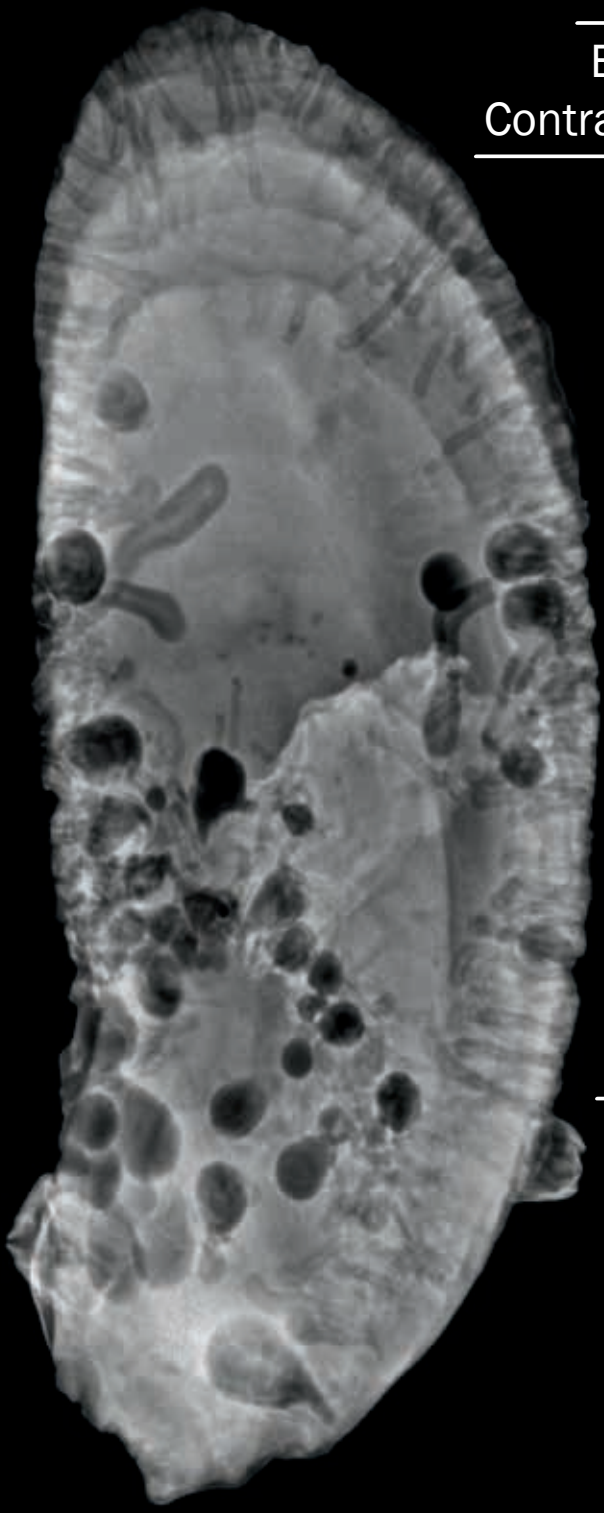

Emerging Diseases
Contrastive Examination

EPI RESEARCH DAY BOOK OF ABSTRACTS

FEBRUARY 2015

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Letter from the Director

Welcome to the eighth annual EPI Research Day! As you look through the abstracts in this book, and see the 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 UF investigators from outside of Gainesville, as well as investigators and collaborators from other institutions.

We are honored to have two outstanding speakers for our afternoon session.

Dr. Andrew Camilli, a Howard Hughes Medical Institute investigator in the Department of Molecular Biology and Microbiology at Tufts University School of Medicine, will be speaking on his work on the Vibrio cholerae life cycle, with a focus on his recent work with V. cholerae-specific bacteriophage and their role in modulating intestinal infection. He is joined by Dr. Myron Levine, former Director of the Center for Vaccine Development at the University of Maryland, Baltimore School of Medicine. Dr. Levine is a member of the Institute of Medicine/National Academy of Sciences, and a former President of the American Society of Tropical Medicine and Hygiene; he has been instrumental in development of vaccines for cholera, typhoid, rotavirus, and malaria.

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

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

Schedule of Events

9:00 AM - 10:00 AM	Registration, Breakfast, and Poster Setup *CGRC 1st, 3rd, and 4th floors*
10:00 AM - 1:00 PM	Poster Session *Presenters, please stand by your posters*
12:00 PM - 12:45 PM	Lunch *CGRC 1st floor Lobby*
12:45 PM - 1:00 PM	Keynote Assembly *CGRC Auditorium 101*
1:00 PM - 1:15 PM	Welcome *Dr. Thomas A. Pearson, Executive VP for Research and Education* Introductions *Dr. J. Glenn Morris, Director, EPI*
1:15 PM - 3:30 PM	Keynote Speeches
3:30 PM - 4:00 PM	Poster Removal

(1:15-2:15)

Dr. Andew Camilli, Ph.D.

Professor of Molecular Biology and Microbiology and Howard Hughes Medical Institute
Tufts University School of Medicine

"Study of the *Vibrio cholerae* Life Cycle and Ways to Prevent Infection"

(2:15-3:15)

Dr. Myron M. Levine, M.D., D.T.P.H

Associate Dean for Global Health, Vaccinology and Infectious Diseases
Simon and Bessie Grollman Distinguished Professor
Founder and Former Director, Center for Vaccine Development
University of Maryland School of Medicine

"Vaccines and Vaccine Development – Ebola Changes the Paradigm"

01. ASSESSMENT OF THE EFFECT OF INGREDIENTS ON PATHOGEN SURVIVAL IN COOKIE DOUGH

Shuang Wu - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; Alan Gutierrez - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; Soohyoun Ahn - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Cookie dough is often recognized as a potential vehicle for Salmonella. Food Safety concern for cookie dough has been further raised due to the recent E. coli O157: H7 outbreak associated with commercial product. It is believed that the nature of cookie dough of high fat and sugar contents and relatively low water activity can provide protection for pathogens to survive during storage. To determine the survival of foodborne pathogens in cookie dough and assess the impact of common cookie dough ingredients on survival of pathogens. Commercial cookie dough was inoculated with Salmonella Enteritidis and E. coli O157:H7 (106 CFU/g), stored at 4°C and -18°C, and the survival of pathogens was determined for 8 weeks. To determine the effect of ingredients on pathogen survival, cookie dough samples were prepared with various fat, sugar and salt contents, inoculated with S. Enteritidis or E. coli O157:H7 (106 CFU/g), and their survival was determined for 8 weeks. After 8 weeks, 2.42 and 2.35 log reduction for Salmonella and 2.23 and 1.99 log reduction for E. coli O157:H7 were obtained for commercial cookie dough at 4°C and -18°C, respectively. Cookie dough prepared with seven different recipes of cookie resulted in reduction of both pathogens, ranging from 0.73 to 1.45 log CFU/g. Our results indicate that pathogens in cook dough products can survive through their recommended storage conditions, and changing the content of a single ingredient hardly affects their survival rate. Our study suggests that that refrigeration/freezing and monitoring the ingredients concentration do not reduce the survival of pathogens.

Following good manufacturing practices is a fundamental step to eliminate initial introduction of pathogens in cookie dough, and, consumer education on risk of consuming raw cookie dough would be critical to eliminate future outbreaks.

02. CHOLERA TRANSMISSION IN OUEST REGION OF HAITI: DYNAMIC MODELING AND PREDICTION.

Alexander Kirpich - Department of Biostatistics, University of Florida; **Thomas A. Weppelmann** - Department of Environmental and Global Health, University of Florida; **Yang Yang** - Department of Biostatistics, University of Florida; **J. Glenn Morris, Jr.** - Department of Medicine, University of Florida; **Ira Longini** - Department of Biostatistics, University of Florida

We present a comprehensive, stochastic, compartmental model for cholera transmission. In our model we consider “short cycle” close human contact transmission and “long cycle” environment-to-human and human-to-environment transmission. This novel dynamic model incorporates both the cholera incidence and environmental data that are typically available from surveillance, and we apply our model to data collected in the Ouest region of Haiti. The model has separate compartments for infectious symptomatic and asymptomatic cases, reflecting different courses of the disease and different infectivity levels. We separately model the environmental compartment based on the knowledge of the biology of the disease, available environmental data, and vibrio shedding from humans.

Based on the model trend and the observed incidence we conclude that the epidemic of cholera “stabilized” in Haiti on the third year and became endemic. Model estimates suggest that the concentration of toxigenic V. cholerae in the environment is still high, and the proportion of the population susceptible to infection remains high, providing a setting in which continuing epidemics are likely to occur.

03. COMPARATIVE GENE EXPRESSION PROFILE BETWEEN V. CHOLERAE N16961 AND ITS GROWTH ADVANTAGE STATIONARY PHASE (GASP-700) PHENOTYPE

Mohammad Jubair - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **J. Glenn Morris, Jr.** - Department of Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Afsar Ali** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

To adapt to hostile and stressful survival conditions, bacterial species employ diverse phenotypic changes either by stochastic mechanisms or by adopting a “growth advantage stationary phase” (GASP) phenotype. We have recently reported that, in response to nutrient starvation in filtered sterilized lake water microcosms (FSLW), V. cholerae N16961 exhibited a novel “persister “ phenotype exhibiting long helical cells with bipolar flagella (as early as 24 h of growth in FSLW) that transitioned into very small cells with peritrichous flagella by 700 days of growth . Upon storage of persister cells in glycerol broth at -80^{o}C, the persister cells lost their ability to render into typical cells (a hall mark of persister cells); however, we found that stored cells become non-motile while promoting biofilm formation specific to FSLW. We termed these cells as growth advantage stationary phase (GASP) of V. cholerae. To better understand the GASP phenotype and compare the gene expression profile between GASP-700 (700 day old stored culture) and N16961 in FSLW microcosms, we compared the transcriptome profile of GASP-700 and fresh V. cholerae N16961 strains grown for 24 h in FSLW to V. cholerae N16961 cells persisting in L-broth for 24-h in otherwise identical growth conditions. The gene expression studies were performed using a custom made microarray containing 60 nucleotide (nt) oligomers designed from all ORFs present in two chromosomes of V. cholerae N16961. Data obtained from this study suggests that, a total of 190 genes were in common and

more highly expressed by both GASP-700 and N16961 grown in FSLW compared to N16961 grown in L-broth. Furthermore, 387 and 195 genes were expressed by GASP-700 and N16961 as stage specific manner, respectively, when compared to the L-broth grown N16961 strain. Similarly, 66 genes in common were repressed by GASP-700 and N16961 in the FSLW microcosm compared to N16961 grown in L-broth; in addition, 165 and 54 genes were repressed by GASP-700 and N16961 as stage specific manner, respectively, when compared to the N16961 grown in L-broth. Our data indicate that the GASP-700 phenotype has differential physiologic and metabolic adaptive responses. We propose that, in addition to persister phenotype, GASP-700 of V. cholerae may contribute to environmental persistence and cholera transmission.

04. DISTRIBUTION AND GENETIC STRUCTURE OF V. CHOLERAE IN SEAWATER AND OYSTERS FROM APALACHICOLA BAY, FLORIDA

Lei Fang - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; Michael Hubbard - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; Eva Chase - University of South Florida; Cheryl Whistler - University of New Hampshire; Andrew Kane - Department of Environmental and Global Health, Emerging Pathogens Institute, University of Florida; Valerie J. Harwood - University of South Florida; Anita Wright - Department of Food Science and Human Nutrition, University of Florida

Background: An outbreak of cholera in Florida in 2011 was attributed to the consumption of raw oysters contaminated by toxigenic Vibrio cholerae O75. Little is known about the ecology of this pathogen; therefore, levels of V. cholerae were examined in Apalachicola and Tampa Bays. The genetic relationship of environmental strains to the outbreak strain was examined.

Methods: V. cholerae levels were determined by most probable number from 138 water and 60 oyster samples collected at 15

sites in Apalachicola Bay and two sites in Tampa Bay, Florida. Distribution of V. cholerae was compared to other pathogenic Vibrios. V. cholerae populations were analyzed with respect to various environmental parameters. The genetic relationship of environmental populations to the outbreak strain was determined by multi-locus sequence typing (MLST).

Result: V. cholerae was not widely distributed throughout the bay and was more often associated with near shore and lower salinity sites, while V. vulnificus and V. parahaemolyticus showed much wider distribution over time, location, and sample types. Regression analysis indicated that salinity and conductivity were inversely correlated with abundance of V. cholerae in both water and oyster samples ($p<0.001$), while dissolved oxygen showed a positive correlation with V. cholerae in oyster samples ($R^2=0.410$, $p=0.0368$). Virulence potential of environmental isolates of V. cholerae appears to be limited due to absence of genes for cholera toxin and toxin co-regulated pilus; however, other virulence-associated genes were identified in most strains. MLST demonstrated the diversity of V. cholerae strains from Florida, but also showed that V. cholerae O75 was most closely related to strains from Apalachicola Bay and to classical V. cholerae, as compared to strains from Tampa Bay or El tor lineages.

Conclusions: V. cholerae showed more restricted distribution compared to other pathogenic Vibrios. Although occurrence corresponded to lower salinity, these sites were also in closer proximity to human impact and prevalence could be due to other factors. Risk from human exposure to contaminated oysters appears minimal, as these sites are not open to harvest, and all environmental isolates lacked the necessary virulence factors that are required for more severe cholera disease.

05. DIVERSITY AMONG SALMONELLA ISOLATES FROM AQUATIC WILDLIFE ASSOCIATED WITH IRRIGATION PONDS IN SOUTHERN GEORGIA

Amber Ginn - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; Zhiyao Luo - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; Elizabeth Antaki - Department of Veterinary Medical Sciences, University of California, Davis; Yingjia Benson - Department of Veterinary Medical Sciences, University of California, Davis; Peiman Aminabadi - Department of Veterinary Medical Sciences, University of California, Davis; Anita Wright - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; Michele Jay-Rusell - Department of Veterinary Medical Sciences, University of California, Davis

Agricultural water is implicated as a risk factor for produce contamination by Salmonella enterica, and this study surveyed Salmonella populations associated with aquatic wildlife from irrigation ponds (n=5) located in southern Georgia, U.S.A. Amphibians and reptiles, as well as pond water and sediments, were sampled, and Salmonella was identified in 17.7% of the aquatic wildlife specimens sampled, compared to 28.2% of water and sediment samples from the same region. Diversity of Salmonella strains was examined using repetitive extragenic palindromic PCR (DiversiLab rep-PCR), traditional serotyping, multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE) and antibiotic resistance profiling. Strains that showed >95% similarity in banding patterns by rep-PCR were grouped into DiversiLab types (DTs). Salmonella isolates derived from aquatic wildlife (n=44) were distributed into 19 DTs, with 10 of DTs containing only one isolate, while the two largest populations of closely related DTs contained 7 and 8 isolates each. DTs of aquatic wildlife strains were compared to those of reference strains (n>500) from both environmental and clinical origin. Most (n=28 or 63.6%) of wildlife strains showed closest similarity to isolates from environmental sources, including pond

isolates from this study and isolates from the Suwannee River or lakes in Florida. Strains unique to wildlife in this study were also identified (n=11or 25%), as they showed 95% DT similarity to water/sediment isolates from the same pond was 24.8%, suggesting the possible impact of the aquatic wildlife on irrigation water. Serotyped isolates from aquatic wildlife (n=41) exhibited the most prominent serotypes as Muenchen (n=11 or 25.6%), Montevideo (n=8 or 18.6%) and Newport in (n=4 or 9.3%). MLST analysis found 15 different sequence types while PFGE analysis found 20 different pulsotypes. Antibiotic resistant profiles showed 97.6% resistant to streptomycin, 69.0% resistant to only 1 antibiotic, 28.6% resistant to 2 antibiotics, and 2.4% resistant to 3 antibiotics with similar resistance profiles between pond water/sediment isolates and those of animal origin. Overall, Salmonella from aquatic wildlife in this region represented a diverse population that was more likely to resemble strains from environmental rather than clinical origin.

06. HIGH-FREQUENCY RUGOSE EXOPOLYSACCHARIDE PRODUCTION BY VIBRIO CHOLERAE STRAINS ISOLATED IN HAITI

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Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **J. Glenn Morris, Jr.** - Emerging Pathogens Institute, University of Florida; **Afsar Ali** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

In October, 2010, epidemic cholera was reported for the first time in Haiti in over 100 years. Establishment of cholera endemicity in Haiti will be dependent in large part on the continued presence of toxigenic V. cholerae O1 in aquatic reservoirs. The rugose phenotype of V. cholerae, characterized by exopolysaccharide production that confers resistance to environmental stress, is a potential contributor to environmental persistence. Using a microbiologic medium promoting high-frequency conversion of smooth to rugose (S-R) phenotype, 80 (46.5%) of 172 V. cholerae strains isolated from clinical and environmental sources in Haiti were able to convert to a rugose phenotype. Toxigenic V. cholerae O1 strains isolated at the beginning of the epidemic (2010) were significantly less likely to shift to a rugose phenotype than clinical strains isolated in 2012/2013, or environmental strains. Frequency of rugose conversion was influenced by incubation temperature and time; appearance of the biofilm produced by our prototype Haitian clinical rugose strain (altered biotype El Tor HC16R) differed from that of a typical El Tor rugose strain (N16961R) by confocal microscopy. On whole genome SNP analysis, there was no phylogenetic clustering of strains showing an ability to shift to a rugose phenotype. Our data confirm the ability of Haitian clinical (and environmental) strains to shift to a protective rugose phenotype, and suggest that factors such as temperature influence the frequency of transition to this phenotype.

07. IN SITU ANTIMICROBIAL ACTIVITY OF CHITOSAN NANOPARTICLES

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Antibiotics have been key elements to sustain human and animal health. However, the essential role of antibiotics has been challenged by the occurrence of antimicrobial resistance (AR). For example, metritis and mastitis are commonly treated by antibiotics, but the treatment failure rate is about 30% in dairy cattle due to AR. The rise of AR is a tremendous concern for public and animal health. Chitosan nanoparticles (CN) have been developed as a natural antimicrobial agent, and in vitro results provide promising use for disease treatment. However, antimicrobial activity of CN in situ remains unclear. The purpose of this study was to evaluate antimicrobial activity of CN in fluids from dairy cows with metritis and mastitis.CN was prepared by cross-linking of chitosan solution, and the size of CN was measured by nanoparticle analyzer (Zetasizer nano series, Malvern, Worcestershire, UK). For in situ antimicrobial activity of CN, cow uterine fluids and milk samples were collected from animals with metritis and subclinical mastitis, respectively. Antimicrobial activity of CN was evaluated by the enumeration of naturally infected pathogens in the fluids. CN treatment effectively reduced the concentrations of pathogens in the matrices of cow uterus and milk. The antimicrobial activity varied depending on matrices and CN concentrations. In LB broth, 0.1% CN completely killed E. coli O157:H7 during 2 h of incubation. In milk, naturally infected pathogens were completely killed in 4 h with 0.1% CN. In cow uterine fluid, although the growth of naturally infected pathogens was inhibited at 0.1%, higher concentration (0.6%) of CN was required to kill pathogens, suggesting the antimicrobial

activity of CN is inhibited in the uterine fluid. In addition, CN resistance was not detected in E. coli O157:H7 after treatment, suggesting CN provides insight for potential use for antimicrobial resistant microorganisms. The data demonstrate that a natural antimicrobial agent CN retains antimicrobial activity in different matrices that provides encouraging solution to enhance animal and public health, especially targeting antimicrobial resistant microorganisms.

08. INCREASED ISOLATION FREQUENCY OF TOXIGENIC VIBRIO CHOLERAE O1 FROM ENVIRONMENTAL MONITORING SITES IN HAITI

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Since the identification of the first cholera case in 2010, the disease has spread in epidemic form throughout the island nation of Haiti; as of 2014, about 700,000 cholera cases have been reported, with over 8,000 deaths. While case numbers have declined, the more fundamental question of whether the causative bacterium, Vibrio cholerae has established an environmental reservoir in the surface waters of Haiti remains to be elucidated. In a previous study conducted between April 2012 and March 2013, we reported the isolation of toxigenic V. cholerae O1 from surface waters in the Ouest Department. After a second year of

surveillance (April 2013 to March 2014) using identical methodology, we observed a more than five-fold increase in the number of water samples containing culturable V. cholerae 01 compared to the previous year (1.7% vs 8.6%), with double the number of sites having at least one positive sample (58% vs 20%). Both seasonal water temperatures and precipitation were significantly related to the frequency of isolation. Our data suggest that toxigenic V. cholerae 01 are becoming more common in surface waters in Haiti; while the basis for this increase is uncertain, our findings raise concerns that environmental reservoirs are being established.

09. PREVALENCE AND DYNAMICS OF CEFOTAXIME RESISTANCE IN BEEF CALVES

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Cephalosporins contribute 14% of total dispensed antibiotics in the US. The third-generation cephalosporins are therapeutically used in human and veterinary medicine. Food animals are blamed for origin of antibiotic resistance, but animal factors that affect the antibiotic resistance haven't been studied in detail. We evaluated several factors that affect the dynamics of cefotaxime resistance in beef calves which have never received any cephalosporin antibiotic in their life. We tracked cefotaxime (a third-generation cephalosporin) resistance in a cohort of 300 beef calves soon

after their birth for one year. Four samples were collected around 3 months apart from a multi-breed beef calf population derived from Brahman and Angus cattle. This study utilized a combination of culture-based and nucleic acid-based methods for the detection and enumeration cefotaxime resistant bacteria from the fecal samples. Fecal swabs were collected, processed and inoculated in culture media containing the cefotaxime at the same concentration at which it is used in clinical cases. Data were analyzed by logistic regression methods using STATA software. Although the beef calves were never exposed to any of the cephalosporin antibiotics, still the prevalence of cefotaxime resistance (CefR) was high in the beef calves. The cefotaxime resistance was 61.07%, 50.7%, 68.57%, and 6.25% in March, June, August, and December sampling, respectively. Cefotaxime resistance was not significantly associated with animal factors like breed, sex, castration, or weight gain. However, the effect of climate could be evident by the lowest prevalence of cefotaxime resistance in December sampling, which could indicate antibiotic use in animals is not the only factor that influences antibiotic resistance. This study will help to develop intervention strategies to control antibiotic resistance in beef cattle and to understand root causes of emerging resistance to modern therapeutics.

10. PREVALENCE AND DYNAMICS OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI (STEC) IN BEEF CALVES

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Each year Shiga toxin-producing Escherichia coli (STEC) are responsible for 2.8 million acute illnesses around the world and almost 30 deaths in the US. Since cattle are the primary reservoir for STEC, lowering the prevalence of this pathogen in farm animals may reduce STEC outbreaks in humans. However, factors that modulate the colonization and persistence of STEC in young beef cattle remain unknown. This study evaluates the role of animal and environmental factors influencing the shedding of STEC in beef cattle such as breed, age, sex. A cohort of 300 beef calves from a multi-breed beef calf population derived from Brahman and Angus cattle was sampled four times every three months after birth. This study utilized a combination of culture-based and nucleic acid-based methods for the detection and enumeration of STEC from the fecal samples. Data were analyzed by regression methods and McNemar's test for matched pairs using STATA software. The herd prevalence of STEC in March was 59.8%, which was significantly higher compared to any of the other sampling times (39.5% in June, 20.3% in August and 20.7% in December). The stx2 genotype was predominant in the herd, whereas stx1/stx2 was the lowest in all samplings. However, there was no significant association between breed group, sex of the calf or average weight gain with the STEC shedding. We observed STEC shedding was significantly affected by animal age. In summary, beef calves shed high level of STEC at an early age and it decreases as the animal grows and reaches a lower level of ~20% in the beef herd. This study provides insight that animal age is a significant factor that influences the prevalence of STEC.

11. RAPID DETECTION OF ESCHERICHIA COLI O157:H7 IN ALFALFA SPROUTS USING LIQUID CRYSTAL-BASED IMMUNOASSAY

Jieun Jung - Konkuk University; **Shuang Wu** - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; **Hyun-Dong Paik** - Konkuk University; **Soohyoun Ahn** - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida

E. coli O157:H7 a major foodborne pathogen that has posed serious problems for food safety and public health. Although E. coli O157:H7 has been associated with various types of food, alfalfa sprouts are considered one of main food vehicles for E. coli O157:H7. A rapid and sensitive detection assay for this pathogen is critical to control outbreaks and ensure food safety.

Purpose: The aim of this study was to evaluate a novel liquid crystal-based system as a rapid and sensitive assay for detection E. coli O157:H7 in alfalfa sprouts. The liquid crystal-based immunoassay for E. coli O157:H7 was developed using immunomagnetic beads (IMB) and liquid crystal. When E. coli O157:H7 is present, formation of E. coli O157:H7-IMB aggregates distorts liquid crystal matrix and causes the bending of light, which is then detected by Crystal Diagnostics Xpress system. The assay was tested for its sensitivity using artificially inoculated alfalfa sprouts, and its specificity was tested with various non-O157 shiga toxin-producing E. coli (STEC) strains and common foodborne pathogens.

The developed immunoassay was able to detect E. coli O157:H7 with detection limits of 105 CFU/mL without any enrichment. However, when 6 hr sample enrichment step was added, the assay could detect E. coli O157:H7 as low as 1 CFU in 100 g of alfalfa sprouts. The total assay was completed within 30 min. The developed assay was highly specific to E. coli O157:H7, and did not show any cross-reactivity with non-O157 STEC or with other common foodborne pathogens.

The novel immunoassay based on liquid crystal technology shows a great potential as a rapid and sensitive detection method for E. coli O157:H7 in food.

12. SIMULTANEOUS DETECTION OF MAJOR SHIGA TOXIN-PRODUCING ESCHERICHIA COLI SEROTYPES IN GROUND BEEF BY IMMUNOMAGNETIC BEAD-BASED FLUORESCENT ASSAY

Alan Gutierrez - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Jieun Jung** - Konkuk University; **Tyler Austin** - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Hyun-Dong Paik** - Konkuk University; **Soohyoun Ahn** - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

The USDA Food Safety and Inspection Service (FSIS) considers raw beef products contaminated with E. coli O157:H7 and big 6 Shiga toxin-producing Escherichia coli (STEC) serotypes including O26, O45, O103, O111, O121 and O145 to be adulterated, and mandates testing of domestic and imported beef for these STEC serotypes in order to mitigate food safety risks associated with these pathogens. It is estimated that STEC causes 176,000 infections each year in the US, and therefore a rapid and simple detection assay that can simultaneously detect the presence of these STEC serotypes is highly desirable. The purpose of this study was to develop an immunomagnetic bead-based assay for the simultaneous detection of E. coli O157:H7 and big 6 STEC serotypes in ground beef. Immunomagnetic beads functionalized with antibodies to target STEC serotypes were loaded into a microplate and used as a bead-based array platform for STEC identification, and the presence of target STEC was determined by measuring fluorescence signal from detection antibodies. Twenty-five grams of each ground beef sample was inoculated individually with target STEC serotypes at concentrations from 100 to 105

CFU/g. The samples were enriched in 225 milliliters of tryptic soy broth and incubated at 37° C. Each sample was collected at different times (6, 9, 12, and 18 h) and tested with the developed assay. The developed immunoassay could detect target STEC serotypes as low as 2,000 CFU/mL without any enrichment. The assay can be completed within 4 hrs, and no cross-reactivity was observed. When ground beef samples contaminated with STEC were tested, the assay was able to detect 1 CFU/g of STEC with 9 hr-enrichment. This study demonstrated that the developed immunomagnetic bead-based immunofluorescent assay has great potential to simultaneously detect multiple STEC serotypes in ground beef present at low concentrations.

13. A SYSTEMATIC REVIEW AND META-ANALYSIS TO MEASURE SERO-PREVALENCE OF INFLUENZA A (H9N2) VIRUS INFECTION IN HUMANS

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Introduction: Influenza A H9N2 subtype is prevalent among domestic poultry in many countries and have a potential to cause pandemics. The objective of this study was to assess the overall burden of H9N2 infections among the poultry-exposed human populations worldwide using a meta-analysis of the published serological data.

Methods: Considering the period January 1997 to December 2013, we performed a systematic search using the PubMed,

AGRICOLA, and Cab abstracts databases for the following terms, all in the "explode" function: "influenza H9N2" AND "serological surveys" OR "seroprevalence" OR "sero-prevalence" OR "seroepidemiology" OR "sero-epidemiology". Related studies in all languages were all considered. Articles were selected if they were population-based (surveillance reports, cross-sectional or prospective) and contained laboratory serological data. Herein we report and compare positive hemagglutination inhibition (HI ≥1:160 titer), positive microneutralization (MN ≥1:80 titer) assays, and seroconversion (4-fold rise in HI or MN titer over time) as a standardized case definition for seropositivity with the reports from the studies. We calculated non-adjusted seroprevalance through a random effect meta-analysis model. We calculated heterogeneity by Pearson's chi-squared test, which was determined using I2 index statistics to estimate the proportion of total variation.

Results: We identified 22 studies for the final review and meta-analysis. Most of these reports were from Asia (86%) and cross-sectional surveys in nature (82%). The majority of the study participants were ≥18 years of age and almost all of them had a history of poultry or wild bird exposure. Meta-analysis demonstrated that the overall HI seroprevalence calculated using the antibody cut-off reported by the studies was 9.6% [95% confidence interval (CI): 08 – 12%, I2 = 98.5%], which was higher compared to the standardized case definition (≥1:160) for seropositivity (seroprevalence: 6.6%, 95% CI: 4 – 9%, I2 = 98.6%). For the MN meta-analysis, the overall reported seroprevalence was 3% [95% CI: 2 – 5%, I2 = 87.8%], which was higher compared to the standardized case definition (≥1:80) for seropositivity (seroprevalence: 0.2%, 95% CI: 0.0 – 0.5%, I2=29.4%).

Conclusion: Findings from the meta-analysis suggest that few poultry-exposed humans are infected with H9N2. However, should H9N2 viruses further adapt to human hosts and develop more virulent characteristics, they may spread quite rapidly among man causing significant morbidity and mortality. Efforts should be increased to conduct more aggressive surveillance for H9N2

strains such that genetic changes might be identified in time to provide pre-pandemic warnings.

14. COMPETITION BETWEEN LP AND HP AVIAN INFLUENZA IN A TWO PATCH SYSTEM

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Over the last decade, epidemiology of avian influenza has undergone a significant transformation. Not only have we seen an increase in the number of outbreaks of the deadly strain known as high pathogenic avian influenza (HPAI), but the number of birds infected, and the cost of control has risen drastically. Live poultry farms play a huge role in the bird to bird transmission of avian influenza. We develop a two patch model to determine the competition between LPAI and HPAI strains. We define the two patches as live poultry markets in which the patches are connected though migration. We use a system of differential equations to analyze the existence-stability of the equilibriums, and established results for the critical threshold R0. We observed that migration plays a key role in determining whether LPAI and HPAI can invade.

15. ENHANCED INFLUENZA VIRUS INFECTIVITY THROUGH SUPPRESSION OF TOLL-LIKE RECEPTOR ACTIVITY BY SINGLE-WALLED CARBON NANOTUBES

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Despite recent advancement in manipulating nanomaterials and their growing application in a wide variety of fields, sound

understanding regarding toxicity associated with potential exposures is still urgently needed. Single-walled carbon nanotubes (SWCNTs), allotropes of carbon with a cylindrical structure that share a resemblance to asbestos, raise concerns regarding long-term adverse health effects associated with inhalation. This underscores the critical need to comprehend how SWCNTs impact the respiratory system. While numerous toxicological studies have focused on fibrosis, cancer, and exacerbation of asthma, the ability of SWCNTs to modulate infectivity of pathogens has been minimally explored. Our recent work has indicated that SWCNTs increase influenza virus infectivity in small airway epithelial cells (SAECs) and suppress anti-inflammatory and -viral genes. To decipher the molecular mechanisms driving viral infectivity, we investigated whether SWCNTs mediate TLR3, a receptor that recognizes viral dsRNA as the first line of defense. Our hypothesis was that SWCNTs reduce TLR activity, resulting in inhibition of downstream anti-inflammatory and -viral genes mediated by transcription factors NF-kB and/or IRFs. For these studies, SAECs were exposed to Poly (I:C), a TLR3 agonist, singly and following pre-treatment with SWCNTs. TLR3 activity and gene expression were measured by luciferase reporter assays and qRT-PCR, respectively. The results demonstrated that SWCNTs did not alter TLR3 activation alone, but suppressed TLR3 activity by Poly (I:C) via NF-kB and IRFs in a dose-specific manner. SWCNTs also repressed genes induced by Poly (I:C), including IFIT2/3, CCL5 while further stimulating IL8. Collectively, these data suggest that SWCNTs suppress the innate immune response to viruses in lung cells, rendering them more susceptible to infections. Our study highlights a novel mechanism of SWCNT toxicity.

16. FIRST CONFIRMED CASE OF MIDDLE EAST RESPIRATORY SYNDROME IN FLORIDA, ORANGE COUNTY, FLORIDA, MAY 2014

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Background: First reported from Saudi Arabia in September 2012, Middle East Respiratory Syndrome (MERS) is a viral respiratory illness caused by a coronavirus called MERS-CoV. Most individuals with confirmed MERS-CoV have had severe acute respiratory illness (e.g., cough, fever, shortness of breath). The World Health Organization has reported 945 lab-confirmed cases of infection with MERS-CoV with a case fatality rate of 37% as of January 5, 2015. So far, all cases of MERS have been linked to countries in and near the Arabian Peninsula. Based on previous investigations, people with underlying health conditions (e.g., diabetes, cancer and chronic kidney, lung and heart disease) are more likely to become infected with MERS or have a severe manifestation. The virus is primarily transmitted through close contact, such as living with or caring for an infected person for an extended period of time. Investigations are ongoing to better understand how MER-CoV spreads from person to person. Based on current information, the incubation period for MERS is 2-14 days. On May 2, 2014 the United States confirmed its first case of MERS-CoV in a healthcare worker with recent travel to Saudi Arabia.

Introduction: On May 9, 2014, an infection control practitioner (ICP) at a local hospital notified the Florida Department of Health in Orange County (FDOH-Orange) of a potential MERS case. The patient presented with a ten-day history of worsening fever, chills,

cough, sore throat and myalgia and reported to the emergency department due to concerns of "coronavirus" since he works at a hospital in Saudi Arabia. Following consultation between public health and hospital officials, it was agreed upon to test for MERS-CoV. As per an established MERS lab algorithm, a serum sample collected from the patient was interpreted as negative for MERS-CoV. Out of an abundance of caution, the Centers for Disease Control and Prevention (CDC) was further consulted and the patient was kept in isolation and an induced sputum sample was collected on May 10. On the same day, the Bureau of Public Health Laboratories in Tampa (BPHL-Tampa) reported that the sample was positive by PCR for MERS-CoV; this was confirmed by the CDC on May 11, 2014. This was the second reported confirmed case of MERS-CoV in the United States.

Methods: An outbreak investigation was initiated on May 9, including active and passive surveillance via monitoring ESSENCE (the Department of Health's statewide syndromic surveillance system), establishing a DOH-Orange hotline, distributing media releases, sending messages through the Health Alert Network system, calling contacts and conducting home visits. The case was interviewed on multiple occasions throughout the course of the investigation by the same interviewer and a detailed history was taken to identify contacts and his epidemiological risks. The case was isolated in a negative air pressure room in the hospital until all specimens tested negative for the virus on two consecutive days. A Patient Under Investigation (PUI) was defined as a person who was clinically compatible for MERS and also had one or more epidemiological risk factors for the virus. PUIs were asked to quarantine at home with a mask in a separate room until clinical samples produced a negative result. The case's clinical samples were collected by the hospital and samples from PUIs were collected by DOH-Orange epidemiology staff. These clinical samples were couriered to BPHL-Tampa and typically tested on the same day. If non-close contacts consented, a serum sample was collected and shipped to the CDC for testing at a minimum of 14 days from last exposure. HCWs attending the confirmed case were independently quarantined by their hospital administration.

Non-close contacts were not asked to quarantine, unless they developed symptoms consistent with MERS. An electronic database, EPI INFO7™, was established to manage all identified contacts.

Results: Initially, the case-patient reported that his job requires very limited contact with patients. However, he later revealed that he visited a friend in the hospital in Saudi Arabia on April 20, 2014 who was ill with respiratory symptoms and subsequently died from MERS. The case-patient was identified to have potentially exposed other people to MERS-CoV in four general locations: 1) during airline travel from Saudi Arabia to Orlando, 2) household contacts and visiting friends, 3) hospital outpatient waiting room while accompanying a relative for an unrelated medical reason, and 4) an emergency waiting room before being evaluated for potential MERS-CoV. In total 211* contacts were identified, including 32 (15%) close contacts and 179 non-close contacts; overall, 14 of these contacts (3 close and 11 non-close) met the criteria for PUI. All specimens from PUIs were polymerase chain reaction (PCR) negative for MERS-CoV. The case-patient continued to test positive for MERS-CoV 15 days post symptom onset. In total, 29 clinical samples were taken from the case-patient; four (3 sputum and 1 serum) were PCR-positive for MERS-CoV.

Conclusions: This investigation highlights the critical role that HCPs and public health practitioners play in the role of considering a diagnosis of MERS-CoV in persons who develop respiratory symptoms 14 days post travel to the Arabian Peninsula. Sputum samples were the most sensitive for viral detection of MERS-CoV. The lack of secondary infections in this investigation is significant for future case contact investigations. In combination with findings from previous case investigations, refinement of the risk definition for contacts may reduce the burden on public health responders both in terms of contact identification, follow-up, and laboratory testing.

17. LIGHTING UP CD8 T CELLS: IMPLICATIONS FOR RESPIRATORY VIRAL VACCINE DEVELOPMENT

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CD8 memory T cells play a critical role in protection against repeated exposure to respiratory viruses. Memory T cell development in the lung is still not fully understood in terms of the nature and source of the molecular signals that establish and maintain the memory state. LIGHT is a tumor necrosis factor family member expressed by immune cells such as activated T cells, dendritic cells, monocytes and granulocytes. It is known to interact with two receptors HVEM & LTβR, leading to differential outcomes. However, the role of LIGHT expressed specifically by CD8 T cells during respiratory viral infection is not known. Using adoptive transfer of LIGHT-deficient CD8 TCR-transgenic T cells responding to Ag in the context of respiratory vaccinia virus (VACV) infection, we found that LIGHT signaling in CD8 T cells had a small impact on the level of primary expansion, with at most 30-40% fewer CD8 T cells accumulating in the lungs at the peak of response to VACV infection. In striking contrast, very few effector CD8 T cells that lacked LIGHT survived the contraction phase to differentiate into long-lived memory cells in the lungs. Furthermore, we found that HVEM, but not LTβR, functions as a trans-activating binding partner for LIGHT expressed on CD8 T cells. These results underscore the importance of LIGHT in the memory CD8 T cell development and suggest that targeting LIGHT-HVEM interaction will help in designing CD8 T cell based vaccines against respiratory viruses.

18. COMPARATIVE TRANSCRIPTOMICS BETWEEN THE CNS OF HORSES INFECTED WITH WEST NILE VIRUS AND THE APICOMPLEXAN PARASITE SARCOCYSTIS NEURONA

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Equine protozoal myeloencephalitis (EPM) and equine West Nile (WN) encephalitis are two most commonly diagnosed equine neurological diseases in North America and they are mutual primary differential diagnoses. EPM is more frequently caused by the coccidian parasite *Sarcocystis neurona* and West Nile virus (WNV) is a mosquito-borne zoonotic arbovirus.

In the EPM study, we first identified parasite presence from four central nervous system (CNS) locations including cerebrum, thalamus, pons, and cervical spinal cord from six EPM diseased horses. Secondly, we cut 30 to 50 ~30mg tissues from each parasite presented location and then tested parasite load and CD3 expression from each tissue. Eventually, total 12 tissues with high parasite load, CD3 expression, and RNA integrity number from four locations of six EPM diseased horses and corresponding normal control tissues were selected for 4x44,000 custom equine CNS microarray.

Equine WN encephalitis microarray experiment was performed by using the same microarray. Six thalamus tissues were collected for microarray from six non-vaccinated experimental intrathecal challenged horses who developed grave West Nile encephalitis (100% nonsurvivorship) and six location matched tissues were selected as control from six normal horses.

Both EPM and WNV microarray data were log2 transformed and quantile normalized to remove inherent technical biases. Normalized data were further analyzed using JMP genomics (SAS

Institute) by a 5% false discovery rate (FDR) to identify differentially expressed genes (DEGs). DAVID was used for Gene Ontology analysis and Ingenuity Pathways Analysis (IPA) was used to build the pathways among DEGs.

4068 probes were significantly expressed from EPM experiment and 5126 probes were significantly expressed from WNV experiment. Among 4068 (5126) probes, there were 1959 (2925) unique equine genes identified by BLAST to equine RefSeq database with Expect Values (E-value) below 10^{-4} and alignment coverage above 85 percentage and there were 1716 (2612) human homologs with E-value at 10^{-4} and with alignment coverage at 75% cutoff.

Regarding to EPM (WNV) study, 1118 (2294) DEGs with more than 1.5 fold change were analyzed by IPA. Antigen presentation pathway and dendritic cell maturation were the common canonical pathways among both top five pathways. In the diseases and disorders and molecular and cellular functions categories, most EPM and WNV DEGs were related to Neurological Disease ($p<0.001$) and Cell Death and Survival ($p<0.01$). Biomarker comparison analysis was performed and there are 14 unique biomarkers for EPM, 44 unique biomarkers for WNV, and 24 common biomarkers.

19. HELMINTHS OF RACCOONS (PROCYON LOTOR): ARE HELMINTHS SENSITIVE TO HEAVY METAL POLLUTION? – A WILDLIFE HEALTH PERSPECTIVE

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Anthropogenic pollutants disrupt biodiversity on a global scale. The raccoon (*Procyon lotor*), a widely distributed omnivorous species, is known to harbor several ubiquitous helminths species, whose occurrence and transmission occur in complex parasite life cycles through multiple invertebrate and vertebrate hosts. Anthropogenic stressors, such as heavy metal pollution, may directly reduce parasites or certain intermediate hosts, potentially influencing the transmission and abundance of parasites. This study investigated the relationship between abundance of helminths and raccoon exposure to heavy metal contaminants. We compared helminth loads and physiological parameters (i.e., morphometry, complete blood cell count, histopathology and metal content of the liver) of raccoons inhabiting contaminated (with Cr, Cu, Zn, As, Se, Cd, Pb, Hg) and uncontaminated sites of the U.S. Department of Energy´s Savannah River Site (SRS) in South Carolina. Between summer and fall 2013, we captured 15 raccoons in an area contaminated with metal pollution from coal fly ash accumulation, and 11 raccoons from a comparable uncontaminated site nearby. Multiple regression models showed that, of eight metals analyzed, copper (β_1 1.6245 t-value 2.778 $p<0.05$), arsenic (β_1 1.7285 t-value 4.445 $p<0.05$), selenium (β_1 0.9762 t-value 2.121 $p<0.05$) and lead (β_1 1.43932 t-value 2.441 $p<0.05$) were elevated in raccoons from the contaminated site compared with animals from the uncontaminated site. Among the 26 raccoons sampled there were four parasites that could be identified to species: Two nematodes: *Placoconus lotoris*, *Physaloptera rara*; one cestode: *Atriotaenia procyonis*; and one acantocephalan: *Macracanthorhynchus ingens*. Increased copper (β_1 -1.2610 t-value -3.857 $p<0.05$) concentrations were correlated with highly parasitized raccoons (i.e., helminth loads ranging from 0 to 32 individual parasites per raccoon). No other adverse health affects were observed in the raccoons exposed to higher levels of contaminants, however. Reported metal levels in raccoons at SRS were comparatively below those believed to cause adverse effects in mammals. It appears that raccoons are sensitive bioindicators of certain metals. Although we did not detect differences in health parameters as measured by body size, hematology and histopathology, we did detect elevated levels of helminths in

animals from the contaminated site. Even though the health effects are unclear, changes in parasite load may be sensitive indicators of heavy metal exposure in raccoons.

20. LOOK DEEP INTO MY SHELL: SHELL PARASITISM AND RADIOGRAPHY OF APALACHICOLA BAY OYSTERS

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Oyster health and condition assessments are part of ongoing, community-based efforts focusing on Apalachicola Bay, a heritage oyster fishery along Florida's northern Gulf coast. Baseline and restoration monitoring research, to better understand environmental and management factors, has been in effect since the fishery decline in 2012. Assessments included observations of shell parasitism by boring clams (*Diplothyra*), polychaete worms (*Polydora*) and sponge (*Cliona*). Radiographic visualization provided a far more accurate assessment of prevalence and ranked severity, than direct visual observations (that typically underestimated shell parasite severity up to 10-fold). Radiography also revealed that *Polydora* infection occurs primary from the shell edge, not from other external aspects of the shell. Further, size bin analyses indicated that colonization of live oysters with

Polydora preceded colonization by *Cliona* and *Diplothyra.* Elevated salinity conditions, associated with drought and reduced water flow into the Bay, favor the presence of these shell parasites, weakening the live shell and making the oyster host more susceptible to predation by drills and crabs. Parasitized shell that remains on the reefs as cultch material likely degrade more quickly due to enhanced surface area. Shell parasitism and *Perkinsus marinus* histological observations from stained mantle tissue were positively associated with oyster height ($p<0.05$) from different sample locations within the bay. Health and condition indices, and size class-related recruitment and mortality, are discussed relative to management considerations. Support for these studies was provided, in part, through the National Institute for Environmental Health Science (U19 ES020683), NOAA/Florida Sea Grant (NA10OAR4170079) and UF IFAS.

21. POPULATION GENETIC STRUCTURE OF THE LATE BLIGHT PATHOGEN, PHYTOPHTHORA INFESTANS, IN MEXICO

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Phytophthora infestans, the causal agent of the late blight in potato and tomato, causes annual losses of more than $6 billion worldwide. In the central highlands of Mexico, P. infestans undergoes regular sexual reproduction in contrast to the asexual reproduction that characterizes this pathogen in the United States. The biology of these populations together with multilocus genetic data points to Central Mexico as its center of origin. The diversity and population structure of P. infestans in Mexico has not been characterized using the simple sequence repeat (SSR) markers

currently being used to monitor P. infestans in the United States, Europe, and elsewhere. We genotyped P. infestans isolates sampled from several states in Mexico at 12 microsatellite loci to examine genetic diversity within and among populations using analysis of molecular variance, Bayesian clustering, and discriminant analysis of principal components. We found significant genetic variation both within and between states. The Michoacán population is distinct from the Toluca population and is also characterized by an asexual population on tomato. Understanding the genetic structure and diversity of P. infestans in Mexico, using the same set of hypervariable markers as used to study P. infestans in other countries, will contribute to our understanding of the contemporary global diversity and migration of this problematic pathogen.

22. THREE CLONAL LINEAGES CHARACTERIZE THE POPULATION OF THE COCOA PATHOGEN, PHYTOPHTHORA MEGAKARYA, IN NIGERIA

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Phytophthora pod rot (black pod disease) is a major constraint to cocoa production in Africa, particularly endemic to Central and West Africa cocoa producing countries. This disease is caused predominantly by Phytophthora megakarya, a devastating, invasive, aggressive and highly virulent pathogen, which frequently causes loss of cocoa pods up to 60-100% especially if left uncontrolled. Breeding for resistant cultivars is a priority to reduce use of the fungicides currently used to control the disease. However, there is little knowledge of the population structure and diversity of the pathogen, which is critical for breeding resistance that will be effective and durable. We obtained SNPs by genotyping-by-sequencing from 95 isolates of P. megakarya collected from cocoa pods in Nigeria. We found two distinct genetic groups that appear to comprise three clonal lineages. The largest clonal group was dominant throughout the collection area,

whereas the others were found only in the southwest cocoa-growing areas near the border with Cameroon. We found no evidence of recent sexual reproduction. Phenotyping will reveal whether the different genetic groups are associated with differences in virulence.

23. WHOLE GENOME SEQUENCE OF THE EMERGING OOMYCETE PATHOGEN OF MAMMALS, PYTHIUM INSIDIOSUM

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The oomycete genus Pythium comprises more than 250 described species, most of which are saprobes or facultative plant pathogens that cause seed rot and damping-off, root, stem and fruit rot, foliar blight, and postharvest decay. Pythium insidiosum is the only Pythium species that infects mammals; it is the causal agent of pythiosis, a deadly disease of horses, dogs, cattle and other mammals in tropical and subtropical regions, including the southeast United States. Pythiosis also affects humans in Southeast Asia, first reported in Thailand in 1985. P. insidiosum sporulates on plant material in vitro and is thought to propagate on aquatic plants in the environment, indicating that it could be a pathogen of both animals and plants.

P. insidiosum ATCC 200269 strain CDC-B5653, was originally isolated from necrotizing lesions on the mouth and eye of a 2-year-old boy in Memphis, Tennessee, USA. Sequencing reads were generated with two platforms: Illumina MiSeq and the PacBio SMRT system. We obtained 14 millions Illumina MiSeq reads four 300-bp paired-end libraries (generated by the UCLA Sequencing & Genotyping Core). A total of 356,001 PacBio reads were generated from four SMRT cells using a 10-kb insert library (generated by the UF ICBR NextGen DNA Sequencing Core). Read quality was assessed with FastQC. For de novo assembly, we applied SPAdes version 3.1.0, run on the University of Florida's high-performance HiPerGator supercomputer. This produced a final assembly of 45.6 Mb contained in 8,992 contigs with an average coverage of 28X, N50 of 13 Kb, maximum contig length of 148 Kb, and 57% G+C content. These values are comparable with those obtained for other Pythium species and using other technologies.

We used Augustus version 3.0.1 to predict genes ab initio, using a gene model previously described for Pythium. This genome contains 225 tRNA and 18,045 putative protein-coding genes. Reciprocal BLAST analysis with other seven Pythium genomes indicated that P. insidiosum shares 5,922 orthologs with these genomes and has 649 taxon specific genes. We found 233 orthologs shared only by P. insidiosum, P. aphanidermatum and P. arrhenomanes, consistent with estimates of Pythium phylogeny based on ITS sequences. Further analysis will focus on genes and gene families that distinguish the P. insidiosum genome from those of plant pathogenic Pythium.

24. BAYESIAN ESTIMATION OF LEVOFLOXACIN PHARMACOKINETICS IN PATIENTS WITH TUBERCULOSIS (TB)

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Background: Levofloxacin has been used for the treatment of TB for over a decade. However, limited information exists regarding levofloxacin pharmacokinetics (PK)/pharmacodynamics (PD) in patients with TB. The objective is to use a prior population pharmacokinetic model to estimate levofloxacin individual AUC's for patients with sparse PK data. The estimated AUC's will be used in subsequent PK/PD analysis

Methods: We utilized two data sets for the analysis. The first is a rich data set previously modeled using NPEM (Peloquin et al, 2008). In that study ten TB patients received a 1000 mg dose and were sampled at 0, 1, 2, 4, 8, 12, 18, and 24 h at steady state. The second data set is comprised of clinical data from the infectious disease pharmacokinetic lab. It includes 58 patients with a total of 123 samples. Most of the patients had two samples, at two and six hours post dose at steady state. Initially we remodeled the rich data set using parametric software (Monolix 4.2). We used the same structural model as in the previous publication. To assess if the model can predict AUC's using only two samples collected around two and six hours, we performed an internal Bayesian validation. The final model was used as Bayesian priors to predict the individual PK parameters for patients in the rich data set using only two and four or two and eight hour samples. Then we estimated the AUC for each patient and compared it to the observed AUC. Lastly, the final model was used to predict the concentrations in clinical data set using a Bayesian approach. To assess the predictability of the model we

estimated the correlation between predicted and observed concentrations and/or AUC's. We also calculated bias and precision, where bias is the mean prediction error and precision is the root mean squared prediction error.

Results: The rich data was modeled using a one compartment model with linear elimination and first order absorption. The final estimates for ka, V/f and Cl/f were 4.49 hr-1, 76.2 L for males and 56.5 L for females and 6.61 L/hr respectively. The coefficient of variation for ka, V/f and Cl/f were 185%, 24.6 % and 42.8% respectively. The average AUC in the population was 169 ug.hr/ml. For estimating the AUC using only two samples collected at two and four or two and eight hours, the predicted AUC's matched well with the observed AUC(R2 = 0.99, bias= 8.2 ug/ml, precision = 16.7 ug/ml using only two and four hours, R2 = 0.99, bias = 0.41 ug/ml, precision = 5.84 ug/ml using only two and eight hours). The final model was then used as Bayesian priors to predict the concentrations for patients in the clinical data set. The average concentrations in the clinical data set were 9.8 ug/ml. The model predicted the concentrations with good accuracy and precision (R2 = 0.92, bias= 0.21 ug/ml, precision=2 ug/ml).

Conclusion: The model showed good predictability and can be used to estimate PK parameters for patients with sparse data.

25. DESCRIPTION OF THE POPULATION STRUCTURE AND GENETIC DIVERSITY OF M. TUBERCULOSIS AMONG PATIENTS OF HAITIAN ORIGIN LIVING IN FLORIDA

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Background: Haitians have one of the highest tuberculosis (TB) case rates in Florida. We investigated the genetic diversity among M. tuberculosis (Mtb) strains, occurrence of strain emergence, i.e.

strains that are spreading faster than the background transmission rate and the determinants of recent transmission among Haitians living in Florida.

Methods: All culture confirmed TB cases reported to the Florida Department of Health (FDOH) are genotyped using spacer oligonucleotide typing (Spoligotyping) and mycobacterial interspersed repetitive units (MIRU) typing. We analyzed data on 451 TB cases of Haitian origin reported to FDOH from January 1, 2004 to August 1, 2014. We used the international database SPOLDB4 for strain family and shared international types (SIT) assignment. We computed the recent transmission index (RTI (n-1)), and the genetic diversity using SpolTools. We assessed strain emergence using the DESTUS program and applying a transmission and mutation model specific to spolygotypes. In multivariate regression, we measured the demographic and clinical determinants of recent transmission.

Results: Of the 451 strains, 129 (28.6%) belonged to the H lineage, 119 (26.4%) to the LAM lineage and 86 (19.1%) to the T lineage. SIT50, H3 sub-lineage, was the most common spolygotype with 56 strains (14.3%). Fifty nine spoligotypes were identified as orphans, i.e. unique to our sample. The dataset was characterized into 107 genotypes with average cluster size of 4.2, RTI (n-1) =0.76, and virtual heterozygosity of 0.042. Adjusting for false discovery rate and multiple comparison tests, SIT50, H3 sub-lineage, was identified as emergent (θ=44.03, p=5.72×10-5, q=0.00612). Controlling for age, gender, years in the US, lung cavitation, HIV status, and data collection period, age group 5 – 24 years (AOR=3.14, CI: 1.10, 8.95; p=0.0319) was the strongest predictor of recent transmission as compared to those 65 years and above

Conclusion: SIT50 from the H3 sub-lineage seems to be emerging in Haitian communities in Florida, largely driven by exogenous infection among patients 5 – 24 years old. Renewed efforts to track and treat TB patients of Haitian origin are warranted to curtail the spread of this Mtb clone.

26. CHANGING PHYSIOLOGICAL SUITABILITY LIMITS OF MALARIA TRANSMISSION IN AFRICA UNDER CLIMATE CHANGE.

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We mapped current and future suitability for malaria transmission in Africa using a published model that incorporates nonlinear physiological responses to temperature of the Anopheles gambiae mosquito vector and the malaria parasite. We found that a larger area of Africa currently approaches the ideal temperature for transmission than previously supposed. Under future climate projections, we predicted a net increase in area marginally suitable for malaria transmission, but a net decrease in highly suitable area. Combined with population density projections, our maps suggest that the location of the most people at risk for severe, year-round transmission will shift from coastal West Africa to the Albertine Rift between Democratic Republic of Congo and Uganda, and seasonal transmission suitability will shift toward sub-Saharan coastal areas. Mapping temperature suitability places important bounds on malaria transmissibility and, along with population, precipitation, economic, and ecological factors, can indicate where resources may be best spent on malaria control.

27. CHIKUNGUNYA VIRUS IN WILD CAUGHT AEDES AEGYPTI MOSQUITOES IN HAITI

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Chikungunya, an acute febrile mosquito-borne disease caused by the Chikungunya virus (CHIKV), was first discovered in 1955 in Africa. It's thought to have originated in Central Africa, where zoonotic cycles have been described and has spread globally in the last several decades, causing massive outbreaks in the Indian Ocean region, India and Asia. Several cases of imported CHIKV have been reported in parts of Europe, South America and North America. However, the first evidence of local transmission of CHIKV in the Americas was documented in late 2013 on the island of Saint Martin in the Caribbean and in May 2014 the Ministry of Health, Haiti (MSPP) confirmed 17 CHIKV cases. Following this outbreak in Haiti, we started capturing Aedes aegypti and Ae. albopictus in the homes of school children suspected of CHIKV infection in the Gressier/Leogane communes in Ouest department of Haiti. The children were tracked back to their homes where mosquitoes were captured by aspirator for indoor resting mosquitoes and by Biogents sentinel trap with BG-Lure to capture

host-seeking mosquitoes outdoors. The aspirator catches was done from 7.00am to 12.00pm while the BG sentinels were set up from 7.00am to 6.00pm for 4 consecutive days. Captured mosquitoes were killed by exposure to low temperatures (minus 20°Celsius), and then sorted by species and sex using morphological keys and then stored in minus 70 degrees Celsius freezer for future testing by real-time-polymerase chain reaction. Pools of mosquitoes (2-5 mosquitoes per pool) per household were tested for the presence of CHIKV using the real-time-PCR assay. A total of 2249 adult mosquitoes were caught using both the aspirator method and the BG sentinel traps in and around sixty one households of school children suspected of CHIKV infection. Of these, 7% (160/2249) were Aedes albopictus, 20% (460/2249) were Aedes aegypti and the rest were Culex sp, Psorophora sp, Anopheles sp and others. Ae. aegypti occurred at much higher numbers than Ae. albopictus, although the two species were both present in almost every household. The RT-PCR testing of the mosquitoes found 10 pools out of 59 pools positive for the CHIKV. All the positive pools were Ae. aegypti. None of the Ae. albopictus were positive. The data presented is the first confirmation of CHIKV infection in wild caught Ae. aegypti mosquitoes in the New World.

28. CHIKUNGUNYA VIRUS INFECTIONS IN FLORIDA, 2014

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Background: In December 2013, the first local transmission of chikungunya virus was reported in the Americas. Since then, the United States has seen an increase in chikungunya cases among travelers returning from endemic areas, particularly the Caribbean and South America. In June 2014, Florida reported the first local transmission of chikungunya virus in the continental United States.

Methods: Cases were reported and interviewed by the Florida Department of Health; in addition to the initial interview, cases were interviewed for chronic symptoms three, six, and twelve months after onset as appropriate. The Council for State and Territorial Epidemiologists case definition for chikungunya virus was used to classify cases as either probable or confirmed. Surveillance related to local introductions of chikungunya virus included 50-100 meter cluster investigations around a patient's residence, enhanced syndromic surveillance, and medical record review. Awareness was increased through media coverage, reverse 911 dialing, and targeted mailings.

Results: Florida reported 11 locally-acquired and 435 imported chikungunya fever cases from January 1 to December 29, 2014. All 11 locally-acquired cases and 205 (47%) imported cases were confirmed, most by polymerase chain reaction (PCR). All 11 locally-acquired cases were Florida residents while imported cases included 404 Florida residents and 31 non-residents. Among imported cases, the most common reason for travel was to visit friends and relatives, reported by 285 (76%) of those responding. The most common country of exposure was Puerto Rico, reported by 114 (26%) patients, followed closely by Haiti, reported by 107 (25%) patients. The 11 locally-acquired cases were reported in four South Florida counties: one case in Broward, two cases in Miami-Dade, and four cases each in Palm Beach and St. Lucie. Three of these counties, Broward, Miami-Dade, and Palm Beach, have reported 203 (47%) of the 435 imported cases. Overall, the 446 cases reported in Florida were 59% female, 45% white, and 52% non-Hispanic. The median age was 50; 22% of cases were 65 or older and 8% were 18 or younger. Of the 349 cases with onset from January 1 to September 30, 2014, 217 could be contacted again for follow-up interviews; 92 (42%) of these cases were still experiencing symptoms three months after onset.

Conclusions: A large proportion of Florida's chikungunya cases are still experiencing symptoms three months after infection. Awareness of the situation in Florida can help inform surveillance activities and control efforts throughout the United States.

29. DENGUE SEROPREVALENCE SURVEY IN YUCATAN, MEXICO.

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Background: Dengue fever, the most common arboviral disease globally, is a serious public health threat and has been a major cause of disease burden in the Yucatan state of Mexico since 1979, with all four serotypes currently circulating in the region. Despite this, information on present disease burden and transmission of the dengue virus is lacking in the Yucatan. It is essential to understand the dengue immunologic profile and immune status of a population that will likely be the target of the post-licensure dengue vaccine.

Methods: We conducted an age-stratified seroprevalence survey from April to December 2014 in three cities of the Yucatan (Merida, Progreso and Ticul) with known differences in transmission risk according to dengue surveillance from 2010 to 2013. Using IgG indirect ELISA on a random sample of 1,671 individuals.

Results: The overall dengue seropositivity was 73.1%. The seroprevalence results varied between cities and age groups. We need to capture the history of dengue by city, age group, and others variables to better understand dengue risk and

transmission in the Yucatan. A cohort study in the same three cities will be conducted following the results of this survey to further examine the baseline epidemiology, transmission dynamics of the dengue virus and immunologic profile of this population before a vaccine is introduced.

Conclusion: Serotype specific seroepidemiologic studies are necessary to estimate the serotype and age-group specific forces of infection.

30. DETERMINANTS OF RISK OF MALARIA PARASITEMIA IN BUNKPURUGU-YUNYOO DISTRICT, NORTHERN GHANA, INCORPORATING REMOTE SENSING AND SURVEY DATA

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Ghana's malaria control strategy prioritizes the northern savannah regions due to persistent hyperendemicity. In Bunkpurugu-Yunyoo district, previously reported household malaria surveys were conducted serially in 3 rainy seasons and 3 dry seasons in 2010-13, covering 11,945 children under five from 179 communities. In spite of high coverage for insecticide-treated bed nets (>75% use in each RS) and indoor residual spraying (IRS) with pyrethroid pesticides in years 2 and 3 (>98% households sprayed), investigators found unexpectedly high and geographically heterogeneous malaria prevalence. To better define and explain this local heterogeneity, this study enhanced the survey dataset with remotely sensed data, then analyzed by ecologic zones, delineated as urban (Zone 1, n=1131); rocky uplands (Zone 2,

n=5234, >750 ft altitude); transition (Zone 3, n=3456, 550-750 ft), and riverine plains (Zone 4, n=2124, <550 ft). The rainy season odds ratios for microscopic malaria parasitemia in children living in Zones 2, 3, and 4, as compared with Zone 1, were respectively 3.9 (95% CI: 2.8-5.4), 7.6 (95% CI:5.7-10.3), and 11.1 (95% CI: 8.0-15.6; all p values here and in the following <0.0001). Zone 1 prevalence of parasitemia never exceeded 25%. In contrast, Zone 4 prevalence across the 3 years was 65.6-72.1% in the rainy season and 39.8-54.9% in dry season. Among 17 variables with statistically significant odds ratios (OR) for malaria risk, 12 exhibited a zonal gradient favoring reduced risk in Zone 1 vs. Zone 4, with Zone 2 intermediate. In the rainy season these included lower wealth quintile (OR=3.6; 95% CI:2.7-4.7), caregiver's lack of education (OR = 2.7; CI: 2.1-3.3), ethnicity (OR = 3.9; CI: 3.2-4.8), lack of health insurance coverage (OR 3.0; CI=2.4-3.6), higher vegetation index (OR=1.6; CI: 1.1-2.3), lower human influence index (OR 5.2; CI:3.8-7.1); and >3 km distance to nearest health facility (OR=2.4; CI:1.9-3.1), among others. Dry season findings were similar. No consistent zonal gradient was found for the malaria control measures (ITN use, ACT use, IRS). Findings suggest that, in spite of high coverage with ITNs and pyrethroid-based IRS, high malaria prevalence in northern Ghana may be found in locations where reduced socioeconomic status and isolation coincide with low-lying terrain. Such areas may require additional and/or modified methods for vector and parasite control. Further geospatial analysis of this unusually rich dataset is ongoing at EPI, and will help to inform malaria control efforts in the challenging West African savannah setting.

31. EMERGENCE OF LOCALLY-ACQUIRED CHIKUNGUNYA IN FLORIDA

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Background: Chikungunya fever is transmitted through Chikungunya virus infected Aedes aegypti and albopictus mosquito bites. Symptoms include fever, joint pain, and rash. The disease is rarely fatal, but can cause temporary disability. Chikungunya virus (CHIKV) was introduced into the Caribbean in late 2013. Four locally acquired infections have been reported in Florida since July 17, 2014.

Methods: Infections with CHIKV were identified through active and passive surveillance. Travel history, onset of illness, symptoms, and risk factors for infection were gathered. A locally-acquired Chikungunya case was defined as an individual who had laboratory evidence of Chikungunya infection (by RT-PCR or ELISA), residence in Palm Beach County (PBC) during illness onset, and no recent history of travel outside PBC. Home isolation of case-patients during viremic phase was implemented. Mosquito Control was notified to complete an environmental assessment and treatment around the case-patients home. A field investigation was conducted within 100 meters of each case-patient's home. The objectives of the field investigation were to identify and test symptomatic and asymptomatic persons, evaluate risk factors, conduct environmental assessment and provide recommendations to eliminate mosquito breeding sites. Following

identification of the second locally acquired case-patient, testing was targeted to only symptomatic persons.

Results: Through active and syndromic surveillance, three locally-acquired case-patients were identified between July 17 and August 13, 2014. There was no clear epidemiological link established through geo-spatial relationships during incubation and infective periods among imported and or locally-acquired case-patients. During four separate field investigations on 7/24/2014 through 8/9/2014, 78 households were visited, of which six were vacant and no one was home at the other 28. No symptomatic persons were identified. Eight blood samples were obtained and none tested positive for CHIKV. The field teams identified mosquito breeding sites on some properties and drained and covered the stagnant water. Education on preventing further mosquito-borne illness was shared verbally and through printed materials provided to every household.

Conclusions: This investigation did not find any evidence to suggest that further local transmission had occurred. Isolation of cases-patients during the viremic phase likely prevented transmission among household members and local community members.

32. EMERGENCE OF SOUTH AMERICAN EASTERN EQUINE ENCEPHALITIS: POSSIBLE ENZOOTIC HOSTS AND HUMAN EPIDEMIOLOGY

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The arboviral disease South American eastern equine encephalitis virus has emerged newly in Panama, causing the first documented outbreak in humans. Its transmission cycle is not well understood. In order to better understand risk factors and ecological features associated with SA EEEV and VEEV exposure, we conducted a small mammal and human cross-sectional in Darien, Panama. Of various animals surveyed, rodents (short-tailed cane rat, Bolivar rice rat, black rat, Tome's spiny rat) had the highest SA EEEV seroprevalence. The short-tailed cane rat had the highest seroprevalence and was the most abundant species trapped. It was found most commonly on farms, pasture land, and in villages. Human SA EEEV seropositivity was associated with cattle ranching, farm exposure, and having corrugated metal walls. Having shrub nearby was protective. Together, these findings suggest that the spheres of exposure for SA EEEV likely includes the house (village), farm, and pasture land. These findings allow for future focused vector and host studies.

33. HIGHLY MULTIPLEXED POINT-OF-SAMPLING DETECTION OF NEW PATHOGENS

Steven Benner - Foundation for Applied Molecular Evolution

The "gold standard" for detecting the presence of a pathogen in a sample is the detection of its nucleic acids, either DNA or RNA (collectively xNA). xNA sequences define the pathogen, and can also indicate its resistance to specific therapies, its immunological serotype, and its recent evolutionary interactions with it previous human hosts. Unfortunately, xNA-targeted assays are often expensive to run, requiring expensive reagents, high levels of expertise, and even large amounts of sample. Scientists working at the nonprofit Foundation for Applied Molecular Evolution over the past few years have created a number of nucleic acid innovations in the hope of changing this. These innovations allow highly multiplex detection of an entire pallet of potential infectious agents for the cost of detecting just one, in complex backgrounds, and near points of sampling. This poster will describe recent activities seeking to detect 22 different arboviruses in mosquito samples, fungi responsible for Valley fever, mutant forms of HIV that confer drug resistance, and the virus that causes Middle East respiratory syndrome (MERS). It will also describe briefly past use of these innovations in xNA-targeted analyses that measure viral loads in HIV and hepatitis-infected patients, SARS, cystic fibrosis mutations, and a respiratory disease panel.

34. INCREASED ARBOVIRUS TRANSMISSION RISK FROM INTERACTIONS BETWEEN COMPETITION AND PESTICIDE EXPOSURE IN LARVAL MOSQUITOES

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Larval mosquitoes occupying container and ephemeral habitats often experience high competitive stress, reducing the number of subsequent adults and affecting their physiology. Mosquito control often includes application of pesticides to larval habitats, altering the number of larvae present and thus modifying competition. The vectorial capacity of the adult population for arboviruses such as dengue virus is a function of the population size and the physiology of the individual adults, affecting survival, susceptibility and biting rate. Thus, the risk of arbovirus transmission may be a complex function of competition and pesticide exposure experienced in the larval environment. We explored these interactions using a modified vectorial capacity model, varying relationships and parameter values linking pesticide exposure and competition to adult population size, daily survival probability, susceptibility to infection and biting rate. Under most conditions examined, there were regions of parameter space where increasing pesticide increased the transmission risk from the adult population. This was driven partly by competitive release, with more adults emerging from high competition containers when treated with pesticide than when untreated due to the relaxation of competition. However, when competitive release was not included there were still regions of parameter space where pesticide exposure increased arbovirus transmission risk. Further

work is needed to determine the likelihood of this occurring in the field and to develop control strategies that minimize this effect.

35. INSECTICIDAL ACTIVITY OF SOME NOVEL FLUORINATED COMPOUNDS

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The continual increase of insecticide resistance in arthropods makes it necessary to identify chemicals that have novel modes of action. An extensive literature search for compounds with insecticidal and mosquito repellent activity have led us to design and synthesize a set of 34 trifluoromethylphenyl amides. These compounds have trifluoromethyl groups located in the ortho-, meta- or para- positions on the phenyl ring and have various substituents attached to the carbonyl carbon, e.g. 2,6-dinitrophenyl, 2-methylphenyl, trifluoromethyl, pentafluoroethyl, 2-chloroethyl, 3-pentyl or n-alkyl groups.

The compounds were evaluated for toxicity against Aedes aegypti larvae, and for toxicity and repellency against adult female Ae. aegypti. Several of the better Ae. aegypti repellents were also evaluated for toxicity against Drosophila melanogaster. Against mosquitoes, four compounds were repellent at concentrations that were comparable to, or up to two times better than, the standard

DEET. Against D. melanogaster, two of the compounds showed full activity against a resistant strain having an altered gamma-aminobutyric acid (GABA) receptor, whereas fipronil did not. In the Ae. aegypti feeding bioassay, compounds were evaluated at 1 □mol concentration with a 1 h exposure, and weak knockdown was observed for 6 compounds (1.1-3.3%) compared to fipronil (2.2%). The same six compounds induced some mortality in larval and adult mosquitoes; however, they were not as potent as fipronil. With the goal of increasing the activity of fluorinated compounds, we synthesized two fipronil-related pyrimidinones and screened them against D. melanogaster and Ae. aegypti adults. These compounds were nearly 6 times as active as fipronil when tested in a glass surface contact assay against susceptible Drosophila, but did show over 1000-fold resistance in a strain (rdl) having an altered GABA receptor. The resistance to fipronil in this strain (rdl) was 10 times lower. The LC50 values of pyrimidinones against Ae. aegypti adults were only 2-3.5 times lower than that of permethrin.

The study has resulted in several structural leads that could facilitate further design to discover new compounds with insecticidal and repellent activity.

36. INTERSPECIFIC LARVAL COMPETITION DIFFERENTIALLY IMPACTS ADULT SURVIVAL IN CHIKUGUNYA AND DENGUE VECTORS

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Mosquitoes often experience intraspecific and interspecific competition among larvae attributable to high densities and nutrient limitation, especially container mosquitoes including Aedes aegypti (L.) and Ae. albopictus (Skuse). Density-dependent effects on larvae impact adult production and adult traits that influence transmission of arboviruses. To improve our understanding of the mechanisms by which density-dependence influences transmission and identify species-specific traits, we

tested the hypotheses: 1) Competitive asymmetry in favor of Ae. albopictus over Ae. aegypti translates to altered adult female survival, and 2) Ae. aegypti adult females are more resistant to life-shortening effects of low humidity conditions than A. albopictus. We gauged the relative impact of inter and intraspecific larval competition on adult survival in high and low humidity regimes (77 and 44% relative humidity, respectively). For A. albopictus, intraspecific but not interspecific competition usually reduced adult survival under both humidity regimes. For Ae. aegypti, both intraspecific and interspecific competition reduced adult survival. Aedes albopictus adult survival was minimally influenced by interspecific competition with Ae. aegypti, consistent with observations that Ae. albopictus is the superior competitor. A species comparison indicated that Ae. aegypti exhibited a survival advantage relative to Ae. albopictus under both low and high humidity conditions. However, similar survival of these Aedes species were observed in some cases depending on conditions experienced in both the aquatic and terrestrial environments. These results demonstrate plasticity in survival rates of dengue and chikungunya virus vectors and the significance of considering the influence of biological interactions during the immature stages and abiotic conditions during the adult stage.

37. INVESTIGATING THE TOXICITY AND SYNERGISTIC ACTIVITY OF CHALCONE ANALOGUES AGAINST AEDES AEGYPTI AND DROSOPHILA MELANOGASTER

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Mosquitoes are hematophagous insects that are both nuisance pests and significant vectors of human and animal diseases. The mainstay for controlling mosquito populations, and therefore disruption of mosquito-borne diseases, is the use of chemical insecticides. The widespread use of chemical insecticides, some having similar biochemical mechanisms of action, has predictably resulted in mosquitoes developing insecticide resistance. Therefore, there is a continued need for the identification and development of new chemistries to aid in controlling mosquito populations. These new chemistries may have either a novel mechanism of action or enhance the toxicity of insecticides that are already available. Toxicity screening was performed against Aedes aegypti, the yellow fever mosquito, using the first instar larval screening assay, and Drosophila melanogaster using an adult feeding assay. Generally, chalcone analogues containing a 2-thiophenyl group showed enhanced activity compared to chalcones analogues containing a 2-furyl group, indicating that the 2-thiophenyl group is important for toxicity. Further, these chalcone analogues displayed a low level of toxicity, and it is possible that these compounds are metabolically inactivated before they can produce a toxic effect. To investigate the metabolic inactivation of the chalcone analogues, adult female mosquitoes were topically treated with piperonyl butoxide (PBO), a well-established insecticide synergist that inhibits the activity of insect cytochrome P450 monooxygenases. However, mosquito toxicity did not increase with PBO pretreatment, indicating that the chalcone analogues were not metabolically inactivated. A select number of chalcone analogues were screened against Ae. aegypti to determine if the analogues were capable of synergizing the toxicity of carbaryl, a carbamate insecticide. Synergistic studies were performed by pre-treating adult female mosquitoes with 500 ng (per mosquito) of the chalcone analogue for 4 hr prior to treating each mosquito with 2.5 ng of carbaryl (the approximate LD25). Three chalcone analogues showed promising activity; however, none of the tested analogues displayed better activity

than PBO. Current studies are focused on determine the biochemical mechanism of action of the observed carbaryl synergism.

38. ISOLATION OF AN ARENAVIRUS, THE TACARIBE VIRUS, FROM HOST-SEEKING AMBLYOMMA AMERICANUM TICKS IN FLORIDA

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Arenaviridae are a family of single stranded RNA viruses of mammals and boid snakes . Twenty-nine distinct mammalian arenaviruses have been identified, many of which cause severe hemorrhagic disease in humans, particularly in parts of sub-Saharan Africa, and in Central and South America. Humans typically become infected with an arenavirus through contact with excreta from infected rodents. Tacaribe virus (TCRV) is an arenavirus that was first isolated from bats and mosquitoes during a rabies surveillance survey conducted in Trinidad from 1956 to 1958. Tacaribe virus is unusual because it has never been associated with a rodent host and since that one time isolation, the virus has not been isolated from any vertebrate or invertebrate hosts. We report the re-isolation of the virus from a pool of 100 host-seeking Amblyomma americanum (lone star ticks) collected in a Florida state park in 2012. TCRV was isolated in two cell lines and its complete genome was sequenced. The tick-derived isolate is nearly identical to the only remaining isolate from Trinidad (TRVL-11573), with 99.6% nucleotide identity across the genome .

A quantitative RT-PCR assay was developed to test for viral RNA in host-seeking ticks collected from 3 Florida state parks. Virus RNA was detected in 56/500 (11.2%) of surveyed ticks. As this virus was isolated from ticks that parasitize humans, the ability of the tick to transmit the virus to people should be evaluated. Furthermore, reservoir hosts for the virus need to be identified in order to develop risk assessment models of human infection.

39. MAGNET2.0: INTUITIVE "EXPLORATION-STYLE" VIEWING OF FUNCTIONAL GENOMIC DATA FOR P.FALCIPARUM

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In the parasitology community the value of easy intuitive interfaces to data is acknowledged, by resource developers and laboratory biologists alike. The primary community resource, EuPathDB, implements genome-browser-like displays and informative plots and its interfaces allow screening for interesting genes/proteins using multiple criteria. Still, there is room for improvement/alternatives, especially with respect to visual integration between data types, navigation and accounting for different "styles" of user interests. The Malaria Genome Exploration Tool MaGnET (www.malariagenomeexplorer.org) was developed in our group to facilitate what we call "exploration-style" analysis of the main different types of available data for its target organism, Plasmodium falciparum. Exploration-style analysis can be viewed as taking "browsing" of data to a next level, through: highly visual, interactive interfaces (viewers), and direct access from each viewer to all others while the selected genes of interest are carried forward automatically. MaGnET's main design differences to classic database interfaces to P.falciparum are (1) its integrated displays that aim to visualise several data types simultaneously (e.g. protein-protein interactions and gene expression) and (2) that users can work with two modifiable selections (each a user-defined set of genes) during exploration (i.e. genes can be added or removed "on the fly", without going back to a search page). MaGnET2.0 (released in 2013, Sharman & Gerloff, Bioinformatics 29:2350-2, 2013) features a new

convenient navigation pane to quickly switch viewers, new modeled protein structures from the PSI's Protein Model Portal, and more. Our tools are freely available for use via the Internet and/or for download. Working with a downloaded version of the software allows users to explore their own data in addition to the public data, while benefitting from the same innovative selection and visualization features. We find that these are helpful to (non-bioinformatician) biologists when forming, and pursuing hypotheses that make non-obvious functional connections between several gene products.

40. MALARIA ELIMINATION IN BOTSWANA, 2013 – 2014: ACHIEVEMENTS AND CHALLENGES

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Introduction: Current malaria elimination strategies focus on countries and regions where disease is relatively uncommon but face several challenges, including the need to alter surveillance strategies as cases decrease and the risk of reintroductions from regions where disease remains endemic. Botswana has made significant strides, reducing the malaria burden since 2000. Malaria prevalence dropped from 0.99% to 0.01% and deaths attributed to malaria declined from 12 to 3 by 2012. Botswana started implementing elimination strategies in 2012. We examine the progress and challenges of implementation (January 2013 to December 2014) of malaria elimination and identify future needs for a successful program in Botswana.

Methodology: Rapid notification and response used available staff and resources at district level, with no designated malaria surveillance officers. Cases were detected through routine passive surveillance system at health facilities. Positive cases were reported to district health management teams to activate district

rapid response teams (DRRT). The health facility and the DRRT were to investigate the cases, household members and other households within 100 meters of case households within 48 hours of notification using rapid diagnostic tests (RDT) and microscopy.

Results: There were 1080 malaria cases reported in Botswana from January, 2013 to December, 2014. All were diagnosed by RDT. Males were more frequently infected (60.2%) than females. Most cases (58%) were reported from Okavango district which experienced an outbreak in 2013. Of the diagnosed cases, only 245 (22.7%) were followed up at the household level due to inadequate capacity at district level with transport and human resources being reported as the main reasons. Eighty one (7.5%) cases were associated with national or transnational movement of patients. Local movement of infected individuals within Botswana accounted for 21 cases while 60 (74.1%) cases were imported from other countries. Most of the transnational movement of malaria into Botswana was into malaria-free districts. Screening individuals in and around index households identified 31 additional, asymptomatic infections.

Conclusion: Botswana has made progress implementing its malaria elimination program. Its experience shows some of the challenges of current elimination efforts. Among them are the substantial movements of human infections within and among countries, and the persistence of asymptomatic reservoir infections. Programmatically, challenges include improving the speed of communicating and thoroughly responding to newly identified cases. The country needs sustainable and further targeted interventions to achieve successful elimination goal by 2015.

41. MALARIA IN HAITI: A FIRST LOOK AT THE PARASITE GENOME

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The improvements in access to vector control, medical care, economic and housing conditions in the Caribbean have eliminated malaria from all islands except Hispaniola. By contrast, in Haiti, which encompasses the western portion of the island, this disease is endemic and becomes epidemic in the rainy seasons. The magnitude 7 earthquake in 2010 left 1.5 million people homeless, destroyed 60% of the government and administrative buildings, and killed 25% of the civil servants in Port au Prince, the capital. This of necessity diverted resources from disease prevention to more urgent concerns, greatly exacerbating the risk of infectious diseases including malaria. In Haiti malaria is caused by Plasmodium falciparum, and its principal mosquito vector is Anopheles albimanus. In a previous study, 1337 febrile patients were tested for malaria infection. Of this sample, 9.6% (129/1337) were test positive for P. falciparum by thick smear and rapid diagnostic test (RDT). Detailed studies of the genetics of the populations of both the malaria parasite and its vector would make available vital information which would facilitate cost-effective malaria control plans. One sample of P. falciparum collected in Leogane, Haiti, was placed in short term culture. Genomic DNA extracted from this isolate was subjected to Whole Genome Sequencing (WGS) with high coverage (average coverage ~ 130x). The resulting dataset was used for mapping and SNP analysis in comparison with the published reference genome of

clone 3D7. Genomic DNA from clone 3D7 cultivated in our laboratory was also resequenced for comparison. This pilot analysis identified ~20,000 mutations in the H4Leo1 sample; by comparison, only 266 were found in 3D7. Mutations were identified in potential vaccine target genes such as CSP, MSP1 and Pfs230. Of interest is also a G437A mutation in the DHPS gene. Mutations at this codon have been previously reported as one among several involved in drug resistance. These data have supplied information on the genetic complexity of the Leogane isolate. Phylogeographic information will be obtained by comparing our sequence data with published sequence data available for malaria isolates from other countries. These are the first data from our project designed to obtain similar data from a representative sample of malaria isolates to be collected in the Ouest and Sud Est Departments of Haiti. Our project goal is to determine the population structure, drug resistance genotypes and phylogeography of the malaria parasite in these regions.

42. P53-MEDIATED RAPID INDUCTION OF APOPTOSIS FUNCTIONS AS AN INNATE IMMUNITY DURING DROSOPHILA VIRAL INFECTION

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Arthropod-borne pathogens account for millions of deaths each year. Understanding genetic mechanisms controlling vector susceptibility to pathogens has profound implications for developing novel strategies for controlling insect-transmitted infectious diseases. Taking advantage of its powerful genetic manipulation tools, Drosophila animal has been developed as an mature infection model to study insect vector susceptibility and innate immunity. In this study, viral infection in adult Drosophila induced a rapid induction of apoptosis within 1–3 hours, which required P53 transcription factor and was mediated by a stress-responsive regulatory region upstream of reaper (IRER). More importantly, the rapid induction of apoptosis was responsible for

blocking viral infection and functioned as an innate immunity. Furthermore, using tissue- specific or stage-specific GAL4 promoted UAS-P53/signal pathways RNAi system, P53 functions during viral infection and upstream signal pathways of P53 will be uncovered. As a supplementary genome-wide screening, we will also utilize next generation sequencing (RNA-seq) to elucidate other immune responses at the early stage of infection or during the systemic infection. New candidate genes will be knockout using CRISPR/Cas9 method to verify their anti-viral functions. This study aimed to establish a systemic Drosophila viral infection research model with combination of several emerging techniques, which will facilitate further study on vector innate immunity and controlling insect-transmitted infectious diseases.

43. PARAMETER ESTIMATION BIAS IN LOGISTIC REGRESSION DUE TO IMPERFECT DIAGNOSTIC RESULTS AND PRACTICAL CORRECTION APPROACHES

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Logistic regression is a widely used statistical model in epidemiology to identify and quantify the effect of potential disease risk factors. Although it is acknowledged that imperfect diagnostic tests distort disease prevalence estimates, little is known about the impact of imperfect tests on effect size estimates of potential risk factors. We show how imperfect diagnostic test results can lead to biased estimates of effect size and propose two methods to correct for this bias: an adjustment factor for parameter estimates derived from the standard logistic regression model, and a Bayesian model that explicitly accounts for imperfect

detection. Using simulations, we illustrate the performance of these bias correction methods, revealing how these methods tend to generate better parameter estimates and substantially improve the 95% confidence interval coverage. We also demonstrate how the proposed methods change inference on disease risk factor by re-analyzing malaria data from Bangladesh. Our methods have the potential for widespread adoption by researchers and offer substantial improvements to current modeling practice in epidemiology.

44. PHYLOGENY OF MURRAY VALLEY ENCEPHALITIS VIRUS

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Introduction: Murray Valley encephalitis virus (MVEV) is a zoonotic flavivirus endemic to Northern West Australia and Papua New Guinea and a member of the Japanese encephalitis serocomplex. MVEV primarily exists in a transmission cycle between Culex annulirostris and birds. It is considered the causal agent of Murray Valley encephalitis (previously known as Australian encephalitis) and humans are generally considered to be dead-end hosts (Marshall et al., 1988). The evolution of MVEV in Australia and PNG has proceeded independently and that circulating Australian MVEV strains are not systematically re-seeded from regions of endemicity in PNG. MVEV is enzootic in northern regions of Australia, and in recent years, its activity has been detected as far south as the Midwest region of Western Australia through occasional widespread outbreaks of disease. The aim of this study was to define the phylogenic analysis of MVEV in Australia and Papua New Guinea, considering relative recent GenBank entries not previously object of precedent phylogenetic studies either under a time-scale either under a population dynamics.

Methods: The dataset consisted in 47 sequences E (envelope) and polyproteingene sequences, downloaded from GeneBank (http://www.ncbi.nlm.nih.gov/genbank/) and isolated in different regions of Northern West Australia, except for the two strains isolated in Papua New Guinea (PNG), and selected on the basis of the following criteria: (1) sequences already published in peer-reviewed journals; (2) known sampling date and location (3) all the available sequences. It was considered the E gene region for the phylogenetic analysis. All MVEV E genesequences were aligned using Clustal X and manual editing was performed with Bioedit to a final aligned length of 462bp after removing gaps. ModelTest version. 3.7 was used to select the simplest evolutionary model that adequately fitted the sequence data. The phylogenetic signal of the dataset was investigated by using of the likelihood mapping analysis of 10,000 random quartets by using TreePuzzle. The Maximum Likelihood tree was performed using PhyML(on line server)with 200 replicates. Statistical support for specific clades and clusters was assessed by bootstrap analysis considering bootstrap values >70%.

The dated tree, was estimated by using a Bayesian MCMC approach (Beast v. 1.7.4, http://beast.bio.ed.ac.uk) implementing a HKY using both a strict and an uncorrelated log-normal relaxed clock model. Chains were conducted for at least 50x106generations, and sampled every 5,000 steps. Convergence was assessed on the basis of the effective sampling size (ESS) after a 10% burn-in using Tracer software v. 1.5 (http://tree.bio.ed.ac.uk/software/tracer/). Only parameter estimates with ESS's of >200 were accepted. The demographic history was also analyzed on the MVEV E gene sequences by performing the Bayesian skyline Plot to give an interpretation of the phylodynamic feature of the dataset.

Results: The Maximum Likelihood tree shows a primary formation of two clades probably under a geographic selection as discussed further. A consensus tree is obtained using a Bayesian MCMC approach for a better confirmation of the ML tree, for a time-scaled phylogenic analysis and phylodynamic study. The estimated mean value of the MVEV E gene evolutionary rate was 0.407 x 10-

3 substitution/site/year (95% HPD: 0.623x10-4- 0.780x10-3). The distribution of the clades and the relative strains is the same for the ML tree. The time scale spreads over 250 years (year 1759) defining different epidemic entries for the clade I in the years 1948, 1966, 1989 and 2001, whereas for the clade II being multiple epidemic entries has been difficult to considered step by step. The analysis of the BSP seems to define a relative constant population until the year 2000, when a strong reduction occurred, probably due to a bottleneck.

Discussion: This articulated phylogenetic study gives different suggestions about the diffusion and the biological history of MVEV in Australia and PNG. As already described the hypothesis of wide-spreading in Australia of MVEV is probably due to two factors: a first contribution of viraemic migratory water-birds and a second contribution of wind-borne infected mosquitoes. In the endemic regions of northern WA the presence of MVEV population is only partially influenced by variation in rain-falling: in wetlands the intensity of rain-falling could have a detrimental effect to Culex larvae, decreasing the vector fitness; in arid grasslands an initial increasing of Aedes can leads to an more extensive viral diffusion.

45. PRELIMINARY SEQUENCING AND PHYLOGENETICS OF WEST NILE VIRAL SPECIES IN THE EQUINE BRAIN

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Since 1999, a highly virulent West Nile virus (WNV) was introduced to North America resulting in more than 41,492 human infections with 4% mortality, 27,075 equine infections, and over 100,000 avian population infections. Genetic analysis of these variants and worldwide expansion of WNV indicate globally, genetic diversification of the virus allowing adaptation to new local transmission cycles. A prime mechanism for this diversification is likely through the production of new viral variants able to adapt to new climates and hosts. These variants then develop new intrahost tissue or cellular tropisms adding to its robustness. This type of genetic diversification of WNV likely has played a major role in the pathogenesis in North American mosquito hosts.

A wide variety of vertebrate hosts are infected with WNV, but only humans and horses develop significant neurological clinical disease. The clinical manifestations of these two hosts are similar. There is limited information regarding viral diversity, tissue tropism and manifestation and severity of disease in mammalian tissues. Although viral variation is required for development of new environmental niches, WNV has demonstrated relatively high genetic conservation for an RNA virus. Tissue tropism generated within the single vertebrate host is a reflection of compartmentalized viral movement. Our overall

hypothesis is that viral escape is encoded at several predictable stages of the virus life cycle with mutations in structural proteins (NS3 and NS5) and non-structural proteins (envelope) favoring blood and neurological niches in the horse. The resulting tropism is unique to the cell type and host resistance is maintained by successful control (or generation) of particular viral variants.

This is a preliminary work comparing sequences of WNV from brains of experimentally infected horses, clinically infected horses, and clinically infected alpacas with WNV NY99 Vero, WNV NY99, and WNV 2002. Based on the phylogenetic tree, the WNV from experimentally infected horses and clinically infected horses are clustered together despite the fact that they are from different origins. The WNV of experimentally infected horses is originated from crow's brain and passaged in Vero, C3/C6 Mosquito cells, and BHK21 while the clinically infected horses is originated from birds and mosquitoes. This is an indication that the WNV has been adapted to the horse brains regardless the origins. The phylogenetic trees also demonstrate viral swarm and compartmentalization of WNV in the Vero cells, horses and alpaca. This preliminary works indicate that further look at viral evolution of WNV in horse is warranted.

46. PYRETHROID RESISTANCE IN THE BLOOD-FEEDING BEHAVIOR OF PUERTO RICAN AEDES AEGYPTI

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Emerging insecticide resistance is a major issue for vector control, especially in disease endemic areas. Resistance is detrimental to a mosquito control program because it is associated with a higher cost in order to achieve a comparable level of chemical control, has the potential to result in disease resurgence. Pyrethroid resistance has previously been documented in Puerto Rican populations of Aedes aegypti mosquitoes. In this study, behavioral differences in blood-feeding activity for pyrethroid resistant and pyrethroid susceptible strains of Aedes aegypti when exposed to pyrethroid-treated cloth are explored. In order to observe the blood- feeding behavior of both pyrethroid-resistant and susceptible laboratory colonies of mosquitoes, these populations are exposed to different concentrations of the pyrethroid-treated uniform fabric. Uniform fabrics are cut and sewn into sleeves with a surface area of approximately 650 cm2 for testing on the forearms of human volunteers. At least six dose levels of each pyrethroid insecticide treatment are prepared in acetone and sewn uniform sleeves are rolled and placed into a sealed 250mL amber jar to absorb the full amount of pre-measured insecticide dose. The uniforms are left to air dry for 15 min to allow the acetone to evaporate. For these assays, the hands are gloved and the sleeve is pulled tightly onto the arm, secured with masking tape at the wrist, and inserted into a stock cage filled with approximately 50 female Aedes aegypti mosquitoes for a 15 minute test. An untreated control sleeve of the same fabric type is paired with each treated uniform sleeve, in order to have a proper basis of comparison since many uniform fabrics have different weave tightness which varies in how easily the mosquitoes can

penetrate the material. Percent bite protection is calculated by utilizing Abbott's formula [{(C-T)/C}*100], where C = the number of mosquitoes blood-fed on a control uniform sleeve, and T = the number of mosquitoes blood-fed on a treated uniform sleeve. Results from these assays show a shift in the dose-response curves for blood-feeding amongst the susceptible and resistant strains, which indicate higher concentrations of pyrethroid chemicals necessary to deter blood-feeding behavior in the pyrethroid-resistant strain of Aedes aegypti. The dose-response curves differ between the two mosquito strains tested, which indicate a difference in efficacy. This research is ongoing and future work will include a larger sample of human volunteers as well as additional pyrethroid-treated uniforms.

47. QUALITY ASSURANCE ASSESSMENT OF WEST NILE VIRUS ILLNESS REPORTING GAPS IN MERLIN THROUGH THE EXAMINATION OF CAUSE OF DEATH DATA IN VITAL STATISTICS

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West Nile Virus (WNV) illness is a vector-borne disease transmitted by infected mosquitos and is endemic to Florida with over 300 locally-acquired cases reported from 2001-2014. Nationally, WNV illness has a case fatality rate of 4% and those with comorbidities have an increased risk of death. Medical providers and laboratorians are required by law to notify the county health department (CHD) of any suspected, probable, or confirmed case of WNV illness. The purpose of this analysis was to identify WNV illness death reporting gaps in Merlin, Florida's reportable disease surveillance system, and to examine whether the vital statistics database (Deathmaster) and Merlin coincide. Data from January 1, 2001 through January 1, 2014 were analyzed to determine if death certificates were issued with WNV infection as the immediate or contributing cause of death in Florida cases not reported in Merlin. Three hundred five cases of WNV illness acquired in Florida were reported in Merlin for the study period. Nineteen WNV illness case patients were reported in Merlin as

having died from their infection. Eighteen WNV-associated deaths were identified in Deathmaster. Fifteen of 18 (83%) WNV-associated deaths found in Deathmaster were reported cases in Merlin of which 10 of 15 (67%) were recorded as deceased in Merlin. The median interval from disease onset to death for the five individuals who were not recorded as dead in Merlin was 49 days compared to 13 days for those who were recorded as dead in Merlin. Three WNV attributed deaths were identified in Deathmaster but were not reported cases in Merlin. Available medical records, physician notes, and laboratory results were reviewed and CHD's contacted. The three deaths identified in Deathmaster with a WNV illness disease code that were not reported in Merlin did not meet the FDOH WNV case definition. In conclusion, the case fatality rate calculated from Merlin data is an underestimate because not all those who died from WNV infection were recorded as having died. In order to improve mortality estimates and have a more accurate impact of WNV illness in Florida, Deathmaster can be used to identify those WNV illness case patients who died subsequent to the investigation being closed.

48. SPATIAL PATTERNS OF MALARIA PARASITEMIA AND SEVERE ANEMIA IN THE BUNKPURUGU-YUNYOO DISTRICT, NORTHERN GHANA DURING OVER SUCCESSIVE RAINY SEASONS

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Malaria is one of the leading causes of global mortality, with an estimated 584,000 deaths occurring in 2013 alone. Worldwide control efforts have reduced the global burden by nearly half since 2000, however there are still significant areas of extreme and

recurrent endemicity. While approximately half of the world's population is vulnerable to malaria, children in sub-Saharan Africa experience disproportionately high rates of infection and mortality. Young children (6-59 months) have been identified as important sentinel populations for describing epidemiological patterns and intervention responses. Malaria parasitemia, determined by microscopy, was recorded in children under 5 years old from 2010 to 2013 in the Bunkpurugu-Yunyoo district of Ghana as part of an intensive indoor residual spraying campaign. These data are now being repurposed to describe spatial-environmental patterns of malaria with high degree of geographic resolution in a highly endemic area. The spatial distributions of these data were compared during the rainy season when malaria prevalence reaches its peak. Patterns of severe anemia in the study region were also analyzed due to the strong correlation with malaria infection and mortality in this geographic region and sample population. A total of 5989 individuals from 223 communities were surveyed over three rainy seasons. Weighted spatial means indicate a higher proportion of malaria parasitemia and severe anemia in the southern portion of the district in each survey and cumulatively across the entire dataset. Kernel density estimation revealed recurring areas of high parasitemia and severe anemia in this portion of the district. Ripley's K analysis identified an overall clustering of malaria parasitemia in the district. These results indicate a non-random spatial-temporal pattern of malaria prevalence and severe anemia in the Bunkpurugu-Yunyoo district. Further analysis of this dataset will tie these spatial patterns to environmental and demographic variables in order to build a geospatial model of malaria dynamics in this area. The high geographic resolution and the extreme endemic state of these data will contribute to the growing global effort of targeting residual regions of high malaria burden.

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

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In 2012 and 2013 3-D structures of the malarial surface protein Pf12 were solved experimentally (Arredondo et al., PNAS 109:6692-97, 2012; Tonkin et al., J Biol Chem 288:12805-17, 2013). These confirmed our prediction of a distant evolutionary relationship between the "6-Cys Domain" antigens in plasmodia with the coccidian SRS (SAG1-Related Sequences) made in 2005, and validated the "first generation" structural models (Gerloff et al., PNAS 102:13598-13603). Fold similarity between surface proteins can be difficult to detect, especially in pathogens, due to highly variable insertions, etc. In this instance the characteristic connectivity between the 6 cysteines is distinct in either family, and variants exist in each. Also their host species and few characterized functions so far seem very different. For example, the malarial gametocyte proteins Pfs48/45 and Pfs230 are transmission-blocking vaccine candidates built from “6-Cys domains”. Instances of this domain are annotated in related species, e.g. Babesia, but not yet outside the Hemosporidia. If anything besides fold resemblance had remained of their common ancestry with the dominant SRS antigen family in the tissue-cyst forming coccidia, this could potentially accelerate target selection for disease intervention.

Looking for clues to their history, we screened 102,878 predicted proteins from Apicomplexan genomes sensitively with merged family HMMs (hidden Markov Models). The more complete map of occurrences of this Apicomplexa-specific β-sandwich fold (>2,500 plausibly aligned domain matches) provides a glimpse of their evolutionary basis, structurally (and possibly functionally). For example we found 9 in Theileria species which currently lack any annotated 6-Cys Domain proteins. Moreover, our searches yielded

a surprising find. We identified an atypical SRS/6-Cys domain homolog in the tissue cyst forming coccidia (Toxoplasma/Neospora/ Sarcocystis) that may resemble the "evolutionary link" we sought, with a predicted "hybrid" disulfide bond pattern w.r.t either family. As functional studies are pending, publicly shared data from high-throughput experiments and molecular modelling help us understand the implications of this discovery, and of the evolutionary relationship between the families. Intriguingly this example may also help illustrate how disulfide bonds can "change position" during evolution.

Updated 3-D structural models are available in our 6-Cys Domain model database (http://pgsh.soe.ucsc.edu).

50. THE POTENTIAL SUITABILITY OF THE LONG-TERM CLIMATE WORLDWIDE FOR HLB AND ITS VECTOR (ASIAN CITRUS PSYLLID) USING TWO CORRELATIVE MODELS

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Two correlative species distribution models (MaxEnt and Multi-Model) were used to predict the global and local potential distribution of huanglongbing (HLB) caused by Candidatus Liberibacter asiaticus (Las) and its vector Asian citrus psyllid (ACP). The species distribution models (SDMs) are able to link the current distribution of a 'species' to long-term climate data to make predictions for new areas (1, 2). The current global distribution of HLB and ACP was gathered from online databases, literature review and personal communications with specialists. The long-term climate data which included 19 bio-climatic variables were sourced from Worldclim website (3). The USA occurrence data of HLB and ACP were not used in model calibration to evaluate the model predictions at independent locations. Both models successfully predicted Florida as highly suitable for both HLB and

ACP establishment, which is in agreement with the rapid spread and current distribution of both HLB and ACP in this region. The model also predicted that a limited area in California is climatically favorable for HLB establishment, but the probability of establishment was predicted to be much lower compared to Florida (4, 5). To increase the confidence regarding the model projections, a dimension reduction method (PCA) was used to investigate the climate similarity of regions with proven HLB presence with the California and Florida climate. PCA analysis showed that the climate in areas around Los Angeles overlapped with the climate of regions where HLB is currently present. On a global scale, HLB predictions from SDMs combined with expert knowledge could be informative for countries such as Australia, New Zealand, and European countries, where HLB has not been reported thus far.

51. TIMELINESS OF ARBOVIRUS REPORTING IN FLORIDA, 2014

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Background: In 2014, Florida had the second highest number of imported chikungunya (CHIK) cases reported in the continental U.S. and was the only state with local transmission of the virus. Florida also has high numbers of imported dengue (DEN) cases and has experienced repeated introductions of DEN every year since 2009. Suspect arbovirus cases are required to be reported to the health department by health care providers within one business day per Florida Administrative Code. Timely reporting of cases is important to ensure proper response and control measures are put in place to prevent local introductions, and that local introductions are detected rapidly when they occur.

Methods: Data from the Florida Department of Health Merlin reporting system was used to analyze the timing of symptom

onset, sample collection, laboratory reporting, county health department (CHD) notification, and mosquito control (MC) notification for CHIK, DEN, and West Nile (WN) illness cases.

Results: As of December 1, there were 394 cases of imported CHIK and 70 cases of imported DEN reported in Florida in 2014. There were also 11 cases of CHIK, 6 cases of DEN, and 15 cases of WN illness that were locally acquired. The CHDs were notified of CHIK cases an average of 20.6 days after onset of symptoms, 4.6 days longer than for DEN and WN. The average amount of time from CHD notification to MC notification was 0.7 days for CHIK, 2.4 days for DEN, and 0.5 days for WN. The average amount of time between sample collection and CHD notification from any source was approximately 10 days for all three viruses. However, initial case notification through laboratory reports (55% of all cases), took longer for commercial laboratories compared with the state public health laboratories. The greatest difference was seen with CHIK cases, with commercial laboratories taking an average of 5.3 days longer to report positive results than the state laboratories. Commercial laboratories reported CHIK test results via paper laboratory reports vs. electronic lab reports, which differed from other arbovirus reporting.

Conclusions: While CHDs reported arbovirus cases to MC quickly, reporting to the CHD was delayed for all arboviruses. The delay seen in CHIK reporting could be due in part to CHIK being an unfamiliar disease for many clinicians, as well as the less efficient reporting methods used by commercial laboratories. Issues regarding delays in timeliness of reporting of arboviruses should be investigated further.

52. TRANSCRIPTOMICS OF DIFFERENTIAL VECTOR COMPETENCE: WNV INFECTION IN TWO POPULATIONS OF CULEX PIPIENS QUINQUEFASCIATUS LINKED TO OVARY DEVELOPMENT

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Understanding mechanisms that contribute to viral dissemination in mosquito vectors will contribute to our ability to interfere with the transmission of viral pathogens that impact public health. The expression of genes in two *Culex pipiens quinquefasciatus* populations from Florida with known differences in vector competence to West Nile virus (WNV) were compared using high throughput sequencing.

A total of 15,176 transcripts were combined for comparison of expression differences between the two populations and 118 transcripts were differentially expressed ($p<0.05$). The fold change in expression of the differentially expressed genes ranged from -7.5 – 6.13. The more competent population for WNV (Gainesville) over expressed 77 genes and down regulated 44 genes, compared with the less competent population for WNV (Vero Beach). The functional analysis showed that the up- regulated gene set contained most of the catalytic activity function and the down- regulated gene set had a notable proportion of transcripts with transporter activity function. Immune response category was shown in only the down-regulated gene set. Several different vitellogenin genes were expressed differentially. Based on the RNAseq data analysis, several candidate genes identified that function in aspects of fatty acid transport or ovary development were investigated using RNAi to determine their functional role in the WNV infection process. Ovary development was compared across the populations and following WNV infection. There were significant differences among the compared groups that suggests that ovary development is related to vector competence in two *Culex* populations in Florida. These results provide novel insight

into the defense mechanism used by *Culex* spp. mosquitoes against WNV.

53. USING COMMUNITY KNOWLEDGE, ATTITUDES AND PRACTICES TO EVALUATE A CHAGAS DISEASE INTERVENTION IN RURAL YUCATAN, MEXICO

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Background: Households in Teya, a rural village in Yucatan, Mexico, received window screens to prevent house infestation by Triatoma dimidiata, the local vector for Chagas disease. T. dimidiata is a peridomestic species that invades households at night to take blood meals. Physical barriers such as insect screens have been identified as a cost-effective, acceptable alternative to insecticide spraying, which has resulted in insecticide-resistant vector populations. Two screens were custom-built and installed by the local carpenter in every household that attended at least two community education seminars about Chagas disease, T. dimidiata and pest management.

Objective: To evaluate the effectiveness of the intervention at influencing community members' knowledge, attitudes and practices related to Chagas disease and pest management, and to identify any cultural and economic barriers to intervention success.

Methods: Semi-structured interviews were conducted in a random sample of households participating in the intervention in Teya, and in a random sample of households in Suma de Hidalgo, a village without the intervention between May and August 2013.

Results: Knowledge pertaining to Chagas epidemiology was significantly higher in Teya than in Suma. A very small percentage of respondents in Teya, and no one in Suma were able to identify the classic signs and symptoms of Chagas disease. Respondents

in Teya had high levels of perceived risk and severity related to Chagas, and almost everyone recognized benefits of insect screens. Very few respondents in either village were able to successfully identify nymph stages of T. dimidiata.

Respondents in Teya report felling safer with the insect screens than they did before the screens were installed. While there is a desire in Teya to purchase additional insect screens to cover all windows and doors, it is a low priority due to economic insecurity.

Discussion: Despite having wide community acceptability and desirability, the economic barrier to hand-made insect screens suggests pursuing scale-up product and distribution strategy is not feasible. Consideration should be given to manufacturing window screens to make them more affordable. Additional consideration should be given to nailing a wire mesh screen around the window instead of providing handmade screens with wooden boarders. These options could improve the product's accessibility to at-risk populations. These findings suggest that new educational materials should be developed to address specific knowledge gaps to improve self-efficacy related to pest management.

Additional pilot studies should be done that test new education materials, types of screens, and screen distribution strategies before an attempt to scale-up.

54. CHARACTERISTICS OF NOVEL KLEBSIELLA-LIKE STRAINS ASSOCIATED WITH NECROTIZING ENTEROCOLITIS(NEC)

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Necrotizing enterocolitis (NEC) is an important intestinal disease affecting preterm infants. It is associated with significant short-

term mortality and morbidity and results in high costs of health care for affected infants. While epidemiologic evidence suggests that antibiotics and feeding associated intestinal dysbiosis contributes to the pathogenesis of NEC no specific infectious agents have to date been identified. We analyzed fecal samples from preterm infants ≤ 32 weeks gestation using 16S rRNA based methods at 2, 1, and 0 weeks, prior to diagnosis of NEC in 18 NEC cases and 35 matched controls. Environmental factors such as antibiotic usage, feeding type (human milk versus formula) and location of neonatal intensive care unit (NICU) were also evaluated. In NEC cases we observed a higher proportion of Proteobacteria (61%) two weeks and of Actinobacteria (3%) 1 week before diagnosis of NEC compared to controls (19% and 0.4%, respectively) and lower numbers of Bifidobacteria counts and Bacteroidetes proportions in the weeks before NEC diagnosis. In the first fecal samples obtained during week one of life we detected a novel signature sequence, distinct from but matching closest to Klebsiella pneumoniae, that was strongly associated with NEC development later in life. From 5 NEC cases in which the novel Klebsiella pneumoniae 16S rRNA signature represented >10% of all sequence reads we have isolated multiple Klebsiella like colonies that match the characteristic 16S rRNA signature. Initial characterization of these isolates suggests that they include the pks-island and exhibit genotoxicity in the COMET assay. We are now planning to perform genome sequencing of isolates to identify unique factors associated with NEC pathogenicity.

55. EXPLORING IN VITRO ANTIPSEUDOMONAL ACTIVITY OF SYNERGISTIC TIGECYCLINE-TETRACYCLINE COMBINATIONS

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Background: Tigecycline (TIG), a broad spectrum glycylcycline antibiotic and tetracycline analog, was given a black box warning by the FDA in 2013 for increased risk of mortality versus comparator therapy, most likely attributable to treatment failure. Recent investigations into TIG have shown that metal-ion chelation increases the unbound concentrations, may increase efficacy and possibly broaden TIG activity.

Methods: In vitro minimum inhibitory concentrations (MIC) of TIG against P. aeruginosa was determined by using serial dilution method in Mueller-Hinton broth in various combinations with EDTA, calcium, and tetracycline (in concentrations less than MIC). Static time kill curves for TIG alone and in combination with 4, 8, and 12 mg/L TET were performed for P. aeruginosa.

Results: For P. aeruginosa, a decrease in TIG MIC from 8 to 0.0625-0.125 mg/L was observed by the addition of EDTA. This enhancement in activity is reversed by the addition of calcium, increasing the MIC to >32 mg/L. The addition of TET in increasing concentrations showed a concentration-dependent improvement in activity with a 2 to 8-fold decrease in MIC. The addition of calcium to TIG-TET combinations reversed the effect. Kill curve data to date has shown enhanced effect of TIG against P. aeruginosa in the presence of TET.

Conclusion: TIG-TET synergy against P. aeruginosa has been observed at clinically attainable concentrations. This novel synergism of two compounds of similar structure is likely resultant of competition for calcium chelation. Subsequent investigations have the potential to lead to increasing TIG's activity at lower doses when given in combination with TET.

56. MEMBRANE BOUND B-LACTAMASE OF BURKHOLDERIA PSEUDOMALLEI

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Objectives: Burkholderia pseudomallei is the etiologic agent of melioidosis, a difficult-to-treat disease with diverse clinical manifestations. β-lactam antibiotics such as ceftazidime are crucial to the success of melioidosis therapy and because of the bacterium's intrinsic polymyxin resistance they are often drugs of last resort. Ceftazidime resistant clinical isolates have been described and the most common mechanism is point mutations affecting expression of critical amino acid residues of the chromosomally encoded Class A PenA β-lactamase. Although PenA is homologous to inducible AmpC β-lactamases, it differs from AmpC-type enzymes by gene organization, lack of substrate inducibility, mode of secretion, and subcellular localization. The objectives of this study were to assess possible effects of secretion and subcellular localization on enzyme activity.

Methods: Site-directed mutagenesis was used to derive mutant enzymes that were still secreted via the twin arginine translocase (TAT) system, but were no longer lipidated and processed via lipoprotein signal peptidase. Subcellular protein location was determined using cell fractionation, enzyme activity and Western blotting. Susceptibility assays were performed to determine substrate profiles.

Results: The PenA signal sequence was mutated such that the protein was still secreted via the TAT system. However, mutation of a crucial lipobox cysteine to a serine residue and a concomitant isoleucine to alanine change at the signal peptide processing site resulted in a protein that was no longer membrane-bound, but rather because of processing by signal peptidase I localized in the soluble, presumably periplasmic fraction. The mutated protein retained enzymatic activity and the substrate profile was indistinguishable from the wild-type enzyme.

Conclusions: Molecular analysis of genetically engineered B. pseudomallei PenA variants showed that mode of secretion and subcellular localization has no effect on substrate profiles. Gene organization context suggests that the enzyme may be involved in peptidoglycan remodeling aside from playing a crucial role in conferring clinically significant β-lactam resistance.

57. MOLECULAR CHARACTERIZATION OF ANTIMICROBIAL EFFLUX IN BURKHOLDERIA PSEUDOMALLEI

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Antimicrobial resistance in Gram-negative bacterial populations continues to emerge as a public health threat in both domestic and international settings. Efflux of antimicrobial compounds is a major cause of drug resistance within this group, and the Resistance Nodulation and Division (RND) efflux pump family represents a highly conserved multidrug resistance determinant. Burkholderia pseudomallei (Bp) is an ideal model for the study of antimicrobial resistance via efflux as it possesses multiple RND operons. Bp's bpeEF-oprC operon encodes an efflux pump that extrudes antibiotics used in the eradication phase of treatment of Bp infections. As Bp is responsible for high levels of morbidity and mortality in endemic South East Asia and Northern Australia, the

study of its resistance factors is vital to improved management of Bp infection.

A molecular approach was taken to characterize control of the BpeEF-OprC efflux pump. Promoter sequences of bpeEF-oprC were identified through 5' deletion assays. Fluorescent primer extension identified 5' transcript ends and gel shift assays located putative binding sites for the regulatory BpeT protein encoded by the divergently transcribed bpeT gene. BpeT's function as a transcriptional activator was demonstrated through correlation of bpeT over-expression to increased minimum inhibitory concentration (MIC) levels of BpeEF-OprC substrates.

The novel regulatory protein BpeS was implicated in control of bpeEF-oprC through selection of resistant bpeS mutants, which were shown to highly overexpress the operon. Interestingly, neither overexpression nor loss of bpeS changes bpeEF-oprC transcript levels, or MIC of pump substrates. This suggests a complex regulatory network remains to be described. The global impact of bpeT and bpeS expression is being assessed by next generation sequencing assays in an effort to further elucidate this regulatory pathway.

Ultimately, this study aims to identify cis and trans regulatory mechanisms governing bpeEF-oprC operon and bpeT gene expression, and the resultant resistance to antimicrobials used in treatment of Bp infection.

58. P. GINGIVALIS EVADES UBIQUITINATION NDP52/P62 MACHINERY AND INDUCES AUTOPHAGOSOME-LIKE VACUOLES FOR INTRACELLULAR SURVIVAL

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P. gingivalis is a major etiological agent in the development of periodontal disease. The ability to survive and replicate in gingival epithelial cells (GECs), which are the first line of defense of oral mucosa, is critical to the success of *P. gingivalis* as a pathogen. Our recent studies showed activation of autophagy is an important mechanism of *P. gingivalis*' survival in GECs where the organism rapidly positions itself in endoplasmic-reticulum (ER) and induces autophagosome-like vacuoles for affluent intracellular life. Nevertheless, the detailed characterization of the intracellular fate of *P. gingivalis* in GECs has yet to be fully elucidated.

Objectives: To study the spatio-temporal fate of intracellular *P. gingivalis* in GECs and examine the role of ubiquitin-binding-adaptor proteins NDP52/p62 for targeting of *P. gingivalis* to lysosomal degradation pathway.

Methods: Primary GECs were infected with wild-type-strain or FMN-green-fluorescent-strain (PgFbFP) over 24-hours. ER-Tracker or Lysosome-marker LAMP-1 were used to determine subcellular localization of the organism in GECs by confocal microscopy. Use of PgFbFP-strain in conjunction with digitonin treatment and anti-*P. gingivalis* red-fluorescent-antibody staining followed by anti-NDP52 or anti-p62 blue-fluorescent-antibody staining permitted

measurement of the co-localization of vacuolar versus cytosolic bacteria with the ubiquitination markers.

Results: Concordant with our recent findings, the confocal analyses displayed steady-state level of co-localizations between *P. gingivalis* and ER (~90%) over 24-hours, whereas ~25% of the bacteria were associated with lysosomes. *P. gingivalis* appeared to predominantly reside in the vacuoles, while small percentage of bacteria was found in the cytosol, distinctly marked by the NDP52/p62. This was statistically significant at $P < 0.01$ *t*-test compared to *P. gingivalis* in the vacuoles.

Conclusion: These results indicate *P. gingivalis* utilize ER-rich-autophagosomes for successful persistence and evade anti-microbial ubiquitin-lysosomal-degradation pathway. This new knowledge may lead to highly targeted therapeutic interventions for controlling *P. gingivalis'* colonization in the oral mucosa by using specific autophagy inhibitors.

59. PENA-MEDIATED CEFTAZIDIME RESISTANCE IN BURKHOLDERIA PSEUDOMALLEI, THE CAUSATIVE AGENT OF MELIOIDOSIS

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Background and Objectives: Burkholderia pseudomallei is the causative agent of melioidosis, a major tropical disease which is endemic in Southeast Asia and northern Australia. Ceftazidime (CAZ) is a primary drug recommended for treating melioidosis. CAZ resistance has recently emerged in several countries. We have determined genetic and molecular basis of CAZ resistance mechanisms in a large collection of B. pseudomallei strains, and developed molecular surveillance for their resistances.

Methods: Fifty-eight B. pseudomallei strains were collected from 24 melioidosis patients who had treatment failures in Northeast Thailand between 1987 and 2007. Next generation sequencing was used to identify mutations in these strains. Allelic replacement and heterologous expression of different penA alleles using the pExKm5 vector is being performed in Bp82, an attenuated select agent-excluded B. pseudomallei strain.

Results: Mutations in penA, a Class A β-lactamase gene, were identified in these CAZ-resistant strains. These mutations are likely site-specific and a consequence of antibacterial therapy. Nine amino acid substitution (AAS) mutations have been identified in penA of B. pseudomallei, six of which are novel. Two AAS mutations, A172T and D240G, are predicted to be involved in the decreased susceptibility to CAZ.

Discussion and Conclusions: CAZ resistance in B. pseudomallei is penA - mediated. Mutations in penA may be used to monitor CAZ resistance in clinical settings.

60. POLYSACCHARIDE DIVERSITY AMONG TIER- 1 SELECT-AGENT BURKHOLDERIA IMPACTS VIRULENCE AND VACCINATION STRATEGIES

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With continued concern of outbreaks in endemic areas and intentional use as biological weapon of mass destruction, we believe that effective vaccines against biothreat agents are greatly needed for public health and biodefense purposes. Burkholderia pseudomallei (Bp) and B. mallei (Bm) the causative agents of melioidosis and glanders, respectively, are classified as Tier-1 (Top Tier) Select Agents by federal health agencies in the United States. Inhalation of these pathogens can result in acute diseases that are highly resistant to most chemotherapy drugs. With high mortality rates, both pathogens cause fatal debilitating disease. Currently, there are no effective vaccines for protection against both threat agents even though several attempts have been made. One major problem is that these vaccines could not provide sterile immunity and long-term protection. We are among those investigators who have been struggling to develop vaccines against these deadly pathogens. We have learned that the ideal vaccines for biodefense purposes should provide protection against all 3 different serotypes of Bp including types A, B, and B2, and also protect against a variant type A of Bm. Types B and B2 are known as atypical serotypes which have different lipopolysaccharide (LPS) structures, and possess virulence levels higher than those of typical strains in mouse models. In addition, our recent study has confirmed that these serotypes have different O-antigen structures that may evade or activate host immune responses differently. It has also been well established that LPS is

a virulence factor and protective antigen of Bp and Bm. LPS from attenuated strains that have the same variant LPS as B. mallei can activate innate and humoral immunity responses, but is also known as a weak immunogen for T-cell activation. Capsular polysaccharide (CPS) in combination with LPS induced high antibody and immune marker production but still did not lead to significant protection from aerosol-initiated glanders. The major concept of our vaccine development is to generate memory immune cells and train them to face various forms of the pathogens; we hypothesize that the best vaccine candidate would be a multivalent vaccine containing CPS, LPS and well-characterized immunogenic proteins. We hope that the biodefense vaccines can also be used for public health for both humans and animals.

61. PORPHYROMONAS GINGIVALIS MODULATES MAJOR NADPH OXIDASE, NOX2, IN PRIMARY GINGIVAL EPITHELIAL CELLS

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Porphyromonas gingivalis (P.g.) is an important etiological agent in severe periodontal disease and has lately been associated with other chronic diseases including rheumatoid arthritis and cancer. P.g. is a successful colonizer of gingival epithelial cells (GECs) and circumvents host-cell machinery to propagate its intracellular survival by abrogating intrinsic apoptotic signaling and modulating/suppressing extracellular ATP (eATP)/P2X7-induced oxidative stress pathways. We have recently defined a bacterial effector, nucleoside diphosphate kinase (ndk), which catalytically depletes eATP thereby promoting bacterial and host cell survival. During the infection, increased eATP-induced cellular reactive

oxygen species (ROS) generated by the mitochondria and NAPDH oxidases (NOX) have been observed. ROS is becoming increasingly recognized for playing important physiological roles in immune response; however it is only recently that these connections have begun to be studied in epithelial cells.

We used qPCR to analyze the expression of NOX isoforms expressed in primary GECs and have observed a three-fold increase in NOX2 expression with eATP treatment. Further analysis into the regulation of NOX2 using subcellular fractionation and western-blot assays of P.g. infected cell lysates indicates that a key cytosolic factor required for the activation of NOX2, p67phox, was modulated by P. gingivalis infection. The p67phox membrane localization was increased at 3h post-infection and returned to basal distribution as compared to untreated control at 24h post-infection. However, the membrane localization of p67phox is only marginally reduced during 24h of ndk-deficient strain P.g. infection. Immunofluorescence analysis during wild-type strain P.g. infection also revealed that membrane localization of Rac1, a major signaling partner of NOX2, was modulated by infection similar to p67phox. We further used qPCR to examine the expression of myeloperoxidase, classically involved in the pathogen defense respiratory burst in neutrophils. Our results revealed that myeloperoxidase is induced during early P.g. infection and subsequently reduced.

Therefore, we suggest that P. gingivalis modulates NOX2 activation by inhibiting complex formation thereby reducing eATP induced ROS in primary GECs and promoting bacterial survival. Our results also display a possible role for P.g. ndk in this process. This study characterizes for the first time eATP-induced NOX2-ROS and myeloperoxidase in epithelial cells and forms connections between the host-cell machinery and bacterial insult.

62. PORPHYROMONAS GINGIVALIS-NUCLEOSIDE DIPHOSPHATE KINASE SUPPRESSES EPITHELIAL CELL APOPTOSIS BY INTERACTING WITH HUMAN HEAT-SHOCK PROTEIN 27

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Porphyromonas gingivalis is a Gram-negative anaerobe that is able to successfully colonize and persist in human oral epithelium and has been implicated in the pathogenesis and progression of periodontal diseases in humans. P. gingivalis produces a plethora of virulence factors that allow it to invade and replicate intracellularly in gingival epithelium, evading innate host cell immune system. Recent studies have shown the ability of P. gingivalis to modulate host mitochondrial apoptotic pathways and to render gingival epithelial cells resistant to apoptosis induced by host danger signals and pro-apoptotic reagents. One of the recently identified virulence factors involved in the anti-apoptotic features of P. gingivalis is a bacterium-secreted effector molecule, nucleoside diphosphate kinase (NDK). In this study, we tried to identify possible target cellular molecules for the NDK modulation of host apoptotic signaling and to construct candidate host cell signaling pathways which could be modulated by anti-apoptotic characteristics of NDK. Through pull-down assay, we identified heat-shock protein 27 (HSP27) as a cellular binding target to P. gingivalis NDK and demonstrated direct phosphorylation of human HSP27 by P. gingivalis NDK. Increased HSP27 phoshporylation was also observed in primary GECs infected with wild type P. gingivalis ATCC33277, compared to the infected cells with P. gingivalis NDK-deficient mutant. We treated P. gingivalis-infected GECs with staurosporine and the result showed that P. gingivalis

NDK successfully suppresses staurosporine –induced cell apoptosis by inhibiting cytochrome c release and caspase 9 activation. Taken together, this study demonstrates the role of P. gingivalis NDK in the anti-apoptosis and unveils HSP27 as a crucial target molecule in the process.

63. COMMUNITY-BASED REPORTS OF CO-MORBIDITY, CO-MORTALITY, AND HEALTH-SEEKING BEHAVIORS IN FOUR MONROVIA COMMUNITIES DURING THE WEST AFRICAN EBOLA EPIDEMIC

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Background: As of December 31, 2014, a total of 20,206 confirmed, probable, and suspected cases of Ebola Virus Disease (EVD) have been reported, with a case-fatality rate of 71% in Guinea, Liberia, and Sierra Leone. The collapse of Liberia's health care system in the wake of the outbreak has diminished capacity for surveillance and treatment for non-EVD conditions. There is currently very little data concerning local patterns of health-seeking behavior in response to the outbreak and influx of humanitarian intervention.

Objective: To identify and describe the distribution of co-morbidity and co-mortality among Monrovians within a collapsed health system.

Methods: 555 household interviews, in the form of "verbal autopsies," were conducted in St. Paul's Bridge(n=256), Fendell(n=53), Gbangay Town(n=69) and Saye Town(n=90), four

Monrovia-area communities from September-October, 2014. Information was also collected for 37 individuals whose community information was missing or unknown. These areas were purposively selected to capture regions that were differentially afflicted by EVD.

Results: 42.5% of all individuals reported going to street drug vendors as their first attempt at receiving health care, regardless of self-diagnosis; this was not significantly associated with gender or age group. Only 4% of sick individuals first used home care to get treatment, regardless of their self-diagnosis, and 20% sought care at the hospital.

Over 50% of children under 5 and 5-15 year olds were self-diagnosed as having malaria, which makes up 35% of all first reported diagnoses. 22% of children under 5 reported having the flu or a cold. 11% of all diagnoses were reported as blood pressure issues such as hypertension, which was mostly prominent in older populations. Only 12 EVD cases were reported, 10 in men and 2 in women, making up 7.1% and 1.4%, respectively.

Discussion: The reported self-diagnoses represent what could be multiple, prevalent co-morbidities among this population. While some of the conditions can be deadly if not appropriately treated by medical professionals, many could be adequately addressed by knowledgeable drug vendors. The informal health care market is an important source of medical information and treatment for sick individuals of all ages and sexes, for a variety of conditions. These vendors may be a useful resource for surveillance and social marketing campaigns during this, and future epidemics.

Anthropological insights into complex health behaviors can provide valuable insights for surveillance purposes, as well as for intervention.

64. CRYPTIC FEMALE MATE CHOICE AS MEDIATED BY SIGMA VIRUS IN DROSOPHILA MELANOGASTER

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Sigma virus is a single-strand RNA virus in the family rhabdovirae. Despite the cost to the host, the virus is able to persist in a drosophila population via vertical transmission. Sigma virus infected males have been observed to outcompete uninfected males. The role of the female parent in this competition is unknown. I hope to discover if selection acts to reduce viral fitness via female mate choice. Infected females will be paired with a male (uninfected or infected) on two separate occasions. I will observe the willingness of the female to re-mate, record the offspring gender and quantity produced, and dissect the female flies' sperm organs to determine the quality of the residual sperm residing within the females after the second copulation (after eggs have been laid). My study will serve to confirm the results of the research paper "Sigma virus and male reproductive success in Drosophila melanogaster", by Rittschof et al. as well as looking closely at the effect of female choice on viral success.

65. CYCLIC MIMETICS OF HIV TAT TO RECOGNIZE TAR RNAS FOR AIDS THERAPY

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In this work, we demonstrated that a designed cyclic peptide mimic of HIV Tat peptide can specifically recognize HIV TAR RNAs with a single binding site, compared to two binding sites for Tat. Our unique ultrafast dynamics approach provides more intricate details of the TAR RNA conformational transition patterns, capturing not only the major sub-populations, but minor sub-populations as well, painting the conformational transition with an ensemble view. Targeting RNAs as new way of therapeutic intervention of viral diseases has been challenging. Design of peptidomimetics is a promising strategy for developing novel drug leads. Although both Tat and L22 bind the TAR RNAs as a beta hairpin structure, cyclization in L22 affords it a more efficient ligand from population shifting perspective. Since such population shift is necessary for specific ligand binding, lessons learned from this study can provide insights into how to better design compounds with desired properties to target similar structures based on distinct dynamic behaviors.

66. ENVIRONMENT AND DEMOGRAPHY INFLUENCE SEROPREVALENCE OF PSEUDORABIES IN FERAL SWINE POPULATIONS OF FLORIDA

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The widespread invasive species, feral swine (Sus scrofa), causes millions of dollars of damage in the United States each year through habitat destruction, crop consumption, and disease transmission. Among the most ubiquitous pathogens carried and transmitted by feral swine is pseudorabies (PRV), a herpesvirus that can kill livestock, pets, and wildlife, including the endangered Florida Panther, for which feral swine are an important food source. Thirty-five to forty percent of feral swine in Florida are seropositive for PRV, and the high density of feral swine in Florida—second only to Texas—makes the disease of particular concern to livestock owners and wildlife managers. This study analyzed 8 years (2007-2014) of serological data from the United States Department of Agriculture (USDA) Wildlife Services to identify relationships between PRV seroprevalence in feral swine and environmental and demographic factors in Florida. Preliminary results suggested that increased urban area was positively correlated with PRV seroprevalence (coefficient = 14.4, $z = 3.69$, $p < 0.001$), and the amount of water was negatively correlated with PRV seroprevalence (coefficient = -3.27, $z = -2.021$, $p = 0.043$). PRV seroprevalence differed among Florida Fish and Wildlife Conservation Commission (FWC) management regions ($X2 = 65.2$, $df = 4$, p 0.100). PRV seroprevalence in feral swine also appeared to be cyclical in time, exhibiting peaks every 4 years (2007-2010 = 29.55-53.14%, 2011-2014 = 32.33% - 48.17%). It appears that land cover and land use are predictive of

PRV seroprevalence. Results have implications for animal health and the management of this invasive species.

67. HIV CONTINUES TO BE AN EMERGING PATHOGEN IN FLORIDA: THE FLORIDA COHORT PROJECT

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Background: Florida has the second highest prevalence of HIV infection in the country, and HIV incidence is increasing each year. In 2013, newly reported HIV infections were distributed among 48% African Americans, 26% Hispanic, 30% white, and 1% other. We sought to create a cohort in Florida with the following objectives: 1) recruit 1500 persons with HIV, and 200 without HIV; 2) create a registry of persons willing to participate in additional research studies, and 3) provide research data that can be used to improve HIV-related outcomes, especially those affected by substance use. This study's objective was to describe the characteristics of the first 200 persons enrolled and to compare them to the overall HIV population in Florida.

Methods: Recruitment began in October of 2014 and currently includes both urban (Orange and Hillsborough Counties) and rural (Alachua, Sumter and Columbia Counties) sites. Participants complete a baseline survey (paper-based or online). Participants' information is further linked to medical chart abstraction and statewide HIV surveillance data.

Results: Of the 202 persons enrolled, 68% were African Americans, 7.4% Hispanic, 24% White, and 1.1% other. African Americans were majority in both rural (55%) and urban (72%) locations. The gender distribution was 53% male, 41.5% female,

and 5.7% identifying as transgender. Substance use reported in the past 12 months included alcohol (62%), cocaine (21.6%), injection drugs (7.2%), non-injection drugs (28.8%) and pain medication (9.6%). Additionally 35.2% reported recent marijuana use in the past 3 months. 90% reported current use of HIV antiviral medications.

Conclusions: Recruitment is off to a good start, with an average of 50 persons per month. Although the initial sample enrolling in the Florida Cohort is diverse, we will need to improve recruitment of Hispanic persons and those who are not already receiving ART.

68. INTERACTION OF DIET AD VIRAL INFECTION ON LIFE HISTORY TRAITS ON DROSOPHILA MELANOGASTER

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Sigma virus, a member of the Rhabidoviridae family, is a vertically transmitted virus and known to induce carbon dioxide sensitivity in Drosophila melanogaster. The main purpose of this study is to investigate the relationship between diet (specifically methionine), sigma infection and other life history traits on the fecundity of D.Melanogaster. Two hypotheses have been formulated regarding this relation. The first hypothesis deals with methionine acting as a stimulator of the insulin signaling pathway which in turn activates the antiviral response in D.melanogaster. Since methionine is an essential amino acid, the second hypothesis states that its presence in diet can induce longevity and higher fecundity. To test these hypotheses, we conducted two different kinds of studies: egg count and ovariole count. For egg count, we created a total of 80 vials consisting of combinations of two genotypes, two dietary conditions(with and without methionine) and two infection statuses(uninfected and infected). The female flies raised in those 8 combinations were then dissected and the number of ovarioles were recorded. Based on these studies, we were able to find out

that the flies raised in the without methionine conditions had higher fecundity and higher ovariole count when infected with the virus. We also found significant relationships between the infection status and fecundity and between the diet/genotype combination and fecundity. This positive correlation favors the insulin signaling pathway hypothesis. In conclusion, we deduce that the methionine activates the insulin signaling pathway and generates an antiviral response in the host cells.

69. KARPOSI'S SARCOMA (KS) TUMORS HARBOR DISTINCT INTRA-HOST HIV-1 SUBPOPULATIONS THAT MAY PLAY A ROLE IN KS PROGRESSION

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Karposi's Sarcoma (KS) is a cancer that initially presents as skin lesions, which can metastasize to visceral organs. Patients co-infected with HIV-1 and HHV8 (the etiologic agent of KS) and on combined antiretroviral therapy (cART) have a 12% chance to develop metastatic KS. HIV-1 evolution in the context of KS metastasis has not been evaluated; therefore, this study's goal was to provide insight into HIV-1 phylodynamics during KS progression. We isolated DNA and RNA from skin and visceral KS tumors and non-tumor sites from 3 HIV-1 patients. While each patient had KS at death, only Patient 3 died due to progressive metastatic KS. 284 HIV-1 env and 300 nef sequences were generated using single genome sequencing. The sequences were subjected to charge, co-receptor, selection, phylogenetic and gene flow/compartmentalization analysis. Within each subject, the presence of well-supported and distinct clades of predominantly R5-using HIV-1 implies the existence of viral subpopulations within and between KS lesions. Viral populations in tumors for Patients 1

and 2 were significantly compartmentalized from non-tumor tissues, whereas in Patient 3 with disseminated KS, no significant compartmentalization was detected, suggesting that gene flow is an important aspect of KS metastasis. Neutrality tests showed that tumor HIV-1 sequences were usually evolving closer to neutral evolution in comparison to non-tumor sequence populations; neutral evolution could occur in reservoirs experiencing little immune pressure. This study suggests that KS tumors can be HIV-1 reservoirs with distinct evolutionary features; moreover, KS tumor HIV-1 reservoirs may contribute to KS progression.

70. PERCEIVED RISK FACTORS FOR THE TRANSMISSION OF PESTE DES PETITS RUMINANTS

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Peste des petits ruminants (PPR) is a viral disease of sheep and goats that is endemic to parts of Africa, the Middle East, and Asia. In the same family, rinderpest is a high consequence viral disease of cattle, which was the first and only animal disease to be eradicated. Shortly after the eradication of rinderpest in 2011, PPR was targeted for global control and eradication. PPR has a high mortality rate and can therefore have a devastating impact on families that depend on small ruminants for food or income. We conducted a survey of PPR experts to identify perceived risk factors for PPR spread, transmission, and maintenance. The survey was conducted via an online form, and supplemented by forms sent as email attachments or paper copies. The supplemental forms were provided for participants located in regions without sustained Internet access. In the first section of the questionnaire, participants were asked in an open form to list and rank the most important risk factors for the transmission, spread and maintenance of PPR. Responses were categorized and their frequencies calculated. Activities associated with movement, such as: husbandry, trade, and grazing and watering

practices were considered to be the primary risk factors for both the transmission and spread of PPR. Activities related to control, such as a lack of vaccination and quarantine were considered important for maintenance. The results of this study provide the unique perspective of experts, who are currently working in the field of PPR. This information provides an important baseline of knowledge, which can be combined with the current knowledge in the literature and lessons learned from the rinderpest eradication. The combination of this information allows for a holistic approach to the global PPR control and eradication strategy.

71. PHYLOGEOGRAPHY OF RANAVIRUS FV3 IN NORTH AMERICA

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Ranaviruses are emerging aquatic animal pathogens that have contributed to the global decline of amphibians. The type species for the genus, Frog Virus 3 (FV3), has resulted in amphibian, reptile, and fish epizootics around the world. To our knowledge,

phylogeographic analyses have not been performed on FV3 isolates. Thus, we investigated the phylogeographic structure of FV3 across its range, to (1) determine patterns among hosts and geography and (2) determine the origin of introductions. Currently, we have gathered genomic sequences from 26 FV3 isolates from fish, amphibian, and reptilian hosts occurring throughout North America. Although data collection is ongoing, we have begun preliminary phylogeographic analyses utilizing non-coding and coding regions of the FV3 genomes. These results will provide insight into ranavirus-host evolution and a better understanding of the temporospatial patterns of FV3 in North America.

72. QUASISTRUCTURES OF HIV-1 QUASISPECIES: THE DYNAMICS OF RNA SECONDARY STRUCTURE IN THE CAPSID-CODING REGION OF HIV-1

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Background: With the recent advances in RNA secondary structure determination, RNA structures have been predicted to occur throughout the genome of HIV-1, including protein-coding regions. Non-coding RNA (ncRNA) structures within HIV-1, such as TAR and PSI, are known to play important roles in regulation, yet ~85% of predicted RNA structures within the HIV-1 genome remain uncharacterized. Chemical probing methods and structural biology have provided valuable insight into RNA folding and thermostability, but in the case of rapidly evolving viruses that exist as a multitude of genetically distinct variants, or quasispecies, the dynamics as well as the selective pressures

experienced by structured RNA in protein-coding regions remain elusive. Phylogenetic methods can be used to elucidate these dynamics and selective pressures and aid in the characterization of structured RNA in protein-coding regions such as HIV-1 capsid.

Methodology and Results: Longitudinal p24 genomic RNA sequences were derived from six HIV-1-infected patients over the course of infection prior to anti-retroviral treatment. Sequence data for each patient were partitioned according to site-specific values corresponding to SHAPE reactivity and pairing probability categories obtained from Watts et al. (2009), and pairing status in the final structure, as determined by Watts et al. Evolutionary rates and transition/transversion rate ratios, frequently differing significantly between pairing states within ncRNA structures, were then determined for each data partition within each patient alignment using PAML (Yang, 1997) in order to determine if global RNA structure and/or local substructures were evolutionarily conserved. No significant differences were observed among pairing categories with respect to these parameters, globally or locally, although one particular region did exhibit trends for these evolutionary patterns. These results indicated that either no RNA structure is present in p24 or that the "true" structure is actually highly dynamic. Sequence alignments for each patient were then divided longitudinally, and mfold (Zuker, 1989) was used to predict a range of relatively low free-energy structures at each time point for each patient. In addition to a diversity of structures at each time point, we observed dramatic changes in the lowest free energy predicted structure followed by periods of stasis and often reversion to a previous lowest energy structure during the evolution of HIV within each patient. This result suggests that the patient-intrinsic viral quasispecies is comprised of a variety of RNA structures, or quasistructures, that adapt as a population in response to different selective pressures likely exerted by the host immune system.

Conclusions: Through the use of phylogenetic methods in combination with previously published data by Watts et al. (2009), we have established that evolutionary patterns that have been used previously to predict ncRNA secondary structure are not

present in p24 capsid RNA, which can be explained by the dynamic range of putative structures owing to the heterogeneous nature of the individual sequences. Hence, the selective pressures to maintain a single RNA secondary structure appear to be different for protein-coding regions, which is important to consider when investigating putative functional or regulatory roles.

73. TRANSGENERATIONAL NUTRIENT EFFECTS IN AEDES AEGYPTI INFECTED WITH DENGUE VIRUS

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Transgenerational effects arise when the environment experienced by parents influences offspring phenotypes. Since transgenerational effects influence offspring life histories and can lead to changes in host-pathogen interactions, studying these effects addresses an intellectual gap in our understanding of mosquito-borne diseases. Transmission requires virus infected blood ingested by the mosquito replicate in the midgut and disseminate through the body and into the salivary glands. Dissemination to the legs is an indicator that the virus has escaped the midgut, a prerequisite for transmission. In this study, Aedes aegypti was used as a model system to determine how the parental larval nutrient environment influences offspring susceptibility to dengue-1 virus (DENV-1) infection and dissemination. Parents and subsequent offspring were reared in either high or low nutrient conditions. To assess whether parental nutrition influenced offspring susceptibility to infection, female offspring were provided with a DENV-1 infected blood meal. Fed mosquitoes were held for 14 days and then dissected into three parts: abdomens, thoraces, and legs. Female offspring were assayed using quantitative RT-PCR for the presence of DENV-1 RNA in the abdomen. Legs of mosquitoes positive for DENV-1 were then tested to determine whether or not the virus was able to escape the midgut and disseminate to other tissues. Contingency table analysis was used to identify transgenerational effects.

Findings suggest that nutrient mediated transgenerational effects occur in this system as related to infection with and dissemination of DENV-1. This research increases our understanding of factors influencing the mosquito immune system and interactions with arboviruses.

74. USE OF ZIP-CODE LEVEL DATA TO IDENTIFY SPATIAL CLUSTERS AND RISK FACTORS OF HIV/AIDS IN FLORIDA METROPOLITAN AREAS

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Background: Florida has been heavily impacted by the HIV/AIDS epidemic. As of 2012, The Florida Department of Health estimates that approximately 130,000 individuals are living with HIV disease in Florida. This research aims to identify spatial clusters of HIV/AIDS prevalence in Florida at a fine zip-code level and investigate underlying risk factors.

Methods: Spatial statistics, such as Moran's I and Getis G have been used to identify clusters of HIV/AIDS in five metropolitan areas in Florida. Further, correlation and spatial regression analysis are employed to reveal associations between HIV/AIDS infection and social, economic, and demographic factors including race, education, and poverty at the zip code level.

Results: High risk clusters were found in central Ft. Lauderdale and northern Miami. Analysis on race revealed that both blacks and whites are positively associated with HIV/AIDS prevalence (R2=0.539 and 0.377, respectively). The Gini Index and the percent of the population in poverty also show positive associations with HIV/AIDS prevalence, with R2 of 0.238 and 0.337, respectively. Lastly, the populations with higher percentages of population with high school diplomas displayed negative associations of 0.213.

Conclusion: The results from spatial clustering analysis help identify high risk areas that need to be targeted by health policies, while the regression modeling supports the prediction of high risk sub-populations. Particularly in Florida, this research suggests that more control efforts should be allocated to ZIP code areas with a high proportion of blacks and whites, large poverty populations, and low educational levels in central Ft. Lauderdale and northern Miami.

75. Δ9-TETRAHYDROCANNABINOL (THC) SUPPRESSES HIV-1 INFECTION OF T CELLS

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Background: Effects by Δ9-tetrahydrocanbinol (THC), a psychoactive and immunomodulatory component of marijuana, on HIV-1 infection in humans are poorly understood. Ex vivo, THC treatment during monocyte differentiation alters resultant macrophage phenotype and reduces HIV-1 receptor expression rendering cells less susceptible to infection. T cell immune functions are modulated by THC as CD4+ T cells, primary cellular targets for HIV-1, express cannabinoid receptors CB1 or CB2. Studies address the hypothesis that THC modulates HIV-1 infection of T cells directly or through an alternate bystander cell such as the monocyte.

Methods: Peripheral Blood Mononuclear Cells (PBMCs) were stimulated with IL-2 and PHA +/- 30 μM THC or ethanol vehicle for 4 days followed by exposure to HIV-1AD. Supernatants were evaluated for HIV-1 (p24), or cytokines (IL-4, IL-10, and IFNγ)

production by ELISA. Distribution of cell populations and HIV-1 receptors were examined by flow cytometry. Expression of selected viral restriction factors was quantified by real-time PCR. Enrichment of CD4+ T cells was performed by magnetic depletion.

Results: THC treatment of PBMCs at the time of infection failed to suppress HIV-1 replication. In contrast, THC treatment of PBMC during IL-2 and PHA stimulation suppressed HIV-1 levels on day 7 by 30% to 95% among 8 donors with no effect on cell division, HIV-1 receptor/co-receptor expression, frequency of stimulated T cell subsets, or expression of viral restriction factors. In PBMCs, THC enhanced supernatant IFNγ (Th1), with limited modulation of IL-4 (Th2) or IL-10 (T reg) production during IL-2 and PHA stimulation. Exposure of enriched CD4+ T cells alone to THC during IL-2 and PHA stimulation reduced IFNγ.

Conclusions: THC mediated alterations in IFNγ expression suggest modulation of Th1 phenotype. THC imparts cell specific effects and may modulate a broader molecular hub, resulting in further immune cell dysfunction. Results underscore the importance of consideration of the full cellular milieu as opposed to individual cell types. Effects of THC in vitro are cell and treatment specific therefore studies of marijuana users with HIV-1 infection are critical to understanding the complexities of chronic virus infection, inflammation, and substance use.

76. A MULTI-PATHOGEN HIERARCHICAL BAYESIAN MODEL FOR SPATIO-TEMPORAL TRANSMISSION OF HAND, FOOT AND MOUTH DISEASE

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Mathematical modeling of infectious diseases plays an important role in the development and evaluation of intervention plans. These plans, such as the development of vaccines, are usually pathogen-specific, but laboratory confirmation of all pathogen-specific infections is rarely available. If an epidemic is a consequence of co-circulation of several pathogens, it is desirable to jointly model these pathogens in order to study the transmissibility of the disease.

Our work is motivated by the hand, foot and mouth disease (HFMD) surveillance data in China from 2008 to 2009. The data set consists of counts of reported cases in 334 prefectures and 53 consecutive weeks and the laboratory test data for a small subset of the reported cases. We build a hierarchical Bayesian multi-pathogen model by using a latent process to link the disease counts and the lab test data. Our model explicitly accounts for spatio-temporal disease patterns. The inference and prediction are carried out by a computationally tractable MCMC algorithm. We study operating characteristics of the algorithm on simulated data and apply it to the HFMD in China data set.

77. AMERICAN COCKROACH TARSAL SENSITIVITY TO PERMETHRIN

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Background: In order to better understand the action of excito-repellents on insects, we investigated the sensitivity of American cockroach (Periplaneta americana) tarsal sensory structures to the commonly used pyrethroid insecticide permethrin, using extracellular recordings on freshly removed legs. Responses to ethanol and acetone solvents, as well as the pyrethroid permethrin were made, in addition to comparisons of responses in pro-, meso-, and meta-thoracic legs.

Method: A stainless steel electrode was placed into the femur as the reference electrode, while a fine tungsten recording electrode was inserted into the base of any convenient tibial spine. The fidelity of each recording set up was achieved by monitoring the synchronized frequency change of nerve discharge caused by a 0.5 Hz tactile stimulation, generated by a glass capillary stimulator to the tarsi, delivered for 10 minutes. The first treatment was application of 0.5 µL of vehicle topically at the terminal pad of the tarsus. Effects were recorded for 10 minutes before treatment with the pyrethroid, permethrin. The same treatment on separate legs was repeated at least three times, to generate the average discharge frequency over each 10 minute post treatment interval from each replicate.

Results: Two-way ANOVA followed by Tukey's test to compared the recordings of foreleg, mesoleg and hindleg before and after ethanol treatments, found no significant difference detected at the level of $p = 0.05$. The mean discharge rate after ethanol treatment was significantly elevated ($p < 0.05$) compared to the no vehicle control, while the rate using acetone as vehicle was not significantly different ($p > 0.05$) from no vehicle control. Response

to permethrin solution in ethanol at doses up to 15 µg permethrin solution in acetone were not significantly different ($p > 0.05$) from acetone controls.

Conclusion: There was no significant difference in sensitivity to ethanol between the different legs of the American cockroach. Thus, it is reasonable to conduct treatments without considering which leg is tested. The results also suggested acetone itself causes less nerve response than ethanol. In addition, the result that 15 µg permethrin causes no significant effect suggests the response dosage threshold would be higher.

78. AUTOANTIGEN-INDEPENDENT VACCINATION PROTECTS NON-OBESE DIABETIC (NOD) MICE FROM ONSET OF TYPE 1 DIABETES (T1D) THROUGH INDUCTION OF REGULATORY T CELLS (TREGS).

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Oral treatment with colonization factor antigen I (CFA/I) fimbriae has previously been shown to be protective in animal models of multiple sclerosis and rheumatoid arthritis. Although CFA/I fimbriae initial mode of action is in a bystander or in an antigen (Ag)-independent fashion, protection was found to be ultimately dependent upon the induction and/or activation of auto-Ag-specific Tregs. Thus, we hypothesized that a Lactococcus lactis vector expressing CFA/I fimbriae (L. lactis-CFA/I) can protect NOD mice from T1D via the stimulation of Tregs. To test our hypothesis,

6 week-old NOD mice were orally dosed with L. lactis-CFA/I and treated every 3 weeks while control groups were given L. lactis vector or Dulbecco's phosphate buffered saline (DPBS). Incidence of disease was reduced by over 45% versus controls (p=0.0116). Histology of the pancreas revealed lower insulitis scores and higher islet counts (p=0.0152) suggesting vaccination preserved β-cell mass. To determine if Tregs were being induced, splenic mononuclear cells were stimulated with anti-CD3 plus anti-CD28 mAbs for 72 hrs at 37°C, and T cells were subsequently examined by flow cytometry. FACS analysis revealed an 8-fold increase in Foxp3+CD25+ Tregs. Additionally, these Tregs were found to express both IL-10 and IFN-γ. To assess whether these could suppress effector T cell proliferation in vitro, CD25+ Tregs from L. lactis-CFA/I-treated NOD mice showed reduced CD25-CD4+ T cell proliferation by 50% (p=0.0375) while analogous Tregs from vector and control mice lacked suppressive activity. Thus, our data demonstrate that oral treatment with CFA/I fimbriae protects NOD mice from T1D and induces a population of Foxp3+CD25+ Tregs capable of suppressing diabetogenic T cells. Research is sponsored by NIH-NIAID 1 R01 AI-078938 and Univ. of Florida Opportunity Fund.

79. PREDICTING THE NEXT EBOLA OUTBREAK: THE ROLE OF BAT DIVERSITY IN FILOVIRUS SPILL OVER

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Some of the world's deadliest diseases and greatest global health challenges include bat-hosted viruses in the family Filoviridae, such as Ebola (Ebolavirus spp.) and Marburg (Marburg marburgvirus). There is an urgent need to understand what conditions lead to the "spill over" of these bat-hosted pathogens to human populations and where these events are likely to occur in the future. The bat species that host these filoviruses have large geographic distributions, but spill over does not occur evenly throughout their ranges. Biodiversity, human population density, and anthropogenic disturbance are broadly considered the primary drivers of zoonotic spill over events, yet the influence of these factors has not been tested for filoviruses across regions of recent outbreaks. We tested how potential bat species richness, human population density, and anthropogenic disturbance (measured as road density) influenced the probability of Ebola and Marburg spill over across the host species' range. Here we show that filovirus spill over from bats occurred in areas with high bat species richness, while neither human population density nor anthropogenic disturbance appeared to influence the probability of spill over events. Filovirus spill overs have devastating effects on people and communities and our results provide an important first step toward understanding and predicting the conditions that increase the risk of future outbreaks of Ebola and closely related diseases.

80. THE EFFECTS OF STILBENE DERIVATIVES ON DELAYED-RECTIFIER POTASSIUM CURRENTS AND MOSQUITO TOXICITY

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It is well established that delayed-rectifier potassium current plays an important role in repolarizing the resting membrane potential on both nerve and muscle cells, and maintains proper electrical excitability. For this reason, our laboratory has been researching this channel as a target for the development of insecticidal compounds. In recent studies, we found that some substituted stilbene derivatives showed good inhibition of delayed-rectifier potassium current. Some of them also showed toxicity to mosquitoes.

For the electrophysiological assays, by using whole-cell patch-clamp, we tested compounds on engineered HEK 293 cell line, which expressed the mosquito Kv2.1 channel, and SH-SY5Y cells, a human derived neuroblastoma cell line. The 2-methoxy-stilbene blocked K+ current on HEK cell with IC50 at 47 μM, while the IC50 of its inactive analog, 4-methoxy-stilbene, was 336 μM. On the other hand, the 2-methoxy-stilbene blocked K+ current on Sy5y cell with IC50 of 237 μM, which suggested it had lower mammalian toxicity.

For the toxicological tests, 9 compounds were tested on Aedes aegypti by using a surface contact assay in glass test tubes. At a 2.9 μg/cm2 concentration, after 24 hours observation, 2 compounds showed the ability to kill mosquitoes. S943916 induced 100% mortality (LC50 of S943916 was 1.1 μg/cm2), and T141631 20% mortality at this concentration. Furthermore, we

also tested S943916 on Anopheles gambiae (susceptible G3 and resistant Akron strains). The IC50 values were 0.82 and 0.92 µg/cm2 respectively. However, S943916 showed an unusual selectivity for mosquitoes. When stilbene compounds were tested on Drosophila melanogaster, they did not cause any appreciable toxicity. Only T141631 induced 5% mortality, and S880159 induced 10%.

Our results suggested that stilbene derivatives were good blockers of delayed-rectifier potassium current, and some of them could be considered for development as insecticidal compounds.

81. THE IMPACT OF LAND USE ON ECOLOGICAL COMMUNITIES AND DISEASE RISK IN SOUTHERN AFRICA

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Changes in land use have the potential to alter both the structure of, and services provided by, ecological communities. One such service that is important to human health is disease regulation and the dynamics of disease transmission. Our study was conducted in Swaziland, a small land-locked kingdom in southern Africa. The landscape of Swaziland is a matrix of industrial sugar cane monoculture, “western-style” cities, “traditional” African villages (with sustenance agriculture and cattle grazing pastures), and protected areas. Rodents are an important pest species, both in terms of damage to crops and as carriers of various pathogens, including leptosoriasis, plague, toxoplasmosis, and tularemia. To look at the effect of land use on rodent community structure, we used Sherman traps to trap rodents in multiple sites in each land use area. We also conducted camera trap surveys looking at the community composition of mesocarnivores in the same land use types. Results indicate that mesocarnivore diversity decreases greatly even at small distances from reserves, and the same is true in the case of rodents, with generalist species like Masotomys natalensis dominating in areas of high disturbance. In the future,

we will analyze blood samples taken from the rodents and screen them for pathogens. Additionally, we have collected mesocarnivore feces from our study sites and we will use DNA barcoding to determine the diet composition of the mesocarnivores sampled, which will then be used to understand mesocarnivores' role in regulating the populations of pathogen-carrying rodents. This information will be helpful in informing management decisions in areas where humans and wildlife may interact frequently.

82. TOXICITY OF THE ISOXAZOLINE FLURALANER TO LARVAL AND ADULT AEDES AEGYPTI MOSQUITOES

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Mosquitoes, such as Anopheles gambiae and Aedes aegypti, are important vectors transmitting mosquito-borne diseases. The purpose of this research is to investigate the insecticidal activity and mechanism of action of isoxazolines, a new type of molecules. In the first phrase of the study, the insecticidal activity of fluralaner (an isoxazoline sold as a veterinary parasiticide) has been tested by bioassay. Fluralaner was quite toxic, and gave a relatively low LC50 (lethal concentration for 50% mortality) and LD50 (lethal dose for 50% mortality) values in larval assay and adult topical assay, respectively. In larval assay, fluralaner has a LC50 value of 1.2 ng/ml on 4th instar larvae. In adult topical assay, the LD50 value of fluralaner after 24 h treatment is 1.3 ng/mg, higher than carbaryl (0.95 ng/mg) and slightly lower than indoxacarb (1.5 ng/mg). In time course studies, the insecticidal activity of fluralaner to adults increased by a factor of around two every other day, suggesting a slowly developing toxicity, and that the large size and lipophilicity of this molecule might influence its penetration into the mosquito body and central nervous system. In adult contact paper assay, the high concentration of 2 mg/paper of

fluralaner could only kill around 12 % of the tested mosquitoes, which is much less active than topical application. More bioassays will be done using various molecules belonging to the isoxazolines class, perhaps evaluating smaller analogs of lower molecular weight having better contact toxicity properties.

83. UF-INTERDISCIPLINARY CENTER FOR BIOTECHNOLOGY RESEARCH (ICBR)

ICBR Staff - Interdisciplinary Center for Biotechnology Research, University of Florida

The UF Interdisciplinary Center for Biotechnology Research was established by the Florida legislature in 1987. Our mission is to enable, strengthen and energize all aspects of molecular life science research at the University of Florida by teaching theory, techniques and applications of modern molecular research and providing research support services. We champion the growth and development of research throughout the Florida university system and jump start research for technology transfer and accelerate molecular life science research success from the lab to the marketplace. The ICBR consists of eight core laboratories: Bioinformatics, Cytometry, Electron Microscopy, Gene Expression and Genotyping, Monoclonal Antibody, NextGen DNA Sequencing, Proteomics and Mass Spectrometry, Sanger Sequencing.

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Dr. Andrew Kane, Director, UF Aquatic Pathobiology Laboratories, and Associate Professor of Environmental and Global Health, studies environmental and oyster health in support of Apalachicola Bay's heritage oyster ÿshery. Images show a radiograph of left and right valves of an Eastern oyster (*Crassostrea virginica*) from Apalachicola Bay. This X-ray provided through UF's Veterinary Forensic Sciences Program, reveals numerous round to pear-shaped boring clams (*Diplothyra smithii*), and tube tunnels of boring polychaete worms (*Polydora spp*) that parasitize the oyster's shell. Elevated Salinity in Apalachicola Bay favors the presence of these parasites that weaken shell integrity, making the oyster more susceptible to predation by drills and crabs.