell line used for evaluation. Thus,

an objective of this study was to determine the role of host cell line in preclinical antiviral assessments.

Methods: The effectiveness of ribavirin (RBV), interferon-alfa (IFN), and favipiravir (FAV) were assessed against the vaccine strain of CHIKV (181 clone 25). Human lung cells (A549), human liver cells (HUH-7), and African green monkey kidney cells (Vero) were infected with CHIKV and treated with varying concentrations of IFN, RBV, or FAV for three days. Viral supernatant was sampled daily and infectious virus was quantified overtime by plaque assay on Vero cells. Cytotoxicity profiles of RBV, IFN, and FAV were evaluated in all three cell lines using the ViralToxGlow assay.

Results: The degree of antiviral activity for each agent was dependent on the host cell line used for evaluation. IFN was most effective against CHIKV in A-549 cells ($EC_{50} = 4.235$ IU/mL) followed by HUH-7 cells ($EC_{50} = 26.58$ IU/mL). IFN failed to provide robust protection in Vero cells ($EC_{50} = 1344.6$ IU/mL). RBV had the greatest antiviral effect in HUH-7 cells ($EC_{50} 2.575$ μ g/mL), followed by Vero cells ($EC_{50} = 99.56$) then A-549 cells ($EC_{50} = 117.1$). RBV activity in HUH-7 cells is likely due to toxicity, as cytotoxicity analyses demonstrated that HUH-7 cells are more vulnerable to RBV-related toxicity compared to A-549 and Vero cells. For FAV, the greatest activity was observed in Vero cells ($EC_{50} 54.60$ μ M) followed by HUH-7 ($EC_{50} = 136.1$ μ M), then A-549 cells ($EC_{50} = 538.0$ μ M).

Conclusion: The results of these studies demonstrate that antiviral effect against CHIKV is influenced by the cell line used for antiviral evaluations. It is imperative to consider not only species but also tissue of origin when selecting cell lines for preclinical drug trials.

73. THE SOCIAL ECOLOGY OF DENGUE PRESENCE AND BURDEN DURING AN OUTBREAK IN GUAYAQUIL, ECUADOR, 2012

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Dengue, an arboviral disease, is an ongoing public health problem in Ecuador and tropical Latin America. Vector control remains the primary method of controlling dengue, necessitating localized control efforts. We examined the presence and burden of dengue in Guayaquil, Ecuador, a coastal port and the largest city in the country (pop. 2,350,915). There were 4,248 clinical cases of dengue fever reported to the Ecuadorian Ministry of Health in Guayaquil during 2012, or a disease incidence of 18.07 cases per 10,000 population. Data provided by the 2010 Ecuadorian National Census were used to test if social-ecological factors were related to dengue transmission. We conducted multimodel selection (criterion=AICc) using the R package “glmulti”: logistic regression search to identify factors related to the presence or absence of cases and a negative binomial regression search to identify factors related to the localized case burden. Risk factors for dengue presence included in the top logistic model (AICc=369.85) included poor housing condition, road access, and households receiving remittances; access to some municipal services was positively correlated with dengue presence. Risk factors

for dengue burden included in the top negative binomial model (AICc=2920.67) included poor housing condition, remittances, education, and demographic variables. These variables are spatially diverse within Guayaquil, thus giving us a better understanding of why some areas within the city have an elevated risk for dengue outbreak. Both models indicate that housing condition is the largest predictor of both dengue presence and burden, while counterintuitive correlations may indicate the effect of population density on dengue transmission.

74. TOWARDS A LYMPH NODE TARGETING NANOPARTICLE BASED VACCINE DELIVERY PLATFORM

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Transmission blocking vaccines (TBVs) are considered as one of the key interventions for global malaria elimination and eradication. TBVs function by blocking the mosquito transmission of both drug-resistant and susceptible parasites from one individual to another. One such well-characterized TBV candidate is the Anopheline Alanyl aminopeptidase N (AnAPN1), a mosquito midgut surface protein which mediates Plasmodium establishment in the mosquito. AnAPN1 antibodies completely block (100% inhibition) the development of naturally circulating isolates of Plasmodium falciparum. However, TBVs exhibit lack of natural boosting, as the target antigen is present inside the mosquito. To circumvent these concerns, this study proposes to develop a “mixed mode” biodegradable nano-/micro-particle TBV delivery system by engineering size-controlled lymph node targeting biodegradable nanoparticles for effective delivery and presentation of AnAPN1 and biodegradable microparticles for sustained release of AnAPN1 to permit continued boosting thus enabling a single dose vaccine producing a superior immune response in the vaccinated individual.

75. TOXICOLOGICAL INVESTIGATION OF PERMETHRIN AND ETOFENPROX RESISTANCE OF TWO LABORATORY STRAINS OF AN. GAMBIAE

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The African malaria mosquito, *Anopheles gambiae*, is the primary vector of malaria in sub-Saharan Africa, and is responsible for the disproportionately high burden of global malaria. Chemical insecticides play a pivotal role in decreasing mosquito populations, and therefore the transmission of malaria. However, the continued overuse of insecticides, primarily pyrethroid compounds, has resulted in mosquito developing insecticide resistance. Two common types of insecticide resistance are an increase in the Phase-I and Phase-II metabolic systems, and target site modification. Knockdown resistance (kdr) is the reduced sensitivity of the nervous system caused by point mutation(s) in the insect voltage-sensitive sodium channel. The presented research will focus on characterizing the toxicity and resistance of two *An. gambiae*, G3 (pyrethroid-susceptible) and Akron-kdr (pyrethroid-resistant) strains, to permethrin and etofenprox. Toxicological studies indicate that resistance with the *An. gambiae* Akron-kdr strain is more evident with permethrin (resistance ratios between 12 and 100) versus etofenprox (resistance ratios between 3 and 36) at three life stages (first-instar larvae, forth-instar larvae, and adult female mosquitoes) with forth-instar larvae being the most resistant. A pre-treatment of piperonyl butoxide (PBO) to adult female mosquitoes of the Akron-kdr strain synergized the toxicity of permethrin and etofenprox indicating that resistance may be the result of a complex between target site modifications (point-mutations) and phase-I metabolism. This indicates that toxicological studies provide the best biomarker for the detection of resistance, compared to molecular techniques,

and etofenprox has decreased resistance compared to permethrin to a resistant laboratory strain of *An. gambiae*.

76. USING A BAYESIAN GEOSTATISTICAL MODEL TO UNDERSTAND LOCAL-SCALE HETEROGENEITY OF MALARIA RISK: THE EXAMPLE OF BUNKPURUGU-YUNYOO DISTRICT IN NORTHERN GHANA

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Bayesian methods have been used to generate country-level and global maps of malaria risk on a large geographical scale but these maps may lack the ability to identify smaller scale heterogeneity and thus may not be ideal for operational prevention and control of malaria. The aim of this research is to apply methods to high resolution malaria data to understand if we can better predict the micro-scale spatial heterogeneity. We will rely on existing malaria parasitemia survey from northern Ghana, consisting of 10,366 children from 438 GPS-located communities sampled between November 2010 and 2013 bi-annually. The Bayesian hierarchical model was chosen to account for individual and community level drivers whilst accounting for both spatial correlation and random variation between communities. It also used both a Gibbs sampler and Metropolis Hastings algorithm. Prior to model runs, Gibbs variable selection was used to determine key variables selected based on availability of spatial information and were chosen to represent both environmental - such as elevation, temperature, NDVI, rainfall - and GIS-derived demographic factors such as distance to health facility, urban centres, roads and water bodies. Model selection revealed elevation, distance to urban centre and distance to health facilities to be important covariates. The geostatistical

model showed that malaria prevalence varied between 19% and 90%, showing a north-east to south-west gradient of predicted risk with the highest prevalence rates found at lower elevation. The general distribution is heavily weighted between the two urban centres, showing lower risk in urban centres compared to rural areas, with some indication that a threshold distance to an urban centre exists for malaria risk. Model predictions revealed high variability in malaria prevalence in areas previously assumed to be homogenous, indicating important short-comings of country level spatial modelling from a programmatic perspective. These types of model can be useful for predicting on ground risk in similar regions for program planning.

77. YELLOW FEVER OUTBREAKS AND THE IMPORTANCE OF GOOD VACCINE AVAILABILITY

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Yellow fever (YF) is an acute arboviral disease caused by infection with the yellow fever virus (YFV). The YFV is transmitted by infected mosquitoes and it is endemic in tropical areas of Africa and Central and South America. There are an estimated 130,000 cases of YF annually, causing 44,000 deaths worldwide each year. There is no specific treatment for YF. Most of the infections are asymptomatic and the symptoms are similar to other flavivirus infections including fever, headache, muscle pain, nausea, and fatigue. Approximately 10% of the patients infected by the virus develop severe symptoms and half of those die within 7 to 10 days.

Since January 2016, an urban yellow fever outbreak started in the district of Luanda, Angola and expanded to all the 18 provinces of Angola. The outbreak was controlled in June 2016, with a total of

3,137 suspected cases reported, including 345 deaths. Given the strong cultural and economic ties of Angola with Brazil, and the history of YF transmission in the region it was expected to start seeing transmission of YF in Brazil. On January 6th, 2017 Brazil reported local transmission of YFV. By February 1st, 2017 Brazil has confirmed YF transmission in five states, with 857 human cases and 150 deaths reported. We analyze the time series of yellow fever incidence from WHO (1980-2016) and the YF vaccination coverage through the years to understand better the transmission dynamics of YF.

78. A BIVALENT O-ANTIGEN POLYSACCHARIDE VACCINE DELIVERED BY ATTENUATED SALMONELLA PROVIDES PROTECTION AGAINST AVIAN PATHOGENIC ESCHERICHIA COLI O1 AND O2 INFECTION

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Avian pathogenic *Escherichia coli* (APEC) are a leading cause of avian airsacculitis and colibacillosis, which is responsible for significant economic loss in the poultry industry. Avian pathogenic *Escherichia coli* has numerous serogroups due to the diverse O-antigen structure, of which, O1, O2 and O78 are the predominant serotypes. O-antigen is also a highly immunogenic and protective determinant. Therefore, the vaccines based on polysaccharide antigens from these three predominant serotypes are attractive to prevent most APEC infections. In our previous work, we have utilized a novel yeast/bacterial shuttle vector to clone the 10.3 kb operon encoding the O1 O-antigen polysaccharide, providing protection against APEC O1 infection when delivered by attenuated salmonella. In this study, we applied the same method to clone an operon encoding O2 polysaccharide, which is larger than 15 kb. The positive plasmid was introduced into an attenuated salmonella strain followed by LPS profile detection and immunological efficacy identification. We

demonstrate that birds immunized with the mixture of S739 (O1) and S739 (O2) induced significant anti-LPS serum IgG and mucous IgA antibody against both APEC O1 and O2, and showed a 66.7% and 73.3% protection against virulent O1 and O2 APEC strain challenge, respectively. The protection efficacy was also evidenced by histopathologic section as well as serum bacterial killing analysis, and opsonisation of bacteria by vaccinated antibodies. The data indicate that the mixture vaccine of S739 (O1) and S739 (O2) is a potential bivalent vaccine against APEC O1 and O2 infection.

79. BAYESIAN SPATIO-TEMPORAL MODELS FOR INFERENCE ON EPIDEMICS USING DATA FROM MULTIPLE SOURCES

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Public health data from multiple sources are becoming increasingly available and are critical components of decision- and policy-making by public health professionals. However, multiple sources may report data at different levels of stratification (e.g., overall totals or totals by age group) and may report with varying levels of accuracy. In this work we develop a spatio-temporal model for combining disease reporting from multiple sources at different strata. The proposed method uses novel constraints to link the multiple data sources and accounts for possible misreporting on one or more data source. Spatial and temporal correlations are also accounted for to provide reliable inference and predictions on an epidemic. We use simulation studies to evaluate the performance gain by combining multiple data sets, and we use the method to provide inference on the cholera outbreak in Haiti.

80. CEFTAZIDIME RESISTANCE IN BURKHOLDERIA PSEUDOMALLEI IN ABSENCE OF EXTERNALLY ACQUIRED RESISTANCE DETERMINANTS

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Burkholderia pseudomallei (Bp) causes melioidosis, an emerging infectious disease syndrome endemic in the tropical regions of the world. Ceftazidime (CAZ) is a cephalosporin, which is effectively used for acute phase melioidosis therapy. Although CAZ resistance (CAZR) is still rare, it does occur in response to therapy and may be more widespread than previously thought. Unlike other Gram-negative bacteria, antibiotic resistance in Bp seems exclusively mediated by chromosomally encoded determinants. Target deletions and enzymatic inactivation are the two major clinically significant resistance determinants characterized thus far in some detail. Deletion of a penicillin binding protein 3 has been documented in clinical isolates that failed CAZ therapy. However, the main player in CAZR is a Class A PenA β -lactamase. Previous studies showed that PenA is exported via the twin arginine translocase system and is an outer membrane (OM)-bound lipoprotein. The latter likely facilitates its OM vesicle localization. Emergence of CAZR not only requires penA mutations that lead to PenA amino acid substitutions in or near conserved domains, but such mutations are frequently accompanied

by a conserved G to A transition near the base of an inverted repeat structure at position -78 upstream of the penA initiation codon. We showed that 1) the G to A transition generates a promoter, which is active in *Bp* and *Escherichia coli* and leads to high-level penA transcription; and 2) the stem loop structure functions as transcriptional terminator. The β -lactamase inhibitor avibactam (AVI) completely restores CAZ susceptibility (CAZS) in the otherwise CAZR isolates than contain PenA structural mutations and in some instances also the promoter mutation. We identified a CAZR Thai clinical isolate, 5041a, that did not contain any mutations in the penA region. Since AVI restored CAZS in this strain, CAZR was likely mediated by a β -lactamase. Unlike strains with mutations in the penA region where the CAZR phenotype is stable, 5041a spontaneously reverted to CAZS at a frequency of 0.25-0.5%. Whole genome sequencing showed that a 33,000 base pair region of chromosome 2 containing penA is amplified in 5041a and that the CAZR of this strain is likely caused by penA gene amplification. Finally, genetic analysis of a CAZR Australian clinical isolate indicated that CAZR is complex and besides target deletions and mutations affecting PenA involves other genes and proteins. Our studies inform strategies aimed at rescuing the activity of existing antibiotics with anti-*B. pseudomallei* activity, as well as efficacy evaluation of new β -lactam antibiotics.

81. CHLAMYDIAL CTL0382 HAS PEPTIDOGLYCAN HYDROLASE ACTIVITY CAUSING LYSIS OF ESCHERICHIA COLI

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Chlamydial species have an essentially complete set of genes required for peptidoglycan (PG) biosynthesis to build a cell wall. The PG structure in Chlamydia is assembled as a ring and is limited to the division plane during the replicative phase and is assumed to be required for cell enlargement, division, and transition between developmental forms. In *Escherichia coli*, cell division and daughter cell separation require hydrolysis of the PG at the division plane that involves at least three different, tightly regulated PG hydrolases. In Chlamydia, the cell division amidase, AmiA, is the only hydrolase thus far shown to break the bond between L-alanine in position 1 of the stem peptide and N-acetylmuramic acid. Chlamydial AmiA also mediates daughter cell separation in an *E. coli* mutant lacking its own amidases. PG hydrolases are also involved in recycling of PG substrates by removing old PG and supplying the substrates to assemble new PG. Here, we report that *C. trachomatis* has an additional potential PG hydrolase, CTL0382. It belongs to the NlpC/P60 family which contains D,L endopeptidase activity that hydrolyzes the D- γ -glutamyl-meso-diaminopimelate linkage in cell wall peptides. We cloned CTL0382 under control of an arabinose-inducible promoter in plasmid pBAD33. Overproduction of CTL0382 in the cytoplasm of *E. coli* had no effect on growth and morphology. However, when targeted to the periplasmic space by fusing the signal sequence from DsbA at the N-terminus, CTL0382 inhibited cell growth and caused active cell lysis in *E. coli* as observed by microscopic examination. These results suggest degradation of the *E. coli* PG sacculus by CTL0382. We are purifying CTL0382 to characterize enzyme specificity and kinetics. We are also

investigating the role of CTL0382 in Chlamydial cell division and PG recycling.

82. COMPARING CHROMOSOMAL REARRANGEMENT IN ORAL INFECTIONS-ASSOCIATED BACTERIA

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Objectives: In this study, we identify and evaluate evolutionary changes in bacteria associated with oral infections, namely periodontitis (gum infection) and dental caries (cavities).

Material and Methods: We selected bacterial strains based on their disease association and the availability of full genome sequences. For periodontitis causing bacteria, we focused on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. For dental caries causing bacteria, we focused on *Streptococcus mutans*. Genomes from each species were aligned against a reference genome using Mauve software. The evolutionary changes in aligned sequences were evaluated, by assessing locally collinear blocks (LCBs), to identify chromosomal rearrangements, reverse insertion, conserved regions, and estimate overall genetic diversity. T-test was used to compare difference in rearrangement between bacteria associated with periodontitis and dental caries.

Results: The total number of currently available genomes was 25 (six *S. mutans*, 10 *A. actinomycetemcomitans*, and nine *P. gingivalis*). The alignment revealed variable level of chromosomal rearrangement among the genomes of each species. Among periodontitis bacteria, *A. actinomycetemcomitans* genomes alignment exhibits extensive LCBs rearrangements, and shorter LCBs (smaller weight) compared

to *P. gingivalis*. Both species exhibited comparable level of reverse and/or insertions. In *S. mutans*, the observed evolutionary changes were significantly lower than those of periodontitis associated bacteria. Nevertheless, diversity between genomic regions was still significant in the *S. mutans* alignment.

Conclusions: Bacteria associated with periodontitis exhibited significantly higher rearrangements compared to bacteria associated with dental caries (p-value=0.006). This difference could be attributed to the stringent periodontal environment and the stress created by variable chemotherapeutic agents used in periodontal disease therapy compared to teeth environment and dental caries therapy. The study demonstrates the considerable evolutionary changes in the evaluated oral pathogens, especially chromosomal rearrangements, and their potential association with specific pathogenic processes.

83. DYNAMIC MODELING OF CHOLERA TRANSMISSION IN OUEST DEPARTMENT OF HAITI

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The challenge with cholera modeling, like with many other infectious diseases, is the course of the disease. The majority of the infections remain asymptomatic and, therefore, unobserved. Underreporting causes a lot of data discrepancies since not everyone who develops symptoms is actually properly reported and recorded. It is believed that external water reservoirs of bacteria and environmental factors such as temperature and precipitation play significant roles in

triggering transmission of cholera and facilitating further spread of the epidemic. All that should be addressed in the analysis.

We present the model that incorporates the incidence and the environmental data that are typically available from surveillance, and apply our model to the data collected in Haiti. When a new epidemic arises it is crucial to react appropriately. Proper public health measures such as vaccination campaigns can curb the epidemic or even stop it completely. We evaluate the effectiveness of various vaccination campaigns in our model by producing the epidemic outcomes under vaccine intervention. We also investigate the behavior of the model over a period of ten years and evaluate the possibility of future outbreaks.

84. EFFECTS OF ANTIBIOTIC TREATMENT ON BACTERIAL COMMUNITIES ASSOCIATED WITH CITRUS TREES BASED ON 16S RRNA HIGH-THROUGHPUT SEQUENCING APPROACH

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The increasing prevalence of antibiotic-resistant bacteria is a global threat to public health, and although agricultural use of antibiotics is believed to contribute to the spread of antibiotic resistance, little is known about its impact on the soil microbiome including “resistome” (antibiotic resistant bacteria + antibiotic-resistance genes). Thus, assessing the effects of the application of antibiotics on microorganisms is of paramount importance to evaluate the risks on

the natural bacterial microbiomes in the environment. In this study, we investigated potential effects of penicillin injection on rhizospheric and endophytic bacterial communities of grapefruit trees in field and greenhouse experiments. Trees were injected with two doses of penicillin G, or water for control trees. Field trees were affected by citrus greening or Huanglongbing (HLB), caused by the α -proteobacterium *Candidatus Liberibacter asiaticus* (Las). We conducted serial sampling over time from petioles and roots from 16 field trees and 18 greenhouse trees for a total of 130 samples that were subjected of 16S rRNA high-throughput sequencing. Bacterial sequences from citrus petioles and roots were taxonomically assigned to 48 bacterial phyla with Proteobacteria accounting for 71% of the sequences. Principal components analysis (PCA) based on weighted-UniFrac distances revealed that each sample source was composed of a unique microbiome community. For each sample source, we evaluated the effect of penicillin injection on the microbial community. We did not observe differentiation of bacterial communities based on treatment in any of the sample sources, only a temporal shift in the communities. Using Linear Discriminant Analysis, we identified particular groups that were significantly more abundant in treatments or in controls, for example, an overabundance of Bacilli in field roots+rhizosphere samples 24 hours after treatment as compared to controls, followed by a shift to overabundance of Actinobacteria on the treated samples compared to the controls on day 7. We have also obtained 16S-data from subcultured colonies exposed to high concentrations of penicillin on dilute S-medium. Among the 41 bacteria taxa recovered from those plates, 18 are described as unculturable in the literature. Overall, our results suggest that the occurrence and abundance of particular groups are affected by penicillin treatments, which is dose dependent. However, the bacterial communities as a whole were not significantly different, which may be due to the great diversity of tree microbiomes. To evaluate the impact on the resistome we will need to evaluate the antibiotic resistance genes (ARGs) their composition and their movement among bacteria groups.

85. EFFECTS OF VANADIUM ON INNATE IMMUNE RESPONSES INDUCED BY STREPTOCOCCUS PNEUMONIA, STAPHYLOCOCCUS AUREUS, IL-1 β AND LPS IN MOUSE AIRWAY EPITHELIAL CELLS.

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The immunotoxicity of V₂O₅-containing air pollutant particulates in the lung has been extensively studied. However, its effect on innate immune molecules such as antimicrobial peptides (AMP) remains to be elucidated. AMPs secreted by airway epithelial cells are part of the initial response to inhaled microorganisms. AMPs, including β -defensins, kill microorganisms and modulate the initial local immune response in the airway. We showed that in vitro, air pollutant particulate matter (ROFA) containing vanadium (VV) inhibited this response in human airway epithelial cells at noncytotoxic levels. Specifically, V₂O₅ and VOSO₄ suppress β -defensin-2 responses to LPS and IL- β in a dose-dependent manner in primary bovine and human airway epithelial cells. We hypothesized that mouse airway epithelial cells would respond similarly. Differentiated primary epithelial cells derived from tracheas (TEC) of C57BL/6 mice (WT), mice lacking the β -defensin-1 gene (KO) and a mouse alveolar type II epithelial cell line (MLE15) were used to model innate immune responses. Triplicate cultures were treated with 10 μ g/ml V₂O₅ or VOSO₄ for 6 hr, washed, then stimulated with medium alone, 100 ng/ml LPS, 100 ng/ml IL-1 β or 1000:1 bacteria:cell for 24 hr. QRT-PCR assessed mBD-3, mBD-14, KC, IL-6 and CCL2 mRNA. In WT and KO mouse TEC, LPS and IL-1 β increased mBD-3 and mBD-14 mRNA, although responses in KO mice were much lower than WT. V₂O₅ inhibited induction of mBD-14 but not mBD-3 in WT TEC by IL-1 β ; it had no effect on their induction by LPS. V₂O₅ enhanced LPS-induced mBD-3 but had no effect on LPS-induced mBD-14 in WT TEC. VOSO₄ induced mBD-3 and mBD-14, yet inhibited *S. pneumonia*-induced mBD-3. IL-1 β and *S. aureus*-induced mBD14 was enhanced by VOSO₄, but KC induction was not affected. In KO TEC, V₂O₅ did not

inhibit IL-1 β induction of mBD-3 and mBD-14 but induced mBD-3 and -14 in WT TEC and only mBD-14 in KO TEC. In MLE15 cells, no mBD-3 was expressed and LPS had no effect on mBD-14, but basal levels of mBD-14 were inhibited by V2O5. Thus, mouse and human primary airway epithelium are similar only with respect to V2O5 suppression of IL-1 β -induced β -defensins, whereas the responses of MLE15 cells do not resemble the responses of human A549 cells. In addition, it appears that the mBD-1 gene coordinates with mBD-3 and mBD-14 in innate immune responses of the airway epithelium. Funded by NIEHS 1R03ES016851 +S1.

86. EFFICIENT PEPTIDOGLYCAN RECYCLING PROTECTS CHLAMYDIA TRACHOMATIS FROM IMMUNE RECOGNITION

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Release of peptidoglycan fragments by intracellular bacterial pathogens and the detection by peptidoglycan-recognizing proteins and NOD receptors stimulate immune response leading to clearance. However, the intracellular pathogen *Chlamydia trachomatis* that causes sexually transmitted infections and infectious blindness, synthesizes significantly less peptidoglycan than other bacterial pathogens. As a result, a very limited or no exposure of peptidoglycan to immune cells allows *C. trachomatis* to persist intracellularly without being completely cleared by the immune system. Peptidoglycan recycling by a bacterial pathogen limits its extracellular release preventing immune recognition and this process seems to be efficient in *C. trachomatis*.

However, the major ATP Binding Cassette (ABC) transporter (mppA/oppB, C, D, F) importing murein tripeptides remains unknown in *C. trachomatis*. We used sequence homology to identify the putative sub-units of a murein tripeptide ABC type transporter in *C. trachomatis*. The transmembrane subunits (oppB, C), and the ATPase

subunits (oppD, F) are annotated in the *C. trachomatis* genome but no mppA homolog exists in the genome. Instead three paralogs (oppA1, A2, A3) are present. Sequence homology of the Chlamydial oppA3 to mppA of *Escherichia coli* and a closer phylogenetic relationship of the Chlamydial oppA3 to the mppA orthologous of different lineages of bacterial pathogens indicate oppA3 to be the actual mppA required to form the complete transporter. We are investigating the Chlamydial mppA-like genes (oppA1, A2 and A3) in *E. coli* mutants lacking mppA. Identification of the true peptidoglycan transport system and its transport kinetics will be useful to design cell-wall recycling inhibitors for the clearance of persisting *C. trachomatis*.

87. EVALUATING THE FSMA STANDARD FOR BACTERIOLOGICAL QUALITY OF AGRICULTURAL WATER FOR PRODUCE GROWING

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Introduction: The Food Safety Modernization Act (FSMA) brought new regulations concerning the bacteriological quality of irrigation water used for fresh produce. Microbial criteria in the form of geometric mean (GM, 50th-percentile of a lognormal distribution) and statistical threshold value (STV, 90th-percentile of a lognormal distribution) calculated from 20 samples of *Escherichia coli* levels are used to ensure adequate water quality.

Purpose: The purpose of this study is to investigate (a) how well lognormal distributions fit empirical data, (b) how estimates of microbial quality are affected by detection limits, (c) if 20 samples are sufficient to characterize the water quality, (d) how sensitive the microbial criteria are to shifts in water quality, and (e) the predictive ability of *E. coli* for the presence of *Salmonella*.

Methods: This study used 540 samples from 6 irrigation ponds (90 samples per pond) measuring *E. coli* concentrations, *Salmonella* presence, turbidity, and other physicochemical parameters. Objectives were analyzed by (a-b)fitting distributions to data using maximum likelihood estimation while considering censoring, (c) analyzing data subsets to simulate limited sampling, (d) simulating shifts in water quality using generated data and measuring the time until microbial criteria reflect the shift, (e) using logistic regression.

Results: Lognormal distributions provided an adequate fit and accounting for censoring due to detection limits increased the spread of fitted distributions. Due to high variability in *E. coli* counts, 20 samples were not sufficient to characterize the water quality and sudden shifts in water quality were not detected using the prescribed sampling scheme for as many as 4 years. *E. coli* was found to be an adequate predictor of *Salmonella* presence, with turbidity as an additional significant variable.

Significance: When bacteriological quality of irrigation ponds has high variability, as in this study, the sampling scheme prescribed by FMSA is not sufficient to ensure water quality.

88. EVALUATION OF SYNERGISTIC ACTIVITIES & EFFICACY OF COMBINATION DRUG THERAPY FOR ANTIBIOTIC RESISTANT INHALATION ANTHRAX IN A MURINE MODEL

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Efficacy was tested using the murine model of inhalational anthrax to evaluate the therapeutic benefit and synergistic activity of various antimicrobial (AM) agent combinations for the treatment of inhalation anthrax resulting from a *Bacillus anthracis* (BA) strain resistant to the first-line AM treatment agent ciprofloxacin (C). Aerosolized spores from a C-resistant derivative of the Ames strain of BA were delivered to 10 groups of 46 female BALB/c mice. At 36 hours postexposure, intraperitoneal therapy was initiated with combinations of two AM plus C. AMs combined with C included: linezolid (L), rifampin (R), meropenem (M), doxycycline (D), clindamycin (Cl) and penicillin (P). Controls received saline or C alone. Ten mice per group were treated for 14 days and observed until day 28 postchallenge. The remainder were serially euthanized by threes at 6-12h intervals; from these, heparinized blood was collected, and lungs and spleens were removed, weighed, homogenized, and plated to determine bacterial load. 3x MIC AM-containing plates were also included to determine emergence of resistance.

Plasma lethal factor (LF) levels were determined by MALDI-TOF mass spectrometry. All AM combinations tested were significantly different from the saline control ($p < 0.0004$), and also from the C control, M/L ($p = 0.004$), M/Cl ($p = 0.005$), M/R ($p = 0.012$), M/D ($p = 0.032$), P/L ($p = 0.026$), P/D ($p = 0.012$), R/L ($p = 0.001$), and R/Cl

($p=0.032$). There were no significant differences in survival between any of the AM combination groups. By 66 hours, plasma LF fell to low levels or below the limit of detection (LOD) for the AM combination groups, but remained high in the two control groups which succumbed to infection. No additional AM resistance was detected.

Multi-drug therapy including a protein synthesis inhibitor can overcome a single first-line AM-resistant infection and also decrease circulating toxin levels. The data suggest that L had the greatest inhibitory effect on LF production, however all: L, Cl, and D reduced LF to below LOD by 36h after initiation of therapy, validating their key role in treatment recommendations.

89. GENETICS OF STAPHYLOCOCCUS AUREUS

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Staphylococcus aureus is a Gram-positive pathogen that is well known for its ability to develop antibiotic resistance and form biofilms, both of which can present a significant challenge to combating infections. Microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) make up a class of surface proteins responsible for host tissue adherence and biofilms formation. These proteins are characterized by repeating regions and N-terminal domains with IgG-like folds that bind ligands such as fibronectin, fibrinogen, and collagen. The repeating regions, combined with the considerable similarity between different MSCRAMMs, complicate the assembly of sequences from short read data. The common structure also confounds algorithms used to annotate sequenced genomes resulting in significant misidentification of the MSCRAMM genes in popular databases such as Biocyc and PATRIC. In addition, the high mutation rates in these

genes result in a plethora of divergent allelic variants, some of which have only been recently discovered to share the same locus (SdrE/Bbp), thereby limiting the value of using of a single common reference to drive alignments. We have developed and applied a systematic approach to assembling MSCRAMM sequences from short reads in a set of 48 isolates.

90. GENOMIC CHARACTERIZATION OF INTRAUTERINE PATHOGENIC ESCHERICHIA COLI FROM COWS WITH METRITIS

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Metritis is a major disease in dairy cows causing animal death, decrease of birth rate, milk production and economic loss. Antibiotic treatment is generally used to treat such disease, but it is associated with a high failure rate (23-35%). The reason of the treatment failure is not clear. Our hypothesis is that pathogens in the uteri carry extended spectrum β -lactamases (ESBLs), which give resistance to ceftiofur, the common antibiotic used to treat such disease. Our study investigated the prevalence of ESBL producing bacteria in uterine samples of cows with metritis and sequenced the isolated intrauterine pathogenic *E. coli* (IUPEC) for further genomic characterization. We found that the IUPEC causing metritis had a high prevalence of ESBLs, which may explain the failure to the treatment. The pathogenicity of these IUPEC isolates was investigated by invasion assay, minimal inhibitory concentration

(MIC) test and antimicrobial susceptibility test. The strains had strong invasion activity in bovine endometrial cell lines. The MIC of cefotaxime against the ESBL carriers were higher than 64 µg/mL. The ESBL positive strains had resistance to at least six antibiotics across multiple drug classes. In addition, these ESBL producing IUPEC had high virulence and multidrug resistance according to bioinformatics, which were similar to the reference human clinical *E. coli* isolates, indicating the possible transmission to human and leading treatment failure in both human and animals.

91. GENOMIC MUTAGENESIS IN CHLAMYDIA TRACHOMATIS USING THE TN7 TRANSPOSON SYSTEM

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The Tn7 transposon system has been successfully exploited to insert genes into a “parking space” at a conserved attTn7 site found in many different bacterial species. This technique facilitates insertion of any gene of interest into a defined location in the bacterial chromosome and, among other applications, has been used for single copy genetic complementation studies. The necessary transposition genes and the sequence to be inserted are encoded in two plasmids: the “helper” plasmid and the “carrier” plasmid. The Tn7 transposase is expressed from the helper plasmid and recognizes Tn7L and Tn7R sequences flanking the target gene of interest on the carrier plasmid resulting in insertion of the desired sequence into the attTn7 site. Tn7 transposition has not yet been demonstrated in *Chlamydia trachomatis* (Ct), the causative agent of bacterial sexually transmitted and ocular infections. The Ct genome contains the attTn7 sequence located downstream of the *glmS* gene. It shows homology to the sequence found in *Burkholderia pseudomallei*, an organism in which the Tn7 parking space method has been successfully employed. This observation suggests that this technique will be successful in Ct. Here we outline the experimental

design and progress to date as we adapt this technique to this obligate intracellular pathogen.

92. INVESTIGATION OF ANTIMICROBIAL RESISTANCE IN UREAPLASMA SPECIES AND MYCOPLASMA HOMINIS ISOLATES FROM URINE CULTURES IN COLLEGE-AGED FEMALES

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Urinary tract infections (UTIs) affect nearly 20% of women aged 15-29 and account for an estimated \$3.5 billion in US costs. Antibiotic resistance (ABR) prolongs UTI treatment, and resistance profiles vary regionally and serve as important considerations in treatment guidelines. Regional studies in the US have identified tetracycline resistance in over a third of *Ureaplasma* spp. isolates, but no studies have evaluated ABR levels in college-aged women with first-time UTI. This study determined the minimum inhibitory concentrations (MICs) of 73 urinary *Ureaplasma* spp. isolates and 10 *Mycoplasma hominis* isolates from college-aged women with first time-UTI against a panel of antibiotics, and evaluated the genetic mechanisms of resistance. Among the *Ureaplasma* spp. isolates, two isolates were resistant: one to levofloxacin and one to tetracycline. All *M. hominis* isolates were sensitive. For the *Ureaplasma* spp. isolates, MIC₉₀s were highest against gentamicin (32ug/ml) and lowest against doxycycline (0.25ug/ml). PCR amplification identified tetM present in the tetracycline resistant isolate, and a S83W mutation within the quinolone resistant isolate. A previous study identified a clinical isolate with a S83W mutation, but was unable evaluate its susceptibility to quinolones. In conclusion, low antibiotic resistance was found in this population of women and phenotypic evidence was provided to support that a S83W mutation confers levofloxacin resistance.

93. MITIGATING RESISTANCE DEVELOPMENT FOR NEW ANTIBIOTIC COMBINATIONS: POST-EXPOSURE RESISTANCE TO TIGECYCLINE MAY BE INCREASED IN COMBINATION WITH TETRACYCLINE

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When combined in vitro, the activity of tigecycline (TIG) and tetracycline HCl (TET) against *Pseudomonas aeruginosa*, is enhanced. Further characterization of this interaction has been examined in time kill curve experiments. When determining adequate dosing for antibiotic combinations, the potential for resistance development is often not considered. The aim of this work was to determine post-exposure resistance to better inform whether resistance should be considered in dose optimization. Static time-kill curve experiments were performed in vitro over 24 hours for *P. aeruginosa* ATCC 27853 with varying TIG and TET exposures, individually and in combination [1]. Presence of resistance was determined in duplicate after 24 hours of 0.25, 1, and 8×MIC TIG or TET exposure, or 0.25, 1, and 8×MIC TIG + 0.75×MIC TET exposure, by plating aliquots on 3×MIC drug-containing agar plates. Plates were incubated for 24 hours before reading. Visible growth was deemed positive for bacterial resistance and lack of visible growth was deemed negative for resistance. Twenty-four hours post-exposure of 0.25 and 1×MIC TIG alone, resistance was observed. There was no observed resistance for 8×MIC TIG alone or any tested exposure of TET alone. When bacteria were exposed to the combination, TIG resistance was observed at all exposure levels, whereas TET resistance remained absent at all exposure levels. Resistance may be of concern at lower concentrations of TIG alone. Although the current experiments suggest that there may be increased resistance to TIG when

administered with TET, further investigations are required to understand the interplay between TIG and TET. In addition, an assessment of drug degradation in the agar plates and during the time-kill curve experiments would be required.

These results may inform future experiments and prompt consideration of resistant bacterial populations when determining an adequate dose, especially in regards to TIG. Acknowledgement: This work supported in part by the NIH/NCATS Clinical and Translational Science Award to the University of Florida UL1 TR000064.

94. MOLECULAR EPIDEMIOLOGY AND PHYLODYNAMIC AND PHYLOGEOGRAPHIC ANALYSIS OF COMMUNITY-ASSOCIATED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS TRANSMISSION: AN EMERGENCY DEPARTMENT POPULATION SAMPLING STRATEGY

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Background: Emergency Departments represent an extensive frontline for infectious disease transmission between community and hospital settings where Methicillin-resistant *Staphylococcus aureus* (MRSA) clones continue to represent a major clinical challenge, due to their tendency to spread within healthcare settings and difficulties in case management. However, questions remain about differences between methicillin-sensitive *S. aureus* (MSSA) infections and MRSA, in terms of nasal colonization and clinical presentation. At a molecular level, the rapid proliferation of Community-associated Methicillin-resistant *Staphylococcus aureus*

(CA-MRSA) is also of concern. Phylogenetic methods with spatial and temporal data can be useful in uncovering transmission patterns.

Methods: From August 2015-2016, patients presenting to the hospital's primary emergency department with an acute skin or soft tissue infection (SSTI) were prospectively enrolled. Both nasal and SSTI site specimens were collected. Geographic, social medical and other patient level epidemiological data were collected. Subjects' addresses were geocoded. All MRSA were spa-typed, with whole genome sequencing (WGS) performed on selected isolates.

Results: 137 cases of SSTIs were included. 63% of SSTIs were located on the extremities of enrolled subjects. 47.8% of subjects were admitted to the hospital and 57.8% reported antibiotic use within 6 months of enrollment. *Staphylococcus aureus* (SA) was isolated from wounds from 90 of these cases: MSSA in 48 (53%) and MRSA in 42 (47%) In an additional 10 cases, SA was isolated from nares, but not from the wound. Only 39 (42%) of the 90 wound cases also had positive nares cultures for SA; rates of nares positivity did not differ significantly between MSSA and MSRA. Isolation of MRSA from the nares was significantly associated with a positive wound culture for MRSA ($P=0.001$, Fishers exact two tail).

Conclusions: Isolation of MRSA from nares significantly predicts MRSA in the wound; however, only about half of patients with SA-positive wound cultures also had positive nares cultures. Data on spa-typing and genome sequencing will help to further elucidate patterns of bacterial subspecies transmission.

95. MOLECULAR EPIDEMIOLOGY OF TOXIGENIC V. CHOLERAE O1 IN HAITI: STRAIN MOVEMENT BETWEEN CLINICAL AND ENVIRONMENTAL SOURCES

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Vibrio cholerae is ubiquitous in aquatic environments, with environmental toxigenic *V. cholerae* O1 strains serving as a source for recurrent cholera epidemics and pandemic disease. Surveillance of the aquatic environment in Haiti following the 2010 epidemic has identified toxigenic *V. cholerae* O1 that are clearly derived from the circulating epidemic strain, as well as potentially indigenous non-toxigenic O1 and non-O1/non-O139 strains. To assess the possible contribution of isolates from surface water sources, a monthly basis fixed environmental sampling frame has been established in 17 sites that represent transects of the Momance, Gressier, and Tapion Rivers, from mountain sources to the sea. No *V. cholerae* O139 strains were isolated. Twenty (5.3%) of 376 samples, from 11 sites, were positive for toxigenic *V. cholerae* O1; while only three samples, from two sites, were positive for non-toxigenic *V. cholerae* O1. Isolation was seasonal, with a significant association with water temperature and rainfall; association with fecal coliform counts was not found. *V. cholerae* non-O1/non-O139 strains were present in over 30% of samples, from virtually all sites. *V. cholerae* non-O1

strains did not show evidence of seasonality, and isolation was not associated with rainfall or temperature. Using whole genome SNP analysis, we explored the phylogenetic relationship among environmental and clinical isolates. The Bayesian phylogeography was used to reconstruct the geographic origin of ancestral lineages. Strains related by direct transmission events, or part of a transmission chain, would cluster in the tree within a well-supported monophyletic clade ($p > 0.9$) and display low genetic heterogeneity. There was evidence of several clusters of closely related clinical isolates. *V. cholerae* O1 isolates tended to cluster by river system with apparent clonal expansion of strains not directly related to clinical isolates. Finally, phylogeography reconstruction also showed evidence of transfer of clinical strains to environmental sources and from the environment back again to humans. Our findings are consistent with the hypothesis that toxigenic *V. cholerae* O1 is present and evolving independently in the environment in Haiti.

96. ONE HEALTH INITIATIVE AND MULTIDISCIPLINARY TEAM APPROACH TO THE INVESTIGATION OF MELIOIDOSIS IN SONGKHLA PROVINCE, 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. Our goal is to develop “One Health” initiative for melioidosis investigation and promote a multidisciplinary melioidosis research in southern Thailand. As a part of the initiative, we have been collecting *B. pseudomallei* isolates from human and animal cases, and soils in Songkhla Province since January 2014. All *B. pseudomallei* isolates identified from patients admitted to three tertiary care hospitals including Songklanagarind Hospital, Hatyai Medical Center, and Songkhla Provincial Hospital were sent to a melioidosis laboratory at Prince of Songkla University for species confirmation. Molecular diagnostics using real-time PCR such as TTS-1 and BTFC&YLF assays have been developed for routinely use to support *B. pseudomallei* identification. 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.

The project is ongoing, and so far we have confirmed at least 80 melioidosis cases from humans and animals, as well as, the presence of *B. pseudomallei* in soils in Songkhla and nearby provinces. The infections were mostly 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, 1323, and 1359 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 infection. Strains with ST3 were found in human and animal cases as well as in the environment. Collectively, we believe that our “One Health” initiative of melioidosis would provide a current situation of *B. pseudomallei* infections in Songkhla and nearby southern provinces that forms an integral part of regional threat assessment of Thailand and Southeast Asia.

97. ORALVANTAGE: COMPREHENSIVE DATABASE FOR DISEASE-ASSOCIATED ORAL BACTERIA

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Introduction: Oral diseases are the most common human infections, which have been associated with a multitude of pathogens. Despite the advancement in pathogen testing approaches, oral health care providers lack tools for instant cross-referencing of clinical presentations with potential pathogens. The progression in treatment practices toward personalized medicine necessitates developing a tool to facilitate matching patient disease risk with relevant infection prevention and eradication protocols. Health care providers and scientists are in need for a tool to facilitate recognizing most isolated and investigated oral pathogens and their associated clinical case presentation. The aim of this study is to generate a database of bacterial genomes associated with oral diseases supported by comprehensive metadata about patients and case presentation.

Methods: Oral bacterial strains specifically associated with microbiome and oral diseases with available full genome sequences were identified by data mining GenBank, the Human Oral Microbiome Database (HOMD), and relevant dental literature. Targeted metadata included patients' information, disease descriptions, and pathogen referential information. A referential database of fully annotated genomes with associated metadata, named OralVantage, was then built using Access® database platform and evaluated for consistency, redundancy, and search ability.

Results: OralVantage contains 412 pathogen full genomes (28 named species, and 10 unnamed oral taxon) associated with all available meta-information. Patient characteristics include age, race, gender, and health status (diagnosis and severity). Bacterial characteristics include date, origin, and site of isolation, genetic/genomic information, and institution information. OralVantage provides cross-reference search capabilities allowing to data mine for available pathogen genomes given clinical presentation and/or patient's criteria, which is merely impossible as a daily practice in dental offices and usually a time consuming and labor intensive even for scientists confronted with the task of looking at poorly annotated GenBank files and extensive literature searches.

Conclusion: OralVantage represents a great advantage for dental practice and scientific research investigations, by making the data mining of genomes and associated metadata of suspected oral pathogens effortless and captivating. OralVantage also allows searching common bacterial strains associated with a specific clinical condition, a process usually requiring broad knowledge in oral microbiology and pathogenesis. In addition to facilitating investigations, the database sheds light on the inherent deficiencies of most metadata currently available for oral microbiome genomic research and provides a model for the development of fully annotated oral microbiome repositories.

98. RETHINKING LIVESTOCK VACCINATION: MODELING THE ENVIRONMENTAL RISK OF ANTHRAX IN UKRAINE TO AID IN POLICY AND ESTIMATE POTENTIAL COST-SAVINGS OF SWITCHING TO TARGETED VACCINATION

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Livestock vaccination is the most effective method for controlling anthrax. In Ukraine, there is a long history of anthrax. Recently, the burden of anthrax has been reduced significantly in Ukraine through sustained vaccination; <1 outbreak per year on average during the last 5 years. However, there are now questions concerning the need for a national livestock vaccination campaign. To address this question, we obtained data on the number of livestock anthrax outbreaks per district in Ukraine during 1979-2015 as well as the number of doses of livestock vaccine administered. We developed environmental risk models using a zero-inflated negative binomial model to estimate the cost of switching to a campaign targeting high risk areas. We used anthrax sero-surveys from wild boar (*Sus scrofa*) to estimate the range of sentinel exposure. From 1979-2015 there were 475 anthrax outbreaks. Results from the zero-inflated model indicated high risk areas in south-central Ukraine. The cost of vaccinating one animal was estimated at \$1.60 USD. During 2010-2015 (34.5 million doses) the cost of vaccination was estimated at ~\$55 million dollars compared to ~\$30 million targeting high and medium risk areas (19.3 million doses). Our findings suggest possible cost-savings of switching to targeted vaccination, however, broad geographic anthrax exposure in wild boar suggest persistence of active anthrax foci across Ukraine. The risk of changing vaccination policy must carefully weighed with the cost-benefits.

99. SEROLOGICAL BASED POINT-OF-CARE DIAGNOSTIC DEVELOPMENT FOR MELIOIDOSIS

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A polysaccharide-based ELISA for identifying serological evidence of *Burkholderia pseudomallei* (Bp) exposure was developed as point-of-care diagnostics. For validation, eighty-four (42 male, 42 female) pre-screened rhesus macaques (*Macaca mulatta*) greater than 3 years of age were challenged with various doses of 4 Bp strains (1026b, K96243, HBPU 101303a, and HBPU 10134a) via head only aerosolization. Serum samples were collected from each monkey at -7, 7, 14, and 28 days in relation to challenge or at terminality. Each sample was screened for reactivity to type A and B lipopolysaccharide (LPS). Purified Bp polysaccharides were probed with NHP serum from 28-day post-challenge survivors in western blots to determine reactivity. NHP serum from macaques exposed to higher virulent strains consistently showed cross-reactivity to LPS but not capsular polysaccharide (CPS). Serum from rhesus macaques 28 days post-challenge showed LPS as the most immunogenic (15/16) followed by CPS (3/16) and no reactivity to the other high molecular weight polysaccharides from Bp. The availability of pre- and post exposure serum offered a unique opportunity for validation and discovery of new point-of-care diagnostic targets. In addition, we further developed a high throughput protein microarray that

containing about 250 known and/or predicted antigenic proteins including species-specific proteins, virulence factors, outer membrane proteins, and geographically differentiating targets. The purified LPS types A, B, and B2 and the major Bp CPS were also included on the chips. Analysis of samples offered insight into the temporal immunological adaptation of the host during aerosol exposure melioidosis.

100. THE RELATIONSHIP BETWEEN HOST GENETIC ARCHITECTURE AND PATHOGEN SUSCEPTIBILITY IN CAENORHABDITIS ELEGANS

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The association between host genotype and the susceptibility to and clinical manifestation of particular pathogens is well-documented, but the specifics of this relationship are rarely understood. A vital first step in better understanding the genetic basis of pathogen susceptibility is analyzing the role of mutation. This study aims to quantify the per-generation input by mutation of genetic variance and covariance for susceptibility of the nematode *Caenorhabditis elegans* to a suite of pathogens, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, and the control *Escherichia coli*. A set of mutation accumulation (MA) lines, which have undergone 250 generations of single hermaphrodite descent, along with their ancestral progenitor, were infected with the suite of pathogens. A chain binomial model was used to estimate the average survival probability of all infected individuals. It is expected that among line variance (VM) of the MA individuals will increase, while the per generation change in mean survival probability (ΔM) will be negative. These findings were confirmed for *P. aeruginosa*, which showed a significant increase in VM for PB306 nematodes, as well as a significant decline in ΔM compared to the ancestral progenitor. Forthcoming analyses will determine if these trends are consistent among the other pathogens.

101. WHOLE GENOME SEQUENCING REVEALS RAPID SHIFTS IN TOMATO BACTERIAL SPOT POPULATIONS IN FLORIDA

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International trade and continued globalization of agriculture has had major effects on plant pathogen populations and their interactions with crop hosts. The long-distance movement of plant material has introduced plant pathogens to new geographic regions, caused the emergence of new diseases, and has made disease management particularly challenging. Over the past 25 years the *Xanthomonas* population causing bacterial spot of tomato in Florida has undergone two major race shifts even though the corresponding resistance genes were not commercially deployed. We aim to reduce the introduction and spread of *Xanthomonas* strains in tomato production by identifying the source of outbreaks and origins of new strains by working with Florida growers and international cooperators. Whole genome sequencing of Florida strains of *X. perforans* has revealed gain and loss of effectors, and homologous recombination with *X. euvesicatoria*. We identified potential targets for R-gene resistance, but also continue to see dynamic, changing bacterial spot populations.

102. ANALYZING EPIDEMIC RISK IN SEED NETWORKS: SWEET POTATO IN NORTHERN UGANDA

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Seed systems, both formal and informal, are a critical component of global food security. Even so, factors that influence the success and failure of seed systems are not fully understood. Seed security is often defined as timely access to quality planting material by all who need it, at a fair price (Sperling 2008, Gibson 2011). Seed systems are complex, highly nuanced networks with a suite of actors that move both material and information. Network Analysis is a quantitative tool that can be used to analyze complex biophysical and information networks (Garrett, 2012; Pautasso & Jeger, 2008). This type of analysis can serve to define key nodes and actors in a system, provide diagnostic metrics, and forecast the risk of network fragmentation and pathogen introduction (Garrett, 2012; Moslonka-Lefebvre et al., 2011; Pautasso & Jeger, 2008). This method allows not only for the description of seed systems, but also for the prescription of policy and intervention. Furthermore, information gleaned from this integrative analytic approach may help inform strategic intervention in times of acute insecurity. In this study we look at sweet potato vine transactions in the Gulu District of Northern Uganda. Sweet potato is a major staple food crop in many African countries and the third most important food crop in Uganda (FAOSTAT 2016). In both 2013 and 2014 sweet potato vine sellers and multipliers were surveyed weekly to obtain detailed information about each of their sales transactions throughout the course of the growing season (April-August). This study uses network analytic tools to: i) Assess key network properties of this informal seed system, ii) determine the availability of diverse planting material to growers in

the system, and iii) model the risk of the introduction of a potential seed-borne pathogen into the system. Several network properties such as coreness and node in-degree appear to influence both access to quality vines as well as vulnerability to introduced risk. Through simulation of epidemics based on known transactions and spatial distance, we were able to identify key nodes for targeted disease management, while maintaining variety availability. This method can serve as an example, with potential to be used across a wide variety of seed systems.

103. COMBINATION THERAPY WITH A NS5B POLYMERASE INHIBITOR (GS-9669) AND NS3 PROTEASE INHIBITOR (VEDROPREVIR) SUPPRESSES GENOTYPE 1B HEPATITIS C VIRUS (HCV) REPLICON REPLICATION AND PREVENTS THE AMPLIFICATION OF DRUG-RESISTANT MUTANTS

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Background: Hepatitis C virus (HCV) is a small, positive-sense RNA virus most commonly spread through blood-to-blood transmission. Although no vaccine exists, the discovery and development of direct acting antiviral (DAA) agents has drastically changed the landscape of HCV treatment. DAA therapeutic regimens have recently demonstrated great clinical success based upon impressive cure rates coupled with fewer side effects and abbreviated care regimens. However, the long-term efficacy of these regimens is dependent on the ability to maximize HCV suppression and prevent the emergence of drug-resistant mutants. Our aim was to evaluate exposure-response relationships for GS-9669 (non-nucleoside NS5B polymerase inhibitor) in combination with Vedroprevir (VDV; NS3 protease inhibitor) against a genotype 1b HCV replicon-bearing cell

line (2209-23 cells). We assessed the influence of drug exposure for each combination on the inhibition of replicon replication as well as the suppression of drug-resistant mutants.

Methods: Sixteen BelloCell bottles were inoculated with 6x10⁷ 2209-23 cells and each agent was administered via continuous infusion. We implemented a full factorial experimental design in which GS-9669 and Vedroprevir were evaluated as monotherapy at three different exposures and as combination therapy at all possible exposure combinations. A no-treatment control was also included. Cells were harvested from BelloCell bottles at various times during therapy and HCV replicon kinetics were quantified by monitoring luciferase activity. HCV RNA was analyzed for defined mutations associated with resistance to protease inhibitors and NS5B polymerase inhibitors by quantitative single-variant sequencing at days 0, 7, and 14 post-therapy.

Results: Antiviral activity was enhanced in all combination regimens relative to monotherapy arms, providing an additional 10-fold reduction in luciferase activity. The same antiviral effect was achieved in all combination regimens regardless of exposure. VDV-resistant mutants were amplified during VDV monotherapy with mutant subpopulations peaking at 85% following an “inverted-U” trend. Mutations associated with GS-9669 resistance were not detected. The amplification of VDV-resistant mutants was completely suppressed in all combination regimens, regardless of the GS-9669 exposure in the combination. This indicates that GS-9669 is highly effective against VDV-resistant mutants.

Conclusions: Combination therapy with GS-9669 and Vedroprevir is effective at suppressing replicon replication and preventing the amplification of drug-resistant mutants against genotype 1b HCV. These results indicate that, at the very least, additivity may be achieved when these DAAs are used in combination. Our findings have important implications for the design and use of combination regimens containing non-nucleoside NS5B and NS3/4A protease inhibitors.

104. COMPARATIVE TRANSCRIPTOMIC ANALYSIS OF FRONTAL LOBE TISSUE FROM SIV-INFECTED RHESUS MACAQUES WITH DETECTABLE SIV IN THE BRAIN

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Some HIV patients develop HIV-associated dementia (HAD), a neurodegenerative disease caused by HIV presence in the brain resulting in cognitive impairment. SIV-infected macaques offer an excellent animal model of HIV infection and HAD, given similar disease progression and incidence of brain infection. CD8+ lymphocyte depletion prior to SIV infection causes an accelerated disease course and higher incidence of brain infection. Study of the gene expression alterations induced by SIV presence offers a potential window into the molecular mechanisms of HAD pathogenesis. We have investigated transcriptome differences detected between SIVmac251-infected macaques with and without detectable virus in specific brain tissues. Total RNA samples from the frontal lobe of six non-CD8+ lymphocyte-depleted, naturally progressing (Mac251-NP) and five CD8+ lymphocyte-depleted (Mac251-DEP) macaques underwent Illumina sequencing. Paired-end reads were mapped to an Ensembl reference macaque genome with

Tophat. Differentially expressed genes (DEGs) were identified using Cuffdiff. Three of the six Mac251-NP and all five Mac251-DEP macaques had detectable SIV in the frontal lobe, as determined by single genome sequencing. 154 upregulated DEGs in macaques with detectable parenchymal virus included those involved in type I interferon signaling pathway, cytokine production, complement activation, macrophage colony-stimulating factor signaling and macrophage markers. Additionally, four significantly upregulated DEGs were Poly [ADP-ribose] Polymerases (PARPs). 5 downregulated DEGs held functions in synaptic transmission, cytoskeletal structure, and ion channel regulation. Our results indicate multifaceted etiology of neurocognitive degeneration linked to SIV presence in the brain, including increased inflammation, macrophage migration, oxidative stress, and calcium homeostasis disruption. We detected decreased expression of genes active in synaptic transmission and ion channel regulation, which would hinder proper neuronal function. As dysregulated PARPs deplete NAD⁺ and ATP in the cell leading to necrosis, the presence of significantly upregulated PARPs with detectable SIV in the frontal lobe provides an additional mechanism of neurodegeneration.

105. COMPLETE GENOME OF GENITAL SQUAMOUS CELL CARCINOMA-ASSOCIATED PAPILLOMAVIRUS FROM A LARGE FLYING FOX (PTEROPUS VAMPYRUS)

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Papillomaviruses are small dsDNA epitheliotropic viruses with a remarkable diversity with 240 fully sequenced human papillomaviruses. Papillomaviruses are approximately 8 Kb in size and contain up to 8 genes, including early genes (E1, E2, E4, E5, E6, E7, and E8) and late genes (L1, L2, & L3). Some of the early genes (E5, E6, E7, and E8) are considered to be potentially oncogenic. Papillomaviruses are cause of warts in many species and one of the most common causes of cancer in woman. We present a case of papillomavirus in a female Pteropus vampyrus, which presented genital warts at clinical examination, which over a period of 2.5 years progressed to squamous cell carcinoma. Clinical lesions and histopathology suggested papillomavirus, however, PCR/sequencing molecular diagnosis was unsuccessful into detect it. Next generation sequencing with an Illumina Miseq was then used to fully. Phylogenetics reconstruction of L1 gene locate novel papillomavirus, here called Pteropus vampyrus Papillomavirus -1 (PvPV1) in the

same clade with Alphapapillomavirus, which include several high risk papillomavirus. This could represent the an animal model for the study for papillomavirus.

106. DEVELOPMENT TIME OF INFECTED DROSOPHILA

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The genus Sigmavirus is a member of the Rhabdoviridae family, which includes seven species of viruses that are detected in fruit flies. *Drosophila melanogaster* Sigma virus (DMelSV) infects the common fruit fly, *Drosophila melanogaster*, through bi-parental vertical transmission. DMelSV reduces the fitness of its host. Viral titer is positively correlated with development time, that is, animals with higher titer take longer to develop. It is possible that some fruit flies may have rid themselves of virus before eclosion. For these flies, increased immune investment resulting in clearance of pathogen may divert resources from other life history traits, including development time. Thus, we might expect animals which have successfully cleared the virus to take even longer to develop than infected, high titer animals. An examination of the development time of various infected genotypes yields data that can potentially display the difference between the cost of infection and the cost of clearance in regards to development time (eggs to eclosion). If the fruit flies undergo clearance and rid themselves of DMelSV, does development time increase when compared against fruit flies that indicate positive infection status? Through the sensitive nature of infected fruit flies to CO₂, we were able to determine which flies retained infection status post-eclosion. An analysis and discussion of the data offers insight into how the cost of clearance affects development time as well as how differences in development time between infected and uninfected flies could be applied to other arboviruses.

107. DIFFERENTIAL EXPRESSION OF BIOMARKERS IN CERVICAL DYSPLASIA IN ETHNICITY-SPECIFIC MANNER

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There has been an increasing need for identifying specific molecular markers for individual's susceptibility to HPV-infection and HPV-mediated dysplasia. By applying DNA microarray, differentially expressed genes were identified in the HPV16-immortalized cells. We tested the identified genes in a cohort of patients to ascertain if there were differences in patients as well. Overall, compared with control subjects, women with CIN2/3 displayed significantly higher levels of EYA2 ($p=0.005$) and IL1-beta ($p=0.05$) mRNA and decreased levels of IL1-beta cytokine ($p=0.02$). When stratified by race, increased expressions of EYA2 ($p=0.002$) and TGF-beta ($p=0.02$) mRNA levels in CIN2/3 were observed in African American group. In white group, we observed increased expressions of IL1-beta ($p=0.02$) in CIN2/3. Gene expression values were found to be correlated for four markers, EYA2 and IL1-beta ($rS=0.3$, $p<0.0001$), SIX1 and IL1-beta ($rS=0.4$, $p<0.0001$), SIX1 and EYA2 ($rS=0.7$, $p<0.0001$) and EYA2 and TGF-beta ($rS=0.3$, $p<0.0001$). Our studies demonstrated that TGF-beta and Eya2 represent a novel cervical dysplasia signal in African American group. Eya2 promotes EMT in cancer cells through interaction with TGF-beta. In white group, IL1-beta signaling plays a role to protect cervical cells to be transformed by HPV. Taken together, our result suggest that there exist differential gene expression pathway for cervical dysplasia based on race/ethnicity at least in transcriptional and cytokine level and these can serve as potential molecular markers for cervical dysplasia before progression to invasive cancer.

108. EFFECT OF RAPAMYCIN ON SIGMA VIRUS IN DROSOPHILA

EFFECT OF RAPAMYCIN ON DMELSV IN DROSOPHILA MELANOGASTER

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Sirolimus, more commonly known as Rapamycin, is a compound produced by *Streptomyces hygroscopicus*. It acts as an inhibitor to Target of Rapamycin (TOR), which is a protein that regulates cell growth, proliferation, translation, and autophagy among other things. Autophagy is the process by which cells naturally degrade cellular components. It has been known to play a role in the innate immune system of flies, and the inhibition of TOR by Rapamycin upregulates the process of autophagy. This project examines the effect of the upregulation of autophagy by Rapamycin on DMelSV (sigma virus) in *Drosophila Melanogaster*. Sigma virus is a vertically transmitted, single-stranded negative sense RNA virus. The effect of Rapamycin on sigma virus is measured by fecundity assay and viral titer. A significant drop in viral titer and a rise in fecundity in the flies treated with Rapamycin is expected, but is not what was observed.

109. EVOLUTIONARY INFERENCES OF THE CONTRIBUTION OF MONOCYTES TO SIV POPULATION DYNAMICS AND THE RELATIONSHIP BETWEEN MONOCYTE SUBPOPULATIONS AND NEUROAIDS DEVELOPMENT

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Viral sequences carry information regarding the evolutionary and epidemiological history of a pathogen, both among and within individual hosts. The long-term fate of specific evolutionary changes depends on the interplay of viral population-level processes such as the effective viral population size (N_e). Understanding these processes and their relationship with the environment, such as the host immune system, can contribute to our knowledge of the complex etiology of AIDS and related illnesses, such as HIV/SIV encephalitis (H/SIVE).

The Bayesian coalescent framework was used to infer SIV N_e over time for gp120 sequences obtained longitudinally from a variety of tissues and circulating cell types within 8 SIVmac251-infected rhesus macaques. Cross-correlation and wavelet spectral analyses were then used to investigate the relationship between individual circulating immune cell sub-populations with overall N_e . Despite similarly dynamic overall N_e observed for all macaques, monocyte SIV N_e exhibited significant host-dependent variation, with resemblance to CD4+ T-cell SIV N_e occurring in only a small number of the macaques and only during certain time intervals. However, overall SIV N_e correlated positively with total circulating classical monocyte counts for macaques with detectable virus in the brain at necropsy and negatively for the macaques without. Furthermore, the slope of these counts over time correlated positively with viral copy number within the brain and was greatest for the macaque with SIVE.

Changes in monocyte SIV N_e and the relationship of these changes with circulating immune cell dynamics were host-specific; however,

classical monocyte expansion in the blood was associated with increased inferred viral transmission in macaques with quantifiable virus in the brain, and the rate of this expansion may correlate with SIVE progression. The results provide evidence of a working model wherein elevated levels of infected monocytes in circulation increase the probability of brain entry and, thus, SIVE development.

110. FIRST REPORT OF KONJAC MOSAIC VIRUS (POTYVIRIDAE, POTYVIRUS) IN CALADIUM (ARACEAE) IN FLORIDA

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Caladium x hortulanum, an aroid native to South America is an ornamental plant valued for its colorful foliage and used as a landscape and potted plant. Lake Placid, Florida produces 95% of the world's supply of caladiums which are vegetatively propagated using tubers. A metagenomic study was conducted to determine what viruses were present in caladiums produced in Lake Placid. The study used Illumina RNA-seq to generate reads from RNA extracted from caladium leaves of 6 cultivars. Analysis of the reads (assembled using Velvet and analyzed with BlastX) indicated the presence of Konjac mosaic virus (KoMV). The full genome of KoMV was recovered using Bowtie. This KoMV isolate from Florida showed 92.8% similarity to the reference genome of KoMV isolated from Japan. A survey of the presence of KoMV in 363 plants across twenty caladium cultivars indicated that KoMV was widely distributed and detected in 14.3% of the tested plants and in 20 cultivars. Twenty-five full length sequences of the coat protein of KoMV were obtained from 11 different cultivars. The genetic distance of KoMV population

was analyzed within and among cultivars, and only slight differences were detected. Phylogenetic analysis indicated that KoMV was probably introduced only once. This is the first evidence for the presence of KoMV in the US. Movement of vegetative material is a common practice and a very effective method to disperse plant pathogens. In addition, viruses can be dispersed readily through the practice of vegetative propagation so it is not surprising that it was found in so many cultivars. The discovery of KoMV was used to produce KoMV-free caladiums using tissue culture and indexing used KoMV-specific RT-PCR.

111. HIV/AIDS: PRODUCING VIDEOS TARGETTING FLORIDA'S AT RISK GROUPS

Nina Stoyan-Rosenzweig - University of Florida; **Michele R. Tennant** - University of Florida; **Hannah Norton** - University of Florida; **Gretchen Kuntz** - University of Florida; **Matthew Daley** - University of Florida; **Margaret Ansell** - University of Florida

HIV/AIDS is no longer a death sentence: rather, treatment has turned it into a chronic disease controlled through medication. However, it still is a major public health issue and especially in Florida. In 2015, this state had the third highest rate of HIV diagnoses, the fourth highest lifetime risk of HIV diagnosis, and 6 Florida cities ranked among the top 25 cities with the highest rates of HIV per 100,000/cumulative number- Miami ranks #1 in the country. Given this concern, the HSC Libraries conducted a year long project, and this poster will highlight an aspect of the HSC Libraries grant-funded project focused on providing reliable information on HIV/AIDS to health professionals, libraries and community members and pairing with community partner successfully to expand outreach efforts. The project included training individuals on use of appropriate AIDS websites, and producing materials for groups who might not be able to access or read the website- these materials included handouts explaining essentials of HIV testing and support groups for those who are HIV positive, and 4 short videos targeting specific at-risk groups in Florida. These videos were produced in partnership with the UF Center for the Arts-in-Medicine to reach

university students, individuals using local free clinics, people over 50, and women who are HIV positive, and they are available on the HSC Libraries YouTube channel. The project includes using surveys and focus groups to evaluate the impact and efficacy of these videos. The poster will include discussion of the larger project, and will focus on methods used to determine video topics, the video production process, and means to evaluate and market the materials to a broad audience.

112. IDENTIFYING HIV INTEGRATION SITES IN HIV POSITIVE PATIENTS ON CART

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In human immunodeficiency virus (HIV) positive patients, HIV preferentially integrates as a provirus into transcribed genes of the host cells' genome. HIV-infected patients have a higher incidence of cancer that has persisted despite ongoing treatment with combination antiretroviral therapy (cART), which effectively eliminates circulating HIV and extends the lifespan of patients. Additionally, genetically identical proviral HIV genomes are found in HIV infected patients undergoing long-term cART and contribute to viral persistence. While the source of these identical HIV variants has not been confirmed, one hypothesis postulates that these variants arise from proliferation of HIV-infected progenitor cells, which produce identical copies of the integrated provirus during mitosis. Previous phylogenetic analysis of env and nef HIV sequences obtained from HC09, an infected patient on cART, revealed clades of identical provirus. Identification of HIV integration sites offers the

opportunity to confirm if the proliferation of these identical HIV variants is due to the clonal expansion of infected cells. Through random shearing of DNA from the HIV-infected patient HC09 and linker-mediated polymerase chain reaction, we sequenced amplicons containing an HIV integration site and a unique breakpoint in the human genome of the patient. The HIV integration site specifies the gene with integrated HIV provirus, while the breakpoint serves to determine if sequences with the same integration site represents clonal expansion or PCR amplification. Our analysis of these sequences revealed over 200 unique integration sites and more than 10 clonally expanded sites. A majority of the detected integration sites mapped to coding regions in the human genome, and a large portion of these genes with HIV integrations are involved in biological regulation processes according to gene ontology classification. Identifying integration sites and studying the origins of identical, proliferating HIV provirus contributes to understanding HIV persistence in patients undergoing cART. Previous studies have indicated that highly expressed genes or genomic regions in close proximity to nuclear pores are likely targets of HIV integration. The discovery of HIV integration in genes that are associated with cellular growth regulation and/or cancer development could indicate a mechanism for the proliferation of infected progenitor cells leading to the presence identical viral variants. Our study provides insight into the origins of identical HIV variants and cancers development in HIV-infected patients undergoing treatment with cART.

113. IMPACT OF PCR-MEDIATED RECOMBINATION ON STUDIES INVESTIGATING HIV/SIV INTRA-HOST MOLECULAR EVOLUTION

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Background: During traditional cloning methods of sequence generation, PCR-mediated recombination can artificially inflate the number of recombinant sequences detected in ex vivo samples. Single Genome Sequencing (SGS), using limiting end-point dilutions where a single copy of cDNA is amplified for sequencing, should eliminate the potential increase in recombination rate due to PCR-mediated events. However, the exact bias introduced by PCR/cloning methods compared to SGS in ex vivo studies is unknown. Herein, we compared results from cloning and SGS experiments on plasma samples to determine the impact of PCR-mediated recombination and polymerase error rate on phylogenetic signal and to support the hypothesis that SGS should be used instead of cloning to obtain reliable HIV/SIV sequences.

Methods: Plasma samples were collected from SIV-infected CD8-depleted rhesus macaques at 21, 61 and 91 days post-infection. Four cDNA samples were chosen: two where recombinants were detected and two where no recombinants were detected in previous cloning/sequencing experiments. New cloning and SGS experiments were done in triplicate resulting in six alignments of ~20 env gp120 sequences per sample. Recombinants were identified using an algorithm based on the PHI test and split decomposition, specifically developed to investigate the presence of intra-subtype

recombination. Sequencing results from both methods were analyzed in depth and compared using chi-square tests.

Results: The number of recombinant sequences found in each experiment was not significantly different (chi-square test, $p < 0.01$). Overall mean distances in cloning experiments were significantly higher than in SGS (t-test, $p < 0.05$) at early time points, but not at later time points. The number of singletons in cloning experiments was also significantly higher than SGS in all cases (t-test, $p < 0.05$); however, the number of parsimony informative sites, an indicator of phylogenetic signal, was essentially the same.

Conclusions: The findings show that in the analysis of intra-host SIV samples collected during early infection, PCR-mediated recombination occurs at a rate not high enough to be significant against the background rate of in vivo recombination. Therefore, the decision to use SGS instead of cloning should not be based on the idea that cloning inherently adds a bias in the phylogenetic signal and number of recombinant sequences, but should be evaluated on the basis of the specific data sets under investigation. For this specific data set, given the short time for evolution due to the accelerated disease progression from CD8 depletion, either experimental method would be suitable for accurate phylogenetic reconstruction.

114. INFECTED LOVE: THE EFFECT OF SIGMA VIRUS ON MALE DROSOPHILA MELANOGASTER COURTSHIP DISPLAY

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Viruses often manipulate the behavior of their hosts to increase viral fitness. However, hosts in turn are sometimes able to perceive viral infection and have evolved to behave differently with infected individuals. The Sigma virus (DMelSV) of the fruit fly, *Drosophila melanogaster*, is a Rhabdovirus that persists in natural populations via biparental vertical transmission. There is evidence that some infected male flies have higher fitness if they are the first mate of the female, and counterevidence that females prefer to not mate with infected males if they have already mated. Females have also been seen to prefer genetically variable and disease-free males by quickly finishing copulation during less desirable matings. Analyzing male fly courtship behavior in infected and uninfected males would allow us to better understand if and how DMelSV affects host matings. Through this we can gain a better understanding of how virulence can persist despite the pressure of natural selection. To determine if male infection status affects courtship behavior, we paired either an infected or uninfected male with an uninfected female in no choice assays. We recorded their courtship behaviors for thirty minutes and tallied the number of times each behavior was displayed by the male. The courtship behaviors we were interested in include wing flap, orientation, chase, licking/tapping, mounting, and copulation. The data are still being processed, but preliminary results suggest that we will not detect a significant difference between infected and uninfected male fly courtship. This suggests that there are other factors that contribute to the differential response of females to infected males. Further research should be done focusing on female mate choice and infection status, as an explanation for this anomaly.

115. INVESTIGATING HOW CHANGING ENVIRONMENTAL CONDITIONS MAY AFFECT THE CHEMOSENSORY ABILITIES OF BENTHIC CRUSTACEANS: THE CARIBBEAN SPINY LOBSTER MODEL

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Coastal ecosystems are some of most important ecosystems on the planet, but they are also on the frontlines of environmental change. They are also often essential or nursery habitat for valuable fisheries such as the Caribbean spiny lobster *Panulirus argus*, which supports the single most valuable fishery in the greater Caribbean. In Florida Bay, extreme seasonal weather events combined with rising temperatures, ocean acidification, and loss of habitat increase stress on spiny lobsters. While we have a growing understanding of the effect of these environmental changes on the survival, growth, and movement of spiny lobsters, the effect on their chemosensory abilities has not yet been documented. Lobsters rely heavily on chemical cues for many biological and ecological activities from mating to predator avoidance and this study aimed to determine the effect of environmental changes (temperature, salinity, and acidification) on behavior and sheltering preference in *P. argus*. In control conditions, chemical cues from conspecifics were used by spiny lobsters to identify suitable shelter and cues from competitors and diseased individuals were used to determine shelters to be avoided. In altered environmental conditions, lobsters did not significantly differentiate between conspecific, diseased conspecific, or competitor shelters. Globally, environmental conditions may change gradually, potentially permitting a degree of adaptation, but extreme events stand to alter the chemosensory abilities of crustaceans. These effects may be more prominent for crustaceans living in shallow nearshore areas where extreme events are more pronounced and more frequent.

116. LONG-TERM INTERACTIONS BETWEEN VIRUSES CAUSING HAND, FOOT, AND MOUTH DISEASE IN CHINA, 2009--2014

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Hand, Foot, and Mouth Disease (HFMD) is a common disease that mainly affects children under five years old. Enterovirus 71 (EV71) and Coxsackievirus 16 (CA16) and some other enteroviruses are common causes of the disease. Large outbreaks of HFMD have been seen in mainland China since 2008. We use the surveillance data for the whole population and the laboratory data for subsampled cases collected by the Chinese Center for Disease Control and Prevention between 2009 and 2014 to study virus-specific epidemics of HFMD. We calculate the correlations between lagged yearly cases adjusted for temporal and spatial effects and use generalized additive models to explore the long-term interactions among different viruses. Our results show signs of cross-immunity between EV71 and CA16.

117. PHYLOGENOMIC CHARACTERIZATION OF A NOVEL SEA OTTER POXVIRUS

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Poxviruses (family Poxviridae) are large, double-stranded DNA viruses that replicate in the cytoplasm of the host cells. The subfamily Chordopoxvirinae infects a wide range of vertebrates including marine mammals within the families Balaenidae, Delphinidae, Mustelidae, Otariidae, Phocidae, and Phocoenidae.^{1,2,3} Recently, a novel poxvirus was discovered in two

orphaned sea otter pups that developed small, superficial ulcerated skin lesions during captive care in 2009 and 2011.³ The pups were from two different Pacific Ocean populations: the northern (*Enhydra lutris kenyoni*) population in Alaska and the southern (*Enhydra lutris neiris*) population in California. Histopathologic examination of the skin lesions in both pups revealed epithelial hyperplasia with affected cells displaying intracytoplasmic eosinophilic inclusions, which were consistent with poxvirus infection. Additionally, the epithelial cells in both pups showed different degrees of ballooning degeneration and necrosis. In the northern sea otter, transmission electron microscopy revealed epithelial intracytoplasmic virions. The virions were brick shaped and had dumbbell electron-dense cores, which were also consistent with poxviruses. Despite being known for more than a decade, the phylogenetic relationships of marine mammal poxviruses are not well established because of the lack of complete genome sequences. Therefore, the purpose of the current study was to sequence the entire sea otter poxvirus (SOPV) genome using an Illumina MiSeq Next Generation Sequencer. The approximately 130,000-bp genome is 31.3% G+C, encodes 114 proteins, and has 2,613-bp inverted terminal repeats. Phylogenetic analysis based on 14 core poxvirus genes showed that SOPV is divergent from other known poxviruses and forms a distinct branch between parapoxviruses and orthopoxviruses, which are able to cause zoonotic diseases. The SOPV genome is the first marine mammal poxvirus to be fully sequenced and is the smallest poxvirus genome known. Sequencing of the SOPV genome is the first step in unraveling the position of a marine mammal poxvirus within the larger Poxviridae tree and provides the necessary sequence to develop future molecular tools for diagnostics and epidemiological studies.

118. PHYLOGENOMIC CHARACTERIZATION OF SQUAMATE ERYTHROCYTIC IRIDOVIRUSES

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Erythrocytic iridoviruses (EIV) have been documented in squamates within the families Gekkonidae, Phyllodactylidae, Scincidae, Cordylidae, Lacertidae, Pythonidae, Colubridae, Viperidae, Varanidae, Iguanidae, Phrynosomatidae, Agamidae, and Chamaeleonidae. Interestingly, similar viral agents have also been reported in more than 20 species of anadromous and marine fishes throughout the Atlantic and Pacific Oceans, as well as amphibians. However, the phylogenetic relationship of these viruses to other iridoviruses remains unclear to date. In this study, we compared the light microscopic abnormalities of infected cells, the ultrastructural morphology and phylogenetic relationship of EIVs to other iridoviruses. Recently, EIVs were partially characterized in a wild Peninsula ribbon snake (*Thamnophis sauritus sackenii*) and captive-bred inland bearded dragons (*Pogona vitticeps*). The Peninsula ribbon snake displayed two types of cytoplasmic inclusions in erythrocytes, polychromasia, anisocytosis, and hypochromasia, while the erythrocytes of the bearded dragon exhibited prominent blue-staining inclusions within normal appearing erythrocytes. Cytoplasmic inclusion bodies within erythrocytes of the Peninsula ribbon snake examined by transmission electron microscopy (TEM) revealed enveloped icosahedral particles morphologically consistent with iridoviruses. The complete genome of the EIV from Peninsula ribbon snake (*Thamnophis sauritus sackenii*; TsEIV) comprises 111,413 bp nucleotides which encodes 115 potential open reading

frames (ORFs). Phylogenetic analysis based on 19 conserved genes shows that the squamate EIVs form a well-supported clade distinct from other established iridoviral genera, and likely represent a novel genus. We propose the genus Hemocytivirus for this new clade of iridoviruses to reflect their predilection for red blood cells.

119. PHYLOGENOMIC DIVERSITY OF NORTHERN HEMISPHERE CETACEAN MORBILLIVIRUSES

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Cetacean morbillivirus (CeMV) is a member of the genus Morbillivirus in the family Paramyxoviridae that include enveloped negative-sense RNA viruses. Morbilliviruses cause notable diseases in both human and veterinary medicine. Over the past 25 years, strains of CeMV have emerged as the most significant pathogen of dolphins. In this investigation, we determined the phylogenomic diversity among six CeMV strains: dolphin morbilliviruses from Mediterranean striped dolphins (*Stenella coeruleoalba*), dolphin morbillivirus from a Gulf of Mexico bottlenose dolphin (*Tursiops truncatus*), porpoise morbillivirus isolated from a North Sea harbor porpoise (*Phocoena phocoena*), and beaked whale morbillivirus (BWMV) from a Hawaiian Longman's beaked whale (*Indopacetus pacificus*). The phylogenomic diversity of the CeMVs were then compared to the six other morbillivirus species: Measles virus, Rinderpest virus, Peste-des-petits-ruminants, Phocine distemper virus, Canine distemper virus, and Feline morbillivirus. Morbillivirus phylogenomic diversity was assessed by constructing Maximum Likelihood phylogenetic trees and nucleotide sequence identity matrixes. The CeMVs formed a well-supported clade with the BWMV as the most divergent member. Although considerable sequence variation was detected among the CeMVs, the magnitude of the difference was suggestive of separate strains rather than novel morbillivirus species. However, recent detections of highly divergent morbilliviruses from the Southern Hemisphere suggest the creation of a new morbillivirus species may be warranted. This study provides: 1) a much needed update to morbillivirus taxonomy, 2) a foundation for future efforts

in developing improved CeMV molecular diagnostics, and 3) a better understanding of the temporospatial dynamics of these globally emerging cetacean pathogens.

120. POTENTIAL EFFECTS OF WOLBACHIA ON MALE TRANSMISSION OF SIGMA VIRUS IN D. MELANOGASTER

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The interaction between viruses and other microorganisms can shed light on the mechanisms of disease transmission. DMelSV (*Drosophila melanogaster* Sigma Virus) is a vertically transmitted rhabdovirus that is relatively common in most wild-type lines of *D. melanogaster* and creates a sensitivity to carbon dioxide in infected individuals. Wolbachia is an extremely common intracellular bacteria that is maternally transmitted and mediates antiviral protection against some RNA viruses. The purpose of this experiment is to determine the relationship, if any, between Wolbachia-infection status and male transmission of sigma. The hypothesis of this research is that, because Wolbachia can protect against RNA viruses, if mothers are infected with Wolbachia, their progeny may have lower sigma infection rates than progeny without Wolbachia-infected parents; thus, that the presence of Wolbachia will lower paternal transmission efficiency of DMelSV.

Seven lines of sigma-infected males (half with Wolbachia and half cured of Wolbachia) were crossed with Wolbachia-infected and Wolbachia-cured females in a factorial cross, generating four treatment groups. Progeny were assayed with carbon dioxide to determine sigma-infection status. In females, there could be a trend between having Wolbachia-negative parents and higher sigma-infection rate of progeny. The effect is less clear in males. The results indicate that Wolbachia does not mediate any significant protection

against DMelSV, but further testing and data analysis are necessary to determine the effect of genetic variation among genotypes on expression of Wolbachia-sigma interaction. The project will be repeated with a male sigma-uninfected control group to control for the effect of cytoplasmic incompatibility and subsequent failure to produce progeny by Wolbachia-infected mothers.

121. POTENTIAL RESERVOIRS OF REPLICATING HIV-1 FOUND IN PATIENTS WHO DIED WITH NO DETECTABLE VIRAL LOAD WHILE ON EFFECTIVE DRUG REGIMENS

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Combined anti-retroviral therapy (cART) used to treat HIV-1+ patients is universally effective in reducing plasma HIV load to undetectable levels and restoring partial immunity. However, viral populations rapidly rebound once therapy is removed, and sometimes patients develop drug-resistant HIV while on cART. Furthermore, HIV-associated co-morbidities, particularly certain cancers and neurological disorders, occur at elevated rates even with effective cART. We hypothesized that tissue microenvironments may

protect localized HIV-infected resident immune cells from cART leading to on-going HIV replication and evolution.

The AIDS and Cancer Specimen Resource (ACSR) provided 50 post mortem tissues from five HIV+/cART+ patients with no detectable viral load. All five (designated Pt6-10) died from cancer and other non-AIDS related causes, and all had neurocognitive disorders. Four patients had both brain and non-brain tissues available for study. Tissues were assessed for HIV using droplet digital PCR (ddPCR) and quantitative PCR specific for gag, the most conserved HIV gene. Following simultaneous RNA and DNA extraction from the tissues, single genome sequencing was used to generate HIV DNA and RNA sequences for two viral genes: env gp120, and nef. Patient-specific sequence alignments were created for the two patients with sufficient sequences from multiple tissues. Maximum-likelihood phylogenies were inferred and multiple statistical tests were performed to investigate viral evolutionary patterns and compartmentalization. HIV env gp120 and nef DNA sequences were generated from 8/28 tissues processed, and three of these HIV+ tissues also contained HIV RNA. We were not able to amplify HIV-1 sequences from brain tissues.

Maximum-likelihood env gp120 and nef phylogenies and statistical testing showed little evidence of complete viral compartmentalization among tissues or between RNA and DNA. These trees contained clades showing potential evidence of on-going evolution from replicating virus, as well as clonal expansion of infected immune cells.

In each tissue, genetic diversity measured in nucleotide pairwise distance and calculated in MEGA7 found that nef sequences have more diversity than env. Ongoing viral expression in a subset of tissues in two patients indicates an uneven level of cART penetration in tissues that may create a privileged environment for persistent HIV replication during cART.

Two distinct patterns of tissue virus evolution suggest that different modes of replication/spread underlie this persistence: cART-resistant

HIV-infected tissue-resident immune cells like macrophages with persistent evolution and migration of HIV infected cells that clonally expand. Interestingly, no HIV-1 was sequenced from brain tissues in these patients with diagnosed neurological disorders.

122. PROTOTYPE FISH VIRUSES - A NEW CLASS OF PATHOGENS & INSIGHTS INTO DSDNA VIRUS EVOLUTION & RECOMBINATION IN EARLY VERTEBRATES

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Analysis of the evolutionary history of viruses relies mostly on molecular analysis of viruses from extant hosts. Comparison of viruses known to infect terrestrial vertebrates to viruses found in distant chordate lineages, such as fish, could provide further understanding of viral evolution in vertebrates over the last 500 M years. We analyzed and compared four prototype double stranded DNA (dsDNA) viruses that infect elasmobranch and teleost fish, including a novel virus from skin lesions in a giant guitarfish *Rhynchobatus djiddensis* (GfCV), a novel virus from a red discus *Symphysodon discus* (RdCV), a virus in marbled eels (AMPyV), and a related chimera known as Japanese eel endothelial cells-infecting virus (JEECV). Investigation using metagenomic analysis, electron microscopy, histopathology, cell culture, in situ hybridization, phylogenetic analysis, and protein structural modeling revealed that all four viruses occupy a previously unknown clade, tentatively named adomavirus. Size, ultrastructure and genome organization are highly novel and distinct from established DNA viral families. Although the genomes exhibit high flexibility in gene organization, all four are united by ultrastructure, a complete circular dsDNA genome, a conserved helicase and a string of homologous open

reading frames that encode putative structural and morphogenetic genes (capsid subunits and maturation protease). Structural and non-structural genes suggest distant recombinant chimerization events involving primitive adenoviruses and polyomaviruses. GfCV also encodes a protein resembling DNA primases of unicellular eukaryotes. These novel viruses provide clues on how this clade evolved and suggest distant recombination could have shaped the evolution of multiple dsDNA viral lineages.

123. QUANTIFYING INFECTIOUS TITER AND COPY NUMBER OF SPRING VIREMIA OF CARP VIRUS BY CORRELATING RT-QPCR RESULTS WITH TCID50 OUTCOMES

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Spring Viremia of Carp Virus (SVCV) is a pathogenic rhabdovirus that has the ability to cause massive die-offs in freshwater aquaculture. Historically, cell culture has been the tool of choice for evaluating SVCV viral load within host tissues. Tissue Culture Infectious Dose (TCID50) dilution assay is a proven and reliable technique used to determine the titer of infectious virus using cultured cells. A TCID50 is calculated by determining where cytopathic effect (CPE) occurs within 50% of the cell culture wells several days post-inoculation with serial dilutions of a sample containing a virus. However, TCID50 assays are labor intensive and results may not be available for several weeks. Alternatively, quantitative PCR (qPCR) has been used to evaluate viral load within host tissues and in environmental samples. qPCR provides the advantage of quick, reproducible results in a fraction of the time without the need for maintaining cell lines and infectious virus. qPCR results can be related to the nucleic acid copy number by generating a standard curve from serial dilutions of the qPCR target. However, viruses can produce copious noninfectious virus particles and these products are amplified along with the nucleic acids from the infectious virus. Therefore, qPCR

data may not accurately predict how much infectious virus is present in a given sample.

In this study, supernatant of a known TCID₅₀ value was serially diluted and the qPCR assay run at each dilution to determine the correlation between SVCV viral titer and copy number. By establishing the relationship between TCID₅₀ outcomes and qPCR data, qPCR results alone can estimate the SVCV infectious titer and virus copy number within mere hours of sample collection. This approach bridges an important gap that has previously hindered clinical diagnostics and research experiments that require prompt and accurate determination of infectious virus titer for SVCV and has established a protocol that can be replicated for other viral pathogens. Furthermore, we show that when water is used in place of the cell culture medium as the initial diluent during the TCID₅₀ assay, the SVCV infectious titer is reduced without any appreciable change in qPCR results. Thus, water has an inhibitory influence on SVCV without any reduction of viral nucleic acid within the dilutions.

124. REDUCTION IN ALCOHOL CONSUMPTION WAS ASSOCIATED WITH IMPROVED HIV VIRAL SUPPRESSION IN WOMEN

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Florida continues to have high rates of new HIV infections, but effective treatment to suppress HIV viral load can reduce HIV transmission. Alcohol use is common among persons living with HIV/AIDS (PLWH), but it is not clear whether interventions to reduce drinking will improve health outcomes among PLWH. We conducted an observational study of women participating in a randomized clinical trial to determine whether reduced drinking after alcohol intervention is associated with improvements in HIV viral suppression (NCT01625091).

Methods: From December, 2012 to August, 2016 we enrolled 194 women with HIV infection who exceed recommended alcohol drinking levels (>7 drinks/week or ≥ 2 binge sections/month). Women were randomly assigned to receive oral naltrexone or placebo for 4 months. Alcohol consumption and HIV viral loads were assessed at 2-, 4-, and 7-months after enrollment. We analyzed data from 166 women who completed the 7 month visit. Women were categorized as "quitting heavy drinking" or not based whether they had reduced to non-heavy drinking (<7 drinks/week) or complete abstinence at the 7-month timepoint. Logistic regression models with propensity score weighting were constructed to determine whether quitting heavy drinking was associated with improved HIV viral load suppression (≤ 200 copies/mL).

Results: Of the 166 women, 11%, 85%, and 4% were Hispanic, black, or white; the mean age was 48 years; and 96% were receiving antiretroviral therapy (ART). The majority of women (76%) had quit heavy drinking at 7 months. At baseline, there was no statistically significant difference in the quitters and non-quitters in HIV viral suppression (67% vs. 60%, $p=0.44$), or having $>95\%$ ART adherence (61% vs 59%, $p=0.86$). However, viral suppression improved over

time in those who reduced drinking and the proportion of women with viral suppression at 7 months was significantly better in than those who quit vs. those who did not quit (74% vs. 54%, $p=0.02$). In the weighted logistic regression analysis, quitting heavy drinking was significantly associated with achieving HIV viral suppression (Adjusted OR: 2.62, 95% CI: 1.02, 6.69), when adjusting for baseline HIV viral load level.

Conclusion: In this sample of heavy drinking women living with HIV, those who successfully reduced drinking were significantly more likely to achieve HIV viral suppression. The findings support continued efforts to screen and intervene to reduce hazardous drinking in persons with HIV, especially those who have not achieved consistent HIV viral suppression.

125. SILENT CIRCULATION OF POLIOVIRUS IN SMALL POPULATIONS

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Poliovirus is an enterovirus that causes the infectious disease called poliomyelitis. The virus can cause fever and flu-like symptoms, and, in more rare instances, acute flaccid paralysis (AFP). One characteristic of poliovirus is asymptomatic transmission amongst individuals who have already had a poliovirus infection. This allows the virus to silently circulate in an endemic population unless a surveillance system is put into place. Eradication efforts have reduced the regions of endemic circulation down to three localities. However, there have been outbreaks in areas in which the virus seemed to be eliminated. There are also areas in which small villages (population < 10,000) are common and in which one or more sub-populations are difficult to reach with a vaccination program. These conditions increase the possibility of silent circulation of the virus,

which is perpetuated by both imperfect or nonexistent vaccination coverage and immunity waning. We have constructed a stochastic model to understand how stochastic effects alter the ability of small populations to sustain virus circulation in the absence of vaccination. In addition, we explored the effects of modifying the AFP detection rates on the duration of a silent circulation in small populations.

126. “I HEARD IT THROUGH THE GRAPEVINE”: INFORMATION FLOW AMONG THE FLORIDA PEPPER INDUSTRY AND ITS CONSEQUENCES FOR DISEASE RECOGNITION AND RESPONSE

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Information is a key component supporting agricultural sustainability. The framework for the National Plant Diagnostic Network (NPDN), established in 2002, planned for a national network of diagnostic centers to help coordinate growers, local diagnosticians, and state regulatory officials in early and accurate recognition and response to plant pathogens. In order to better understand how information pertaining to plant disease is disseminated among the NPDN, local growers and the larger agricultural community, a study has been planned and organized to study information diffusion throughout the state of Florida. This study aims to understand which factors influence communication network participation and decision-making based on shared knowledge. Greater knowledge of how the NPDN functions on a regional and individual farm level will support implementation of policies directed at improving food security.

127. A PRACTITIONER-DRIVEN RESEARCH AGENDA FOR SYNDROMIC SURVEILLANCE

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Syndromic surveillance (SyS) has expanded since 2001 in both scope and geographic reach, and has benefitted from a broad collection of research studies adapted from numerous disciplines. The practice of SyS continues to evolve rapidly. The International Society for Disease Surveillance (ISDS) solicited input from its global surveillance network on key research questions with the goal of improving syndromic surveillance practice. A workgroup of SyS subject matter experts (SMEs) was convened to review and categorize the proposed topics. They identified twelve topic areas in four SyS categories: informatics, analytics, communications, and systems research. The context of each of the topics and their public health implications is detailed. This research agenda can serve as a guide for catalyzing the research identified by public health practitioners as most important.

128. ANALYSIS OF INFECTIOUS DISEASE TRANSMISSION DATA: BINOMIAL CONSIDERED HARMFUL

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One of the primary goals of household surveillance studies of infectious disease is to calculate the secondary attack rate (SAR), the probability of disease transmission from an infected household member A to a susceptible member B during A's infectious period. In a household of size m with a single index case, the number of secondary infections is often treated as a binomial($m-1$, p) random variable where p is the SAR. This assumes that all subsequent infections in the household are transmitted directly from the index case. Because a given transmission chain of length k has probability

pk, it is thought that transmission chains of length $k > 1$ can be ignored when p is small. However, there are $P(m - 1, k)$ such chains, so the probability of k generations of infection within the household can be much greater than the probability of any single transmission chain of length k . In simulations, we show that estimation of the SAR using a binomial model is biased upward and produces confidence intervals with poor coverage probabilities. Chain binomial models or survival analysis can be used to estimate the SAR more accurately.

129. DESIGN CONSIDERATIONS FOR VACCINE EFFICACY TRIALS IN OUTBREAK SETTINGS

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Evaluating the efficacy of a vaccine during a public health emergency presents important logistical challenges. The novel ring vaccination cluster randomized trial design, in which a cluster is defined as the contacts and contacts of contacts of an index case, addressed many of these challenges and was used in Guinea in 2015 to evaluate an Ebola vaccine candidate. One key statistical consideration of this and other vaccine trial designs is determining which cases should contribute to the primary vaccine efficacy outcome. Cases appearing shortly after vaccination may have been infected prior to vaccination or before developing protective immunity. The traditional strategy is to start counting cases after a long fixed, delay period, but this may require dropping a large proportion of the cases observed in the trial. Furthermore, if incidence declines over time within clusters, it may be hard to collect enough countable endpoints. We use an analytical and simulation based framework to provide recommendations on how to select the optimal delay period to minimize bias and maximize study power in the primary vaccine efficacy outcome.

130. DETERMINING MANAGEMENT OUTCOMES IN ECUADORIAN POTATO SEED SYSTEMS

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Bayesian networks (BN) are underutilized for data analysis in agricultural and disease systems. They elegantly capture probabilistic relationships between the multiple variables. We use BN to determine relationships between yield, disease, environmental variables and management choices. Preliminary results from Ecuador demonstrate the importance of seed degeneration for potato yields. Management performance maps could help to prioritize management, policy, funding and capacity building efforts.

131. EFFECTS OF AVAILABLE AREA DEFINITIONS AND ANIMAL MOVEMENT PATTERN TYPES IN RESOURCE SELECTION FUNCTIONS

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Species distribution estimation and mapping are key aspects to several animal management and ecology research directions. Resource selection function (RSF) methods are useful tools for this, and attempt to determine the preferred resources (e.g. environmental characteristics) of the species. Often, this function employs a ‘use’ versus ‘available’ framework, comparing the resources at known presence locations against places that were available but were not used. We were interested in comparing different methods of defining available area, at a second-order selection scale (home range of an individual or social group), while also comparing the effects of how the animals may be moving across the landscape. We compared four available area definitions using various home-range estimators: two versions of a minimum convex polygon (MCP), Localized Convex Hull (LoCoH), and Potential Path Area (PPA). Various available area determination methods have trade-offs in data requirements, computation needs, and biologically meaningful definitions. For the movement type data, we simulated three canonical movement types: nomads, central foragers, and territorials, plus we used real telemetry data of male bison. Various research applications may have different sensitivity requirements,

and the choice of available area definition may best be driven by a combination of considering the research question, the data available, and movement type of the species being studied.

132. HIGH-PERFORMANCE COMPUTING SERVICES FOR EMERGING PATHOGENS RESEARCH

Oleksandr Moskalenko - University of Florida

Overview of the UFIT Research Computing (UFRC) infrastructure and services for large-scale computational analyses, interfaces, data storage, software installation as well as software and biocomputing consulting for Emerging Pathogens research at the University of Florida.

133. MICROBIOME NETWORKS: A SYSTEMS FRAMEWORK FOR IDENTIFYING CANDIDATE MICROBIAL ASSEMBLAGES FOR DISEASE MANAGEMENT

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Network models of soil and plant microbiomes present new opportunities for enhancing disease management, but also challenges for interpretation. We present a framework for interpreting microbiome networks, illustrating how the observed structure of networks can be used to generate testable hypotheses about candidate microbes affecting plant health. The framework includes four types of network analyses. “General network analysis” identifies candidate taxa for maintaining an existing microbial

community. “Host-focused analysis” includes a node representing a plant response such as yield, identifying taxa with direct or indirect links to that node, interpreted as either beneficial or detrimental to plant health. “Pathogen-focused analysis” identifies taxa with direct or indirect links to taxon nodes known a priori to represent pathogens, which can be interpreted as agonists and antagonists, respectively. “Disease-focused analysis” identifies key nodes for both plant and pathogen responses. We illustrate the interpretation of network structure with analyses of two microbiomes: the oak phyllosphere and soil associated with the presence or absence of infection by *Rhizoctonia solani*. Such network analyses can be used to further characterize microbial communities and associated conditions involved in the suppression of plant pathogens, the biofertilization of crop plants, and/or the expression of host resistance in crop plants.

134. MODIFICATION OF HUMAN GUT MICROBIOTA BY RAISIN CONSUMPTION: INDICATING POTENTIAL REDUCTION OF ENTERIC INFLAMMATION AND COLORECTAL CARCINOGENESIS IN HEALTHY ADULTS

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Dried fruit containing phytochemicals and dietary fibers, such as raisins, contribute to shaping a healthy commensal gut microbiota. Maintenance and targeted modification of microbiota composition and activities has potential for improving various health parameters. We performed a feeding study in human volunteers to determine how consumption of three, one ounce servings of sun-dried raisins/day affects composition and activities of fecal microbiota.

Thirteen adults were recruited from faculty, students and staff at the University of Florida to participate in a 14 day raisin feeding study. Fecal samples were collected at baseline, days 7 and 14 of the study period. While adherence to the study protocol was high, participants struggled to maintain an intake of three ounces of raisins/day over the study period. Based on High Throughput 16S rRNA sequence analysis we observed stability in microbiota diversity indexes and in the proportions of bacterial phyla. Individual bacterial signature sequences suggested that the prevalence of up to 16 Operational Taxonomic Units (OTUs) changed during the first week of raisin consumption compared to only 4 OTUs that changed during the second week of raisin consumption. We detected a significant reduction in an OTU closest to *Klebsiella* sp., potentially an enteric pathogen. Our findings suggest that while adding raisins to the diet doesn't distort overall microbiota composition, it may potentially be beneficial to the host by reducing enteric inflammation associated with subclinical infections with an enteric pathogen. Potential health benefits of the observed microbiota changes, including reduction in enteric inflammation, should be determined in future studies in populations for which specific health benefits can be targeted.

135. OOMYCETE COMMUNITIES OF CACAO SOIL DETERMINED BY MASSIVE AMPLICON SEQUENCING OF CYTOCHROME OXIDASE II

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Oomycetes are a group of fungal-like eukaryotes with diverse lifestyles: many are saprophytic in terrestrial or aquatic ecosystems and contribute to nutrient cycling, while others are primarily pathogenic. Studies of soil Oomycetes communities may be used to link their abundance and survival with different environmental conditions or niches. However, most efforts to understand these relationships have been done in temperate regions, whilst potential disease hotspots in the tropics remain understudied. Therefore, we aimed to describe Oomycete species composition in soil samples from cacao farms and contrast the communities from cultivated soil with communities inhabiting the soil of adjacent areas, which included weeds and other cultivated crops. We used high-throughput multiplexed sequencing of the cytochrome oxidase II gene (*cox2*), because this marker has been used in previous studies to identify taxa in large Oomycete collections. Eight farms were sampled, covering three precincts of the Coastal Region of Ecuador. Total DNA was extracted from collected soil samples and used for library preparation. Amplicons of ~580bp were sequenced by Illumina MiSeq 2x300. Resulting reads were quality filtered before conducting OTU picking with usearch method against a custom Oomycete sequence database. Using *cox2* was advantageous to clearly differentiate up to genus level and in some cases, species were identified. The custom database, constructed from available records in Genbank and curated sequences from Phytophthora Database, reduced analysis time and returned better resolution than default databases. On the other hand, PCR bias and significant heterogeneity in read counts per sample undermined downstream analysis. The identified species belonged to Phytophthora and Pythium genera. Known Oomycete plant pathogens, like Phytophthora palmivora, are present both in cacao crops and in the

adjacent interfaces, despite the differences in plant diversity that are characteristic from both types of environments. Our results suggest that the neighboring zones contribute to the ecology of Oomycete pathogens affecting cacao.

136. SHORT-TERM DYNAMICS OF CULTIVABLE BACTERIAL POPULATIONS AND GREENHOUSE GAS EMISSIONS IN RESPONSE TO INDUCED AND NATURAL DISTURBANCES IN ORGANICALLY AND CONVENTIONALLY MANAGED SOILS

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Organically managed (ORG) soil is often considered healthier than conventionally managed (CONV) soil, with greater resistance and resilience to disturbances, as evidenced by reduced oscillations in bacterial populations and activities. Greenhouse gas (GHG) fluxes are mediated by bacterial processes, but variations in GHG emissions have not been related to bacterial oscillations in soil. Two environmentally controlled and two field experiments were set up to compare oscillations in bacterial colony-forming-units (CFUs) and GHG (nitrous oxide (N₂O), carbon dioxide (CO₂) and methane (CH₄)) fluxes after disturbances in ORG and CONV soils. Soil amendment with grass-clover (GC) or cattle manure (CM) resulted in peaks in N₂O and CO₂ emission, followed by CFUs. CH₄ temporarily increased in GC but decreased in CM amended soil. Ratios of CFUs and GHGs in amended over nonamended soils oscillated during three weeks, mostly with lower frequencies and amplitudes in ORG soils. Fluctuations were more irregular in field soils, but significant oscillations were detected after irrigation or intensive rain in summer. Cross correlations between variables hinted at sequences of microbial processes under controlled conditions but not in the field. GHG emissions were higher from ORG soil than CONV soil, indicating that these have to be taken into consideration when estimating soil health.

137. THE EVOLUTIONARY LINK BETWEEN COCCIDIAN SRS PROTEINS AND THE MALARIAL "6-CYS DOMAIN" FAMILY : NEW CLUES FROM MODELED 3-D STRUCTURES

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Fold similarity between surface proteins can be difficult to detect, especially in pathogens, due to highly variable insertions, etc. Bioinformatics and modeled 3-D structures can help, especially when such proteins are notoriously difficult to express and refold in vitro and crystal/NMR structures are pursued over decades.

The malaria vaccine candidate antigens Pfs48/45 and Pfs230 in the "6-Cys" (or PGSH) family are a good example. In 2012 and 2013 our prediction of a distant evolutionary relationship between these antigens in plasmodia and the dominant surface protein family in Toxoplasma, the "SAG1-Related Sequences" (SRS) (Gerloff et al (2005) PNAS 102:13598-13603), was validated by the first representative 3-D structures of the malarial surface protein Pf12 (Arredondo et al., PNAS 109:6692-6697, 2012; Tonkin et al., J Biol Chem 288:12805-12817, 2013). However, while the SRS surface proteins in the tissue cyst-forming coccidia (Toxoplasma/Neospora/Sarcocystis) and the "6-Cys" (PGSH) family in the hemosporidia (Plasmodium/Babesia/Theileria) seem distantly related, their functions and their disulfide bonds differ.

To find clues to their evolution, we screened 102,878 predicted proteins from apicomplexan genomes sensitively with merged family HMMs (hidden Markov Models). This yielded a more complete map of occurrences of this Apicomplexa-specific β -sandwich fold (>2,500 plausibly aligned domain matches), for example we found 9 in Theileria species which currently lack any annotated 6-Cys Domain proteins. Most interestingly, we caught a glimpse of the evolutionary basis of these proteins, structurally (and possibly functionally).

Most strikingly, we find a potential "evolutionary link" protein in the contemporary genomes of coccidian parasites (including the basal lineage *Eimeria*) with unexpected resemblance to the homologs in the other group, the plasmodia/piroplasmida. While functional studies of this "oddball" are underway, this and molecular modeling help explain how disulfide bonds may "shift" during evolution, a phenomenon that is observed in most β -sandwich folds, including immunoglobulin-like domains.

138. THE TYPE I INTERFERON RECEPTOR IS NOT REQUIRED FOR *C. MURIDARUM*-MEDIATED PROTECTION AGAINST A LETHAL HERPES SIMPLEX VIRUS CHALLENGE IN A MURINE SUPER-INFECTION MODEL

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Herpes Simplex Virus type 2 (HSV-2) and the bacterium, *Chlamydia trachomatis*, are common sexually transmitted pathogens responsible for new infections totaling more than 120 million per year in the US. *Chlamydia trachomatis*/HSV-2 vaginal co-infections are seen clinically, suggesting that these pathogens may interact. Therefore, we established an intravaginal super-infection model in which female mice are inoculated with 10⁶ inclusion forming units (IFU) of *C. muridarum*, followed by HSV-2 either 3 or 9 days later. While 50-100% of mice singly-infected with 5 x 10³-10⁵ plaque forming units (PFU) of HSV-2 develop fatal neuroinvasive disease, *C. muridarum*/HSV-2 co-infected mice exhibit greatly reduced viral shedding and <10% mortality. Thus, chlamydial pre-infection protects mice from a subsequent lethal HSV-2 challenge. Type I interferon, IFN- β , binds to the type I interferon receptor (IFNR), elicits a host cellular antiviral response, and inhibits HSV replication in vitro and in vivo. Because previous studies demonstrated that *C. muridarum* infection stimulates genital tract (GT) IFN- β production, we hypothesized that chlamydial pre-infection protects mice from

HSV-2 challenge via the IFN- β /IFNR-induced antiviral response. To test this model, we quantified IFN- β levels in vaginal swab samples from singly- and mock-infected mice. IFN- β was detected in *C. muridarum*-singly infected, but not in mock-infected animals. We then performed vaginal single and co-infections in IFNR knockout (IFNR KO) mice, which produce IFN- β but are deficient in IFN- β -induced antiviral responses. *C. muridarum* pre-infection reduced HSV-induced mortality in both wildtype mice (40% in HSV to 0% in Cm/HSV) and IFNR KO mice (100% in HSV to 40% in Cm/ HSV). HSV pathologic scores and viral shedding were similarly reduced by *C. muridarum* pre-infection. These data indicate that, while chlamydial infection induces GT production of IFN- β , type I IFN-induced antiviral responses are likely not required for the observed protective effect.

139. TRUCK MOUNTED NATULAR™ 2EC ULV RESIDUAL TREATMENT OF ARTIFICIAL CONTAINERS TO CONTROL AEDES AEGYPTI AND AEDES ALBOPICTUS IN NORTH FLORIDA

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Populations of adult *Aedes aegypti* and *Ae. albopictus* mosquitoes are notoriously difficult to target and control, and are key vectors of Zika, dengue, and chikungunya viruses. Larval populations of these species on the other hand are easier to target due to their aquatic nature, and confined development. However, the numbers of artificial and natural containers that can be exploited by these species, coupled with the tendency to skip oviposit can render control methods such as source reduction ineffective. Alternatively, treatment of an area with a residual larvicide may prove to be an effective measure of control in light of the number of potential sources of development in an area. We treated artificial containers placed in a variety of open and protected locations via truck-mounted ULV sprayer to dispense biologically-based Natular 2EC (spinosad) larvicide in a simulated urban setting in North Florida. The containers were then returned to the laboratory for the addition of water and *Ae. aegypti* or *Ae. albopictus* larvae. Efficacy was

measured by the number of adult mosquitoes that emerged from treated cups. We discuss our results in the context of control of these two species in key urban regions of the US.

140. USING SPATIAL VIDEO AND GEONARRATIVES TO SUPPORT FIELD CHOLERA EPIDEMIOLOGY IN THE SLUMS OF PORT-AU-PRINCE

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A multidisciplinary team is collaborating to assess water points and drainage for cholera in four slums of Port-au-Prince. Spatial encoded video and associated geonarratives provide context to the sites and conditions surrounding each water test location. Novel software is used to produce different visual displays and maps of these insights, such as potential sources of contamination and the changing nature of each test location. We will show how this method can enrich traditional epidemiological fieldwork.

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