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#### Letter from the Director

Welcome to the seventh 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, from as far away as Mongolia.

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

Admiral Tim Ziemer is the U.S. Global Malaria Coordinator, with responsibility for the President's Malaria Initiative (PMI), a historic \$1.2 billion, five-year initiative to control malaria in Africa. The PMI strategy is targeted to halve the burden of malaria in 70% of at-risk populations in sub-Sahara Africa, or some 450 million people. He is joined by Dr. Chris Plowe, who has been responsible for pioneering studies on malaria vaccine development and drug resistance. A Howard Hughes Medical Institute investigator and Professor of Medicine at the University of Maryland School of Medicine, Dr. Plowe has conducted multiple studies of malaria in Africa, with publications in Nature, Science, and New England Journal of Medicine.

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 - 1:10 PM Welcome & Introductions

Dr. J. Glenn Morris, Director, EPI

**1:10 PM - 3:10 PM** Keynote Speeches

**3:10 PM – 3:15 PM** Closing Remarks

Dr. David Norton, VP of Research

**3:15 PM - 4:00 PM** Poster Removal

**Keynote Speakers** 

(1:10-2:10)

#### R. Timothy Ziemer

Rear Admiral, United States Navy (Retired) President's Malaria Initiative Coordinator

#### "The President's Malaria Initiative...Why It Has Been Successful"

(2:10-3:10)

#### **Dr. Christopher Plowe**

Investigator, Howard Hughes Medical Institute Professor and Leader, Malaria Group Center for Vaccine Development University of Maryland School of Medicine

# "Malaria Eradication: Prospects and New Tools"

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

**Mustafizur Rahman** - Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida; **Mohammad Jubair** - Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida; Meer T. Alam -Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; Ilya A. Sakharuk - Emerging Pathogens Institute, University of Florida; Mohammad H. Rashid - Emerging Pathogens Institute, College of Medicine, University of Florida; Judith A. Johnson - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, University of Florida; Mahmuda Yasmin - Department of Microbiology and Cell Science, University of Dhaka; 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

Background. For the first time in 60 years, Haiti witnessed epidemic cholera caused by accidentally introduced altered Vibrio cholerae O1 El Tor strain in October, 2010 which continues to inflict Haitians. Continued presence of toxigenic V. cholerae in aquatic reservoirs is a key to promote cholera endemicity. The rugose phenotype of V. cholerae, characterized by exopolysaccharide production that confers resistance to stresses, is considered to be a prototype of environmental persistence. Here we provide evidence that Haitian V. cholerae strains, including clinical and environmental O1 and indigenous non-O1/non-O139 isolates can switch to rugose phenotype. Methods. A total of 35 clinical V. cholerae O1, 15 environmental O1 and 44 environmental non-O1/non-O139 isolates collected during 2010-2012 in Haiti were included in this study. To determine if the

Haitian V. cholerae strains can switch from a smooth to rugose colony phenotype, alkaline phosphate water (APW #3) was used to promote high-frequency conversion and the experiment was conducted both at 37°C and room temperature. Biofilm production of a rugose-producing Haitian V. cholera O1 strain HC16 was measured using absorbance and confocal microscopy. Resistance to chlorine, and oxidative and osmotic stresses were also assessed. Results. A higher number of V. cholerae O1 clinical isolates of 2012 compared to those isolated in 2010 shifted to rugose phenotype (61.9% vs. 7.1%, respectively; p<0.05). The rugose frequency seen with 01 isolates was remarkably higher relative to rugose frequency observed with non-01/non-0139 strains. Incubation temperature 37°C compared to 25°C induced increased rugose production. Genetic analysis exhibited that vpsA is required for the production of rugose exopolysaccharide which confers resistance to diverse stresses. Conclusion. Altered toxigenic V. cholerae O1 El Tor strain introduced in 2010 in Haiti has been evolving with regard to smooth to rugose phenotype conversion which has potential to establish itself as an endemic pathogen promoting cholera transmission in Haiti.

# **02.** PHYLODYNAMICS OF VIBRIO CHOLERAE **01** IN HAITI DEMONSTRATES EVIDENCE OF POPULATION BOTTLENECKS DRIVEN BY POSITIVE SELECTION

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The 2010 Haitian cholera epidemic appears to have started with a single strain in a cholera-naive setting. As of November 2013, 693, 875 cases and 8, 482 deaths have been reported. This outbreak provides a unique opportunity to study the evolution of this global pathogen in humans and the environment. Phylogenetic analysis of genome-wide single-nucleotide polymorphism data enables the investigation of bacterial epidemics and the underlying evolutionary processes at a remarkable resolution. We apply these methods to the analysis of 33 V. cholerae isolates from the Haitian epidemic including 25 clinical and 8 environmental strains isolated in Haiti in 2010 and 2012. Phylogenies were inferred from genome-wide SNPs and analyzed using coalescent-based demographic models within a Bayesian framework. Polymerase chain reaction (PCR) analysis of virulence genes (ompW, toxR, tcpA, ctxA, MAMA, rstA and rstC) was also conducted. We found that V. cholerae has been accumulating mutations throughout the epidemic driven and is therefore a measurably evolving population characterized by sequential population bottlenecks driven by the emergence of genomic variants under increasing positive selection. Reconstruction of ancestral states showed that fixation of nonsynonymous changes occurred along the internal branches of the genealogy leading to each major population bottleneck. Molecular and whole-genome analysis also revealed that an environmental isolate had lost the CTX phage. Overall, our

inclusion of environmental isolates in the analysis demonstrates a complex dynamic of transmission. As the epidemic in Haiti continues, the progressive accumulation of mutations in V. cholerae under selection during human infections could potentially affect virulence, transmission dynamics, and even vaccine efficacy. Overall, these findings have significant implications for V. cholerae transmission and pathogenesis and emphasize the need for continued monitoring of isolates from patients and the environment.

# 03. VIBRIO CHOLERAE PERSISTED IN MICROCOSM FOR 700 DAYS INHIBITS MOTILITY BUT PROMOTES BIOFILM FORMATION IN NUTRIENT-POOR LAKE WATER

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**Background**: Toxigenic Vibrio cholerae, ubiquitous in aquatic environments, is responsible for cholera; humans can become infected after consuming food and/or water contaminated with the bacterium. The underlying basis of persistence of V. cholerae in the aquatic environment remains poorly understood despite

decades of research. We recently described a "persister" phenotype of V. cholerae that survived in nutrient-poor filter sterilized lake water (FSLW) in excess of 700-days. Previous reports suggest that microorganisms can assume a growth advantage in stationary phase (GASP) phenotype in response to long-term survival during stationary phase of growth. Here we report a V. cholerae GASP phenotype (GASP-700D) that appeared to be resulted in from 700 day-old persister cells stored in glycerol broth at -80°C. **Methods**: Motility of V. cholerae strains was tested on motility agar. Gene expression analysis was performed by qRT-PCR. Biofilm formation was measured in Lbroth and filters sterilized lake water (FSLW) by using absorbance and confocal microscopy. The presence of bacterial exopolysaccharide was examined using transmission electron microscopy (TEM). Stress resistance was assessed against H2O2, NaOCl and NaCl. **Results**: The GASP-700D, compared to its wildtype N16961, was defective in motility, produced increased biofilm that was independent of vps and resistant to oxidative stress when grown specifically in FSLW (p<0.05). **Conclusion**: We propose that V. cholerae GASP-700D represents cell populations that may better fit and adapt to stressful survival conditions while serving as a critical link in the cycle of cholera transmission.

### 04. MONITORING OF TOXIGENIC VIBRIO CHOLERAE O1 IN ENVIRONMENTAL RESERVOIRS IN HAITI

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Cholera appeared in epidemic form in Haiti in October, 2010, for the first time in over 100 years. Cases have continued to occur, raising questions whether the microorganism has established environmental reservoirs in Haiti. We monitored 15 environmental sites in the Gressier/Leogane region of Haiti between April, 2012 and March, 2013. Toxigenic Vibrio cholerae O1 El Tor biotype strains were isolated from 3 (1.7%) of 179 water samples and cholera toxin (CT)-negative O1 V. cholerae from an additional 3 samples; all samples with V. cholerae O1 also had non-01 V. cholerae. Isolation of V. cholerae 01 and non-01 was significantly associated with temperature, with V. cholerae 01 only isolated when water temperatures were ≥31°C. Our data substantiate the presence of toxigenic V. cholerae O1 in the aquatic environment in Haiti. If, indeed, these isolations reflect establishment of long-term environmental reservoirs, eradication of cholera from Haiti may be difficult.

### **05.** Whole-Genome Sequencing for National Surveillance of Enteric Diseases

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University of Florida; **Marc Allard** - Food and Drug
Administration

In the US, over a million cases of foodborne illness and >350 death annually are attributed to Salmonella spp. infections. Florida has one of highest incidence rates of Salmonellosis in the nation, reporting 31.3 cases per 100, 000 population in 2011. Due to the low genetic diversity of Salmonella, subtyping methods such as pulsed field gel electrophoresis (PFGE) provide limited resolution needed to discern between related strains. Discrimination between closely related strains is central to public health surveillance efforts including outbreak identification and investigation. Recently, advancements in next-generation wholegenome sequencing (WGS) have enabled delineation between closely related bacterial pathogens. In an effort to characterize the genetic diversity of Salmonella and test the feasibility of a WGS-centric surveillance framework, the Food and Drug Administration is collaborating with state and federal public health laboratories to establish an archived database of Salmonella WGS from clinical, food, and environmental sources. This database will function similarly to PulseNet, allowing public health officials to utilize WGS for real-time outbreak detection, investigation, and food product trace-back. The UF Emerging Pathogen's Institute is currently participating in this pilot group project, sequencing a representative sample of Salmonella isolates from Florida and Georgia. To date, we have sequenced 236 Salmonella isolates using an Illumina MiSeq desktop

sequencer, and in addition to contributing to the national cross-sectional sample of isolates, we are utilizing this opportunity to compare phylogenies based on WGS data with PFGE and other typing methods. We are exploring the demographic history of this pathogen in Florida and Georgia using a longitudinal sample of isolates. Additionally, what is revealed is a complex dynamic of isolates collected from environmental and wildlife sources across north-central Florida in South Georgia. This technology promises to enhance our current national surveillance framework as well as expand our understanding of Salmonella ecology and transmission in Florida.

# **06.** DEVELOPMENT OF A NOVEL CROSS-STREAKING METHOD FOR ISOLATION, CONFIRMATION, AND ENUMERATION OF SALMONELLA FROM IRRIGATION PONDS

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Introduction: The 2013 Produce Safety Rules in Food Safety Modernization Act (FSMA) require regular testing for generic Escherichia coli in agricultural water intended for pre-harvest contact with the edible portion of fresh produce. However, the use of fecal contamination indicators frequently does not correctly reflect distribution of foodborne pathogens such as Salmonella enterica. Therefore, to ensure food safety, novel methods are required for pathogens direct detection and

enumeration in agricultural settings. Purpose: We report the evaluation of different cost-effective methods for quantification, isolation, and confirmation of Salmonella in irrigation pond water and sediment samples. **Methods**: A most probably number (MPN) dual enrichment culture method was used in combination with differential and selective agars, XLT4 and CHROMagarTM Salmonella plus (CSP). The necessity for PCR confirmation was evaluated, and methods were compared by cost and performance measures (i.e., sensitivity, specificity, positive predictive value, and negative predictive value). **Results**: Statistical analyses showed that using XLT4 as the initial selective agar to isolate Salmonella colonies improved recovery compared to CSP agar; however, PCR confirmation was required to avoid false positive results on either agar. Therefore, a novel cross-streaking method utilizing CHROMagarTM agar for individual colony confirmation of Salmonella presence/absence on XLT4 was developed. This method classifies the colony as positive if typical Salmonella morphology is observed on both agars. Statistical analysis showed that this method was as effective as PCR for species confirmation of pure individual strains isolated from enrichment cultures (sensitivity=0.99, specificity=1.00, relative to PCR). **Significance**: This method offers a cost-effective alternative to PCR that would increase the capacity and sensitivity of Salmonella evaluation.

## 07. RISK FACTORS AND TRANSMISSION DYNAMICS OF ESCHERICHIA COLI O157:H7 AMONG CATTLE IN A CLOSED SETTING

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Cattle are considered to be a main reservoir for Escherichia coli 0157:H7, one of major causes of foodborne diseases in humans. Understanding how E. coli 0157:H7 is transmitted among cattle is important in the prevention and control of the disease. In E. coli 0157:H7 epidemiology, supershedding has been reported and defined as cattle that shed high concentrations of E. coli 0157:H7 (≥10, 000 colony-forming cells (CFU)/g of feces) at a crosssection in time. Here, a longitudinal study was conducted on a farm in Wooster, OH from November 2005 to April 2006. 168 cattle confined to 24 pens were followed for the 6 month period. E. coli O157:H7(including subtype A, B, C) was isolated from cattle's fecal samples with the overall prevalence ranging from 2.94% to 31.55% across all pens over time. Exposure to shedding events in either the same pen or neighboring pens significantly increased the probability of catching E.coli O157:H7 by susceptible cattle (OR=1.27, 95% CI 1.14-1.42 and OR=1.08, 95% CI 1.01-1.16 respectively). Supershedders had greater impact than non-supershedders by 26%, especially for E.coli 0157:H7 subtype A. The study offers insights into risk factors underlying the transmission of E. coli O157:H7 among cattle in a closed setting. Based on the findings, an individual based model is under development to further understand how the risk factors drive the dynamics of E. coli O157:H7 transmission.

## **08.** Underlying Mechanismm of Antimicrobial Activity of Chitosan Microparticles

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The emergence of multidrug-resistant microorganisms is a great public health concern and has triggered an urgent need to develop alternative antibiotics. Chitosan microparticles (CM). derived from chitosan, have been shown to reduce E. coli 0157:H7 shedding in a cattle model, indicating potential use as an alternative antimicrobial agent. However, the underlying mechanism of CM on reducing the shedding of this pathogen remains unclear. Here, we studied antimicrobial properties of CM to understand the mode of action against E. coli 0157:H7. We report that CM have antimicrobial activity against through membrane damage that is dose, pH, and contact dependent. In addition to an Outer Membrane Protein A (OmpA). lipopolysaccharide of E. coli 0157:H7 is critical for CM binding, and this binding activity is coupled with a bactericidal effect of CM. Since Shiga toxins, encoded in the genome of bacteriophage, is overexpressed during antibiotic treatment, antibiotic therapy is generally not recommended due to high risk of HUS in patients. Unlikely to the mitomycin C treatment, CM did cause induction of bacteriophage and overexpression of Shiga toxins in E. coli 0157:H7. This work establishes an underlying mechanism of CM, whereby CM exert antimicrobial activity in vitro without production of Shiga toxins, providing significant insight for clinical application.

# **09.** CPXRA NEGATIVELY REGULATES EXPRESSION OF THE SHIGA TOXIN AND THE LOCUS OF ENTEROCYTE EFFACEMENT OF ENTEROHEMORRAGIC ESCHERICHIA

Miguel De la Cruz - Department of Molecular Genetics and Microbiology, Emerging Pathogens Institute, College of Medicine; Jason Morgan - University of South Florida, Department of Biology, Microbiology and Molecular Biology; Riordan James - University of South Florida, Department of Cell Biology, Microbiology and Molecular Biology; Jorge Giron - Department of Molecular Genetics and Microbiology, Emerging Pathogens Institute, College of Medicine, University of Florida

Enterohemorrhagic Escherichia coli (EHEC) is a significant cause of serious human gastrointestinal disease worldwide. EHEC strains produce Shiga toxins responsible for the hemolytic uremic syndrome and contain a pathogenicity island called locus of enterocyte effacement (LEE), which encodes most of the genetic elements required for the production of intestinal attaching and effacing (AE) lesions. The Cpx envelope stress response of E. coli is controlled by a two-component system (TCS) consisting of a sensor histidine kinase (CpxA) and a cytoplasmic response regulator (CpxR). A cpxA mutant affected the adherence of EHEC to human cell and was reduced in expression of stx gene. Both transcription and production of cpxR and CpxR, respectively were observed in the absence of CpxA. High levels of CpxR generated in the cpxA mutant or under the overexpressing NlpE lipoprotein, repressed the EspABD translocator proteins. In addition, CpxR repressed transcription of grlA and ler, which code for the main positive regulators of LEE. The absence of CpxA initiated a complex regulatory cascade involving sigma factors, transcriptional regulators and proteases. CpxR activated rpoH expression, which codes for Sigma 32 factor, and this sigma factor was reported to activate lon transcription. ler and grlA were derepressed in a lon mutant and similar levels were observed in the lon cpxA double mutant indicating a CpxA-CpxR-RpoH-LonLEE complex cascade. By using the Galleria mellonella model, we found attenuation of the cpxA mutant. Our results show the importance of CpxA sensor kinase to control the virulence of EHEC.

## 10. EFFECT OF CHANGES IN VACCINATION COVERAGE ESTIMATES ON IMPACT AND COST-EFFECTIVENESS OF ROTAVIRUS VACCINATION IN INDIA

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Rotavirus is a major cause of mortality in children under five, particularly in India which accounts for 20% of the annual global diarrheal burden. While new low-cost vaccines can provide an opportunity to significantly reduce rotavirus burden, their impact depends on attaining high and equitable levels of coverage. Over the past decade, efforts in India have focused on increasing the coverage of routine vaccinations, especially in states with historically high child mortality, though how well these efforts have addressed vaccine equity and reduction in disease burden is unclear. This research uses data from three surveys spanning four years, to predict the rotavirus vaccination impact and cost-effectiveness in select high mortality regions and states in India. We used a rotavirus mortality risk index, DPT1-3 vaccine and timing data from National Family and Health Survey III (NFHS-

III), District-Level Health Survey (DLHS), and the 2009 UNICEF Coverage Evaluation Survey (CES) as a proxy to model changes in rotavirus vaccine impacts and geographic, socioeconomic and gender disparities in one annual birth cohort of children during the first five years of life in India. Modelled national three-dose vaccine coverage improved from 61% to 75%, corresponding to an increase in benefits from 5.4 to 7.8 deaths averted per 1000 births and average cost effectiveness improvement from \$197 to \$176 per DALY from NFHS-III to CES surveys. However, significant inequities in post-vaccination mortality remain. Our model predicts significant gains and reduced disparities, especially in high mortality regions, resulting in more favorable impacts of national rotavirus vaccine introduction. Future programs targeting improving vaccine access for children by integrating with other effective health programs, enhance the impacts of rotavirus vaccination in India.

### 11. IDENTIFICATION OF 8 COMMON SALMONELLA SEROVARS USING MULTIPLEX PCR

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Salmonella is a significant concern in public health and food safety. It is one of the leading causes of foodborne illnesses in the United States. Rapid and accurate identification is important in pathogen control since it can facilitate epidemiological study and timely implementation of corrective actions, and therefore ensure food safety. PCR is commonly used to detect Salmonella. However, in most cases, it needs another technique to identify the serotype of isolated Salmonella. In this study, we developed a

multiplex PCR assay that can simultaneously identify eight different Salmonella enterica serovars that have been associated with foodborne illnesses: Entertidis, Typhi, Javiania, Typhimurium, Newport, Gaminara, Michigan, and Agona. In this study, fifteen genetic loci were selected, and 17 primers were designed using Bioinformatics software and tested for multiplex PCR development. The final assay was run with 9 primers as two five-plex PCRs. The multiplex PCR could correctly identify the tested 8 serovars based on their unique amplification patterns. The developed assay did not show any cross-reactivity with other common foodborne pathogens or environmental bacteria. When the assay was tested with experimentally contaminated meat samples, the assay could identify the Salmonella serovars without losing sensitivity. It demonstrated this multiplex PCR assay could serve as a simple and rapid method for molecular subtyping of Salmonella enterica in food samples.

## 12. THE CHOLERA PUBLIC HEALTH SURVEILLANCE SYSTEM IN CAMEROON

<u>Moise Ngwa</u> - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Song Liang** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **J. Glenn Morris** - Emerging Pathogens Institute, College of Medicine, University of Florida

**Introduction** In the ongoing 7th cholera pandemic, cross border expansion of the disease is recognized as a threat to global health. Public health surveillance systems are essential for early detection and rapid response to prevent cross border expansion of cholera. Cameroon's public health cholera surveillance system recently underwent major improvements. **Methodology** A descriptive cross-sectional study methodology was used in the settings of the Far North and the Centre regions of Cameroon.

**Results** The hierarchical health system include the peripheral (community, health facility, and health district), intermediate (regional), and central (Ministry of Health) levels. Surveillance core activities (case detection, ...), response core activities (immediate investigation and planned intervention), and support functions (coordination, training, supervision, and resources mobilization) have been integrated at all hierarchical levels. Case definitions: Suspect case is any patient  $\geq 5$  years with severe dehydration or death from acute watery diarrhea. During epidemics, a suspect case is any person  $\geq 5$  years with acute watery diarrhea, with or without vomiting. Confirmed case is suspected case plus isolation of Vibrio cholerae O1 or O139 in the stool. The threshold for an epidemic is one confirmed case. At the community level, cholera is defined as any person  $\geq$  to 5 years with lots of watery diarrhea. **Data flow**: Cholera patients who present at health facilities are recorded in hospital registers. The data is compiled and sent to health districts, who merge and forward to the Regional Delegations of Public Health (RDPH). RDPH de-identifies the data and forward to the central level via internet. Data is then sent to international partners. Immediate reporting is done by mobile phones following the same process. **Major innovations**: The creation of 183 health districts, introduction of mobile phone fleet at the health districts and laptop computers at the RDPH, and the creation of the cholera command and control center (C4) in all ten regions of Cameroon are major innovations of the surveillance system . **Limitations** and Future Directions This study was at the RDPH and in two regions only. Future studies should include levels above and below the regional level and should extend to all 10 regions in the country. Conclusion The creation of 183 health districts, introduction of mobile phone fleet at the health districts and laptop computers at the RDPH, and the creation of C4 in all 10 regions are important milestones in the Cameroon's public health cholera surveillance.

## 13. TEMPORAL PATTERNS OF CHOLERA OUTBREAKS AND THEIR ASSOCIATIONS WITH RAINFALL AND TEMPERATURE

<u>Chelsea Stacy</u> - Department of Health Science, University of Florida; **Yang Yang** - Department of Biostatistics, University of Florida; **Song Liang** - Department of Environmental and Global Health, University of Florida

It has long been recognized that the transmission of cholera is governed by environmental factors. Over the past decades, many studies have been carried out aiming to understand the patterns of cholera transmission and the cholera-environment linkage based on historical outbreak data. Many studies from different endemic regions used different approaches and some of traditional statistical methods did not take into account nonstationary nature of the time-series outbreak data. In this study, we re-constructed time-series data for historical cholera outbreaks, rainfall, and temperature from 13 locations across the world. Studies included cover a variety of locations and ecological settings, primarily in Africa and southeast Asia. The majority of the case data reported in the studies came from the local hospital or health center. We used wavelet approaches to analyze all historical datasets. The analysis indicates that dominant modes of epidemics may vary by region and by different time periods at the same region. Countries in similar regions tend to have modes of similar length dominate for epidemics, however, the timing of the dominance varies. The regions in Africa tend to have longer periods of dominance, while southeast Asia sites tend to be dominated by shorter periods. For associations between cholera and rainfall and temperature, similar patterns - cross-region and within-region heterogeneities were also observed. Both shorter and longer modes were witnessed in most of the locations. regardless of region. The cholera and rainfall associations were often similar to the cholera and temperature associations, suggesting that the two environmental factors have similar effects on cholera incidence.

# 14. IDENTIFICATION AND EFFECT OF EVENTS IN EPISODIC CONTAMINATION OF SURFACE IRRIGATION WATER IN CENTRAL FLORIDA

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The safety of produce can be related to microbial quality of fruit contact water. Current recommendations state, the highest allowed Escherichia coli limit for irrigation waters is 235 MPN/100 ml and a geometric mean (n=5) not to surpass 126 MPN/100 ml. Water exceeding this number should be retested, not used, or used by reducing potential contamination risk of produce. The purpose of this study is to identify and evaluate the influence of events that may result in the increase of episodic contamination of surface irrigation pond water. Total of 500 ml surface water samples from six ponds in Central Florida were collected weekly, and every other day after rain events of 0.8 inches or more within a 24 h, and after intensive surface water usage due to initial planting stage or freeze protection. The samples were collected from 20 cm below surface within a 10 ft radius of the intake water pump or hose. The populations of target microorganism were enumerated via Most Probable Number (CFU/100 ml) in required dilutions with Quantitray 2000 IDEXX. Total coliform and E. coli populations were examined through the activity of  $\beta$ -galactosidase and  $\beta$ glucuronidase, respectively. The activity of β-glucosidase was used for the Enterococci population identification. Animal activity was observed during sampling. Turtles, fishes, frogs, and various types of bird including ibis, heron, sandhill cranes, wood storks, wild and farm ducks, and vultures were observed in and around ponds. The population of tested microorganisms varied for each

sampling time, up to 100 folds. In general, E. coli populations stayed below 235 CFU/100 ml; increasing in four ponds (up to 365.4 CFU/100 ml) during freeze alerts compared to previous and subsequent sampling. Populations of coliform and Enterococci ranged from 103 to 105 and 101 to 103, respectively.

## 15. SURVIVAL OF FOODBORNE PATHOGENS IN READY-TO-BAKE COOKIE DOUGH IN TWO DIFFERENT STORAGE CONDITIONS

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Since the multistate E. coli 0157:H7 outbreak in 2009 associated with commercial pre-packaged cookie dough, cookie dough product is recognized as a novel vehicle for transmission of pathogens including E. coli 0157:H7, the most well known Shiga toxin-producing E. coli serotype. Considering that common ingredients including eggs and chocolate have been associated with pathogen outbreaks, there is potential food safety risk associated with raw cookie dough product. Yet, it has been reported that young adults in the United States frequently consume raw cookie dough against the warning from government agencies and food manufacturers. This study examined the survival of two foodborne pathogens, Salmonella serovar Enteritidis and E. coli O157:H7 in commercial raw cookie dough during its storage of 4 weeks. Cookie dough samples were inoculated with 6.23 cfu/g and 5.09 cfu/g of Salmonella Enteritidis and E. coli 0157:H7, respectively, and stored for 4 weeks at 4°C or -18°C. During the 4 week-storage period, sample was taken at 0, 1, 3, 5, 7, 10, 14, 18, 21, 25, and 28 days after initial inoculation and tested for survival of pathogens. For Salmonella Enteritidis, 1.70 log reduction and 1.19 reduction were observed at 4°C and -18°C, respectively. For E. coli 0157:H7,

 $1.89 \log$  reduction and  $0.67 \log$  reduction were observed at  $4^{\circ}\text{C}$  and  $-18^{\circ}$  C, respectively. The results from this study indicate pathogens can survive for more than 1 month in cookie dough products stored under refrigeration or freezing, even with a low moisture and high fat and sugar content. These results suggest that good manufacturing practices and effective monitoring system should be implemented to prevent cookie dough from pathogen contamination. Also, consumer education for risks associated with raw cookie dough would be highly desirable to reduce any cookie dough-associated foodborne illnesses.

### 16. BEAD-BASED, SIMULTANEOUS DETECTION OF 7 MAJOR STEC SEROTYPES

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Bead-based, Simultaneous Detection of 7 Major STEC Serotypes Abstract Shiga toxin-producing Escherichia coli (STEC) have been associated with foodborne outbreaks. They are found in various food including meat and produce. Due to their deleterious effect, they have become a big concern in food industry and public health. Besides the most recognized E. coli 0157, other non-0157 STEC (026, 045, 0103, 0111, 0121, and 0145), commonly known as "Big Six", have been associated with multiple outbreaks. These 7 STEC serotypes are now considered as adulterants in certain meat products and the testing for their presence is required for these products. Various detection methods for STEC have been developed. However, most of these methods cannot detect multiple serotypes from a single assay and time-consuming. The goal of this study was to develop a sensitive and rapid immunesensor-based multiplexing array system to simultaneously detect and identify 7 major STEC serotypes. High

multiplexing bead-based array was combined with simple immunomagnetic separation for effective target concentration. The mixture of 7 different types of bead sensors, each functionalized with antibodies to each of 7 STEC serotype, was loaded into 96-well microplate and used as an array platform. Presence of target STEC was determined and identified by reading fluorescent signals from binding of fluorescently labeled detection antibodies to target STEC cells. The developed multiplex array was able to detect target STECs at the concentration as low as 103 CFU/mL with minimal crossreactivity within 5 hrs without any enrichment. This result indicates the developed immunosensor-based multiplexing array can be a rapid and reliable method for simultaneous detection and serotype identification of multiple STECs. This array shows great potential to be adapted to automatical testing of food or environmental samples for the presence of STECs.

# 17. IMMUNOMAGNETIC BEAD-BASED FLUORESCENT DETECTION FOR SHIGA TOXIN-PRODUCING E. COLI (STEC) O157 AND THE BIG SIX SEROTYPES

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Shiga toxin-producing E. coli (STEC) are a group of foodborne pathogens that can cause serious illness in humans and even lead to death. O157 is the most commonly identified STEC serotype related to foodborne illness; however, recent outbreaks have shown that numerous non-O157 serotypes are also important threats to food safety. In order to prevent foodborne illnesses by STEC, it is important to develop a sensitive and cost-effective assay that can rapidly detect the presence of multiple STEC serotypes in a single assay. In this study, an immunomagnetic bead-based fluorescent assay was developed for the simultaneous

detection of seven different STEC serotypes: 0157 and the "big six" non-0157 STEC (026, 045, 0103, 0111, 0121 and 0145). For our goal, immunomagnetic beads, functionalized with antibodies to each target STEC serotype, were loaded into a 96-well microtiter plate and used as a bead-based array platform for STEC identification. A sandwich-type immunofluorescent assay was employed and the presence of target STEC was determined by measuring fluorescent signals from detection antibodies coupled with Cy3 dye. The developed bead-based immunoassay was tested with various STEC serotypes, generic E. coli, and other common foodborne pathogens for its specificity. The developed immunoassay could detect target STEC serotypes as low as 2,000 CFU/mL without any enrichment. The assay can be completed within 3.5 hrs, and no cross-reactivity between tested organisms was observed. This study demonstrated that the developed immunomagnetic bead-based immunofluorescent assay can be a useful tool to simultaneously detect multiple STEC serotypes. It is believed that the addition of a short enrichment step will improve the sensitivity of the assay even further. Additionally, the 96-well plate format employed in this assay can provide a great potential for automatic high-throughput screening of large number of samples for food testing.

## 18. ISOLATION OF AN EQUINE INFLUENZA A VIRUS FROM A CAMEL DEMONSTRATES CROSS-SPECIES TRANSMISSION.

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**Background**: Mongolia has encountered repeated outbreaks of influenza A virus infections among its populations of free-ranging horses. The most recent outbreaks (2007-08 and 2011) have been associated with a virus similar to that observed circulating in horses throughout Central Asia and Europe belonging to the Florida Clade 2 H3N8 subtype. During efforts as part of the Centers for Excellence in Influenza Research and Surveillance network (CEIRS) zoonotic influenza surveillance efforts a number of dromedary camels that regularly co-mingle with horses were reported to experience influenza like illness during a time when influenza virus was observed circulating in the horses. **Methods**: Swabs from the nares of Mongolian camels exhibiting upper respiratory illness were collected and tested for the presence of influenza A. Swabs testing positive were passaged in embryonated chicken egg. Allantoic fluid was then shipped to the Emerging Pathogens Institute where real-time RT-PCR was used to confirm the presence of influenza A. Upon confirmation of the presence of influenza A an aliquot of virus was shared with collaborators at the J. Craig Venter Institute where the full genome of the virus was sequenced and phylogenetic analysis completed. Results: A single isolate of Influenza A was obtained from a Mongolian camel exhibiting influenza like respiratory illness. The virus genome of this virus was fully sequenced. Phylogenetic analysis of the viral genome demonstrated identity with equine influenza A H3N8 viruses recently found circulating

in Mongolian horse populations. Additionally it was observed that the HA sequence from this virus carries a two amino acid insertion found only among a subset of recently isolates of equine influenza A H3N8 viruses of the Florida Clade 2. **Conclusions**: The isolation of an H3N8 equine influenza virus in camels indicates that the virus possesses the ability to cross species. The identity with currently circulating viruses indicates that this cross species transmission is likely a recent event. Without additional isolates from camels it is difficult to predict if the viruses possesses the ability to transmit from camel to camel.

# 19. COMPARISON OF COMMERCIAL INFLUENZA A VIRUS ASSAYS IN DETECTING AVIAN INFLUENZA H7N9 AMONG POULTRY CLOACAL SWABS, CHINA

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Avian H7N9 virus emerged in China in February 2013 and has since spread widely among China's poultry, causing 194 human infections and 53 deaths to-date. While there are multiple commercial influenza A assays available and commonly used to detect influenza in humans, they have not been well-studied as tools to specifically detect H7N9 given that the virus is emergent

and access to H7N9-positive human clinical samples is limited. However, we reasoned that if certain commercial influenza assays were effective in detecting H7N9 virus among avian species fecal swabs, they may serve as an additional diagnostic option in H7N9 endemic areas. To address this diagnostic need, we sought to compare World Health Organization (WHO) and US commercial influenza assays in detecting avian H7N9 virus in poultry cloacal specimens. Between April 6 and July 15, 2013, 261 cloacal swabs were collected from commercial poultry in Nanjing and Wuxi City, Jiangsu Province, China. Swabs were screened with the WHO's influenza A and H7N9 real-time RT-PCR (qRT-PCR) assays. A blinded panel of 97 specimens (27 H7N9-positive and 70 influenza A-negative) was then used to compare 3 antigen based commercial assays (Remel Xpect Flu A&B, Quidel Quickvue influenza, and Quidel Sofia Influenza A+B), and 2 molecular commercial assays (Quidel Molecular Influenza A+B assay and Life Technologies VetMAX-Gold SIV Detection Kit). None of these commercial assays were approved for use with poultry specimens. Considering the WHO H7N9 qRT-PCR assay as the gold standard, all assays except the Ouidel Ouickvue influenza assay had high specificity (ranging from 96-99%). Regarding sensitivity, the Life Technologies VetMAX-Gold SIV Detection Kit (100%; 95% CI 87-100%) and the Quidel Molecular Influenza A+B assay (85%; 95% CI 66-96%) performed the best. The sensitivities of the non-molecular antigen detection assays, were either unable to detect small amounts of H7N9 viral RNA or were inhibited by specimen type. The Life Technologies VetMAX-Gold SIV Detection Kit and the Quidel Molecular Influenza A+B assay are comparable in performance to the WHO H7N9 gRT-PCR assay in detecting H7N9 from poultry cloacal specimens. Should H7N9 infections become more prevalent in man, these data support the merit of evaluating these assays among human specimens.

# 20. MODULATION OF TOLL-LIKE RECEPTOR ACTIVITY AND INFLUENZA VIRUS INFECTIVITY BY SINGLE-WALLED CARBON NANOTUBES WITH DISTINCT CHIRAL

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Despite rapid advancement in manipulating nanomaterials and their growing application in various fields, sound understanding regarding their toxicity is urgently needed. Single-walled carbon nanotubes (SWNTs) have raised eminent public concern on causing potential long-term adverse health consequences. Inhalation, being a likely route of exposure, underscores the critical need to comprehend how SWNTs impact the lungs as they possess a superficially resemblance to asbestos. Additionally, few studies have focused on the ability of these particles to mediate pathogenic infections. The aim of this study is to investigate whether SWNTs of distinct chiralities modulate toll-like receptors (TLRs), which recognize pathogens as the first line of innate immune defense in humans. Our hypothesis is that SWNTs alter

TLR activity resulting in modulation of pro-inflammatory cytokines through induction of transcription factor nuclear factor kappa beta (NF-κB). Furthermore, SWNTs of distinct chiral enrichment (SG65, SG76, and CG200) differentially impact TLR activity. Using human embryonic kidney (HEK) cells that overexpress TLR2, TLR3, or TLR7 we have determined that all types of SWNTs (SG65, SG76 and CG200) did not induce NF-kB at non-cytotoxic doses. However, SG65 SWNTs dramatically suppressed TLR2 activation by the natural agonist zymosan while significantly enhancing TLR3 induction by its agonist poly (I:C). Neither SG76 or CG200 SWNTs nor the particle control carbon black (CB) had any significant influence on TLR activity. On the other hand, SG76 SWNT and CG200 SWNTs dramatically inhibited TLR7 natural agonist (imiquimod) activity while SG65 SWNTs and CB had no significant impact on this receptor. In addition, we found that SWNTs promoted the infectivity of influenza A virus H1N1 (IAV) in lung cells. Utilization of near-infrared fluorescence (NIRF) suggests that SWNTs may not influence viral infectivity through strong direct interactions with the virus, but instead modulate genes important to viral replication. Overall, our study is the first to report modulation of TLRs activity and IAV infectivity by SWNTs. The present research lays a foundation for better comprehending the potential health impact of nanomaterials and provides data imperative for evaluating their safety.

# 21. EFFECTIVENESS OF COMMON HEALTHCARE DISINFECTANTS AGAINST H1N1 INFLUENZA VIRUS ON REUSABLE ELASTOMERIC RESPIRATORS

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**OBJECTIVE**. To determine the efficacy of commonly used hospital disinfectants to inactivate influenza virus on elastomeric respirators. **METHODS**. A section of elastomeric respirator was inoculated with 2009 H1N1 influenza virus and allowed to dry. The surface was then disinfected with one of three disinfectant wipes. Positive control respirators were inoculated but not wiped. Negative control respirators were inoculated with sterile viral culture media. Plaque and polymerase chain reaction (PCR) assays were performed on swab samples obtained from the inoculated surface. **RESULTS**. Positive controls showed recovery of 14 to 73 plaque-forming units (pfu) of virus while PCR was positive at 28 to 29 PCR cycles. Influenza virus could not be grown from any respirator treated with either a quaternary ammonium/isopropyl alcohol or bleach-detergent impregnated wipe but 12.5% and 62.5% were positive by PCR, respectively. However, influenza virus grew in cell culture from 75% of the respirators treated with 70% isopropyl alcohol; 83% were positive by PCR. **CONCLUSIONS**. Quaternary ammonium/isopropyl alcohol and bleach-detergent wipes effectively inactivate influenza on the surface of elastomeric respirator material while 70% isopropyl alcohol alone was ineffective.

## 22. A Prospective Study of Romanian Agriculture Workers for Zoonotic Influenza Infections

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**Background**: In this prospective study we sought to examine seroepidemiological evidence for zoonotic influenza virus infection among Romanian agricultural workers. Methods: Sera were drawn upon enrollment (2009) and again at 12 and 24 months from 312 adult agriculture workers and 51 age-group matched controls. Participants were contacted monthly for 24 months and queried regarding episodes of acute influenza-like illnesses (ILI). Cohort members confirmed to have acute influenza A infections permitted respiratory swab collections which were studied with rRT-PCR for influenza A. Serologic assays were performed against 9 avian, 3 swine, and 3 human influenza viruses. **Results**: During the two-year follow-up, a total of 23 ILI events were reported. Two cases were identified as influenza A by rRT-PCR: both were subtyped as human H3N2. During the follow-up period, three individuals experienced elevated microneutralization antibody titers ≥1:80 against three (one each) avian influenza viruses: A/Teal/Hong Kong/w312/97(H6N1), A/Hong Kong/1073/1999(H9N2) or A/Duck/Alberta/60/1976(H12N5). However, none of these

participants met the criteria for poultry exposure. A number of subjects demonstrated 4-fold increases over time in hemagglutination inhibition (HI) assay titers for at least one of the 3 swine influenza viruses (SIVs) during the follow-up periods. However, only the elevated antibody titers against A/Swine/Flanders/1/1998(H3N2) seemed not to be confounded by antibody against human influenza viruses or vaccines. In examining risk factors for elevated antibody against this SIV, swine exposure (adjusted OR=1.8, 95% CI 1.1-2.8) and tobacco use (adjusted OR=1.8; 95% CI 1.08-2.9) were important predictors. Conclusions: While Romania has recently experienced multiple incursions of HPAI among domestic poultry, this cohort of Romanian agriculture workers had sparse evidence of avian influenza virus infections. In contrast, there was evidence, especially among the swine exposed participants, of previous infections with a swine H3N2 virus.

# 23. LITTLE EVIDENCE OF HUMAN INFECTION WITH EQUINE INFLUENZA DURING THE 2007 EPIZOOTIC, QUEENSLAND AUSTRALIA

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**Background**. Equine influenza virus (EIV) is considered enzootic in Europe (except Iceland), Asia, North Africa, and North and South America. When EIV outbreaks occur they may severely impact the equine and tourist industries. Australia faced its first EIV outbreak beginning in August of 2007. The outbreak was concentrated in New South Wales and Queensland, with more than 1400 confirmed EIV infections in horses during the first month. Rapid response from the equine industry and the federal government was successful and Australia was declared free from EIV by the end of 2007. **Objectives**. This cross-sectional study was designed to examine associations between exposure to EIVinfected horses and evidence of EIV infection in humans. Study **Design**. Employing informed consent, during the period October 2007 to April 2008, 100 subjects (89 with horse exposures and 11 non-exposed) were enrolled during equine events. All subjects provided a blood sample and were asked to complete an online questionnaire including health history, animal exposure and demographic information. Sera samples were tested for the presence of antibodies against two H3N8 EIV strains using microneutralization, hemagglutination inhibition, and enzymelinked lectin assays. Results. Evidence for H3N8 infection was sparse, with only 9 study participants having any evidence of H3N8 infection and the seroreactivity seen was low and easily explained by cross-reactions against human influenza strains or vaccines. **Conclusions**. These data provide little evidence to support the premise that EIV infections occurred among humans exposed to EIV-infected horses during the 2007 Australian epizootic.

# 24. VACCINE COVERAGE GAP IN A SCHOOL-LOCATED INFLUENZA VACCINATION PROGRAM: REACHING THE THEORETICAL MAXIMUM PARTICIPATION RATE

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Background: The Alachua County School-Located Influenza Vaccination (SLIV) Program offers free live attenuated influenza vaccine (LAIV) to students K-12. The program's goal is to immunize 70% of school aged children, thus providing community-wide protection from influenza as predicted by computer models. In 2013, the overall immunization rate in Alachua County public schools was 41.16%. Since some students are medically ineligible to receive LAIV due to contraindications (i.e., asthma, egg allergy, chronic medical conditions), the maximum achievable participation rate consists only of individuals that are medically eligible. This study aims to determine the vaccine coverage gap, defined as the difference in the maximum achievable and the current participation rate, in order to develop strategies and interventions to increase program uptake. **Methods**: Three schools were selected for study: one elementary with a 100% Consent Form Return Rate (CFRR). one middle, and one high. No middle or high schools obtained a 100% CFRR; instead, all classrooms with 100% CFRR were included. Alachua County Schools provided the contraindications list for review. The CFRR ("Yes"+"No" consent forms/total population) and the proportion of students intending to participate ("Yes" consent forms/total population) were calculated. The immunization rate (IR) is the actual proportion of students vaccinated. **Results**: In 2013, the IR for elementary, middle, and high schools in Alachua County was 49.22%, 42.94%,

and 27.00% respectively. The CFRR for elementary, middle, and high schools was 83.01%, 73.44%, and 49.92%. Sixty five percent of students intended to participate in the sample elementary school. Contraindications were present in 5.12% (n=24) of students. The vaccine coverage gap was 30.70% (n=144). In the middle school, 60% intended to participate. Contraindications were present in 6.47% (n=11). The vaccine coverage gap was 32.94% (n=56). In the high school, 46% intended to participate. Contraindications were present in 11.98% (n=43). The vaccine coverage gap was 40.66% (n=146). Contraindication rates were low (5-12%), indicating that an additional 30-40% of the population could participate in the program. Within our population, the vaccine coverage gap was largest in high schools. This study was not able to determine precise reasons for nonparticipation. **Conclusions**: Reaching the theoretical maximum participation rate for SLIV programs is of public health importance because it will provide the most protection from influenza for communities. A significant proportion of students who are medically eligible for the SLIV program do not participate. Concerted effort will be required from schools and communities to reduce this vaccine coverage gap.

### 25. IN VITRO MODELING REVEALS THAT HUMAN CORONAVIRUS NL63 HAS A TROPISM FOR HUMAN KIDNEY CELLS

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Human coronaviruses are common causes of acute nasal nasopharyngitis (the common cold). They are thus considered respiratory viruses, and kidney impairment has not reported in descriptions of illnesses by these viruses. Human coronavirus NL63 (HCoV-NL63) was identified as one of three different adventitious agents in a specific batch of primary human renal proximal tubule epithelial cells (RPTEC) that had been purchased from a commercial source. In vitro modeling reveals that HCoV-NL63 replicates to high titers in primary human RPTEC and in renal epithelial cells (HREC), but not in renal cortical epithelial cells (HRCEC). These findings indicate that some human cells are fully permissive for HCoV-NL63. More recently, Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and Sudden Acute Respiratory Syndrome Coronavirus (SARS-CoV), which are zoonotic coronaviruses that are capable of causing fatal illnesses in humans, have been shown to affect both lung and kidney cells. Both SARS-CoV and HCoV-NL63 use angiotensin-converting enzyme 2 (ACE2) as a viral receptor, and ACE2 is expressed in kidneys. These findings raise the question whether HCoV-NL63 affects more than just lung cells when it causes illness in humans.

### 26. ASSESSMENT OF SCHOOL ABSENTEEISM AS A METRIC FOR INFLUENZA ACTIVITY AND VACCINE EFFECTIVENESS

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Introduction: Influenza transmission studies show that school children are disseminators of influenza in a community, due to higher susceptibility for infection and numerous opportunities for congregation within their environments. One-way to monitor influenza among the general population is by using absenteeism surveillance data as a tool to track influenza activity within the student population. The current uses of absenteeism data have been largely inconsistent, with varying methodological approaches. To help us better understand the full utility of absenteeism as a potential source for influenza surveillance, we conducted a thorough literature review to establish the different methodologies and conclude which methodologies are most effective in tracking influenza activity. Methods: A PubMed search was used to was used to conduct a systematic literature review using "influenza + Absenteeism" as search terms. Articles meeting the inclusion criteria: student absenteeism in public schools and absenteeism rate as the dependent variable with a comparison to influenza activity, were included in the analysis. Two independent reviewers evaluated each article using the following metrics: the method of data collection and analysis, as well as the findings. **Results**: Of the 409 papers found from PubMed as of October 1, 2013, 53 met the initial inclusion criteria. Following further review, 17 papers were included in the final analysis. The data suggests three methodological themes persist among the literature: comparing influenza activity to absenteeism rates by employing a threshold indicator method, a significant increase method, or a proportion of students absent. This was accomplished either by comparing the method to current influenza activity indices, such as lab reported data, or by comparing influenza and non-influenza portions of the year studied. Nine of the studies showed a significant result indicating absenteeism's potential utility as an influenza surveillance tool.

### 27. SCHOOL LOCATED INFLUENZA VACCINATION PROGRAMS IMPACT ON PRIVATE PEDIATRIC PRACTICES' VACCINE UPTAKE

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**Background**: In the United States influenza is responsible for more than 200, 000 hospitalizations and an additional 36, 000 deaths per year. School Located Influenza Vaccine (SLIV) programs can aid in the control of influenza in a community, but despite the potential benefits SLIV program have not been fully endorsed. There are concerns that SLIV programs could have a negative economic impact on private pediatric practices, by reducing the number of children immunized for influenza in the offices. We investigated whether there was an effect on vaccine uptake in private pediatrician's offices in Alachua County, Florida where there is a well-established SLIV program Methods: The total number of influenza vaccines administered in all pediatricians' private practices in Alachua County, Florida for the past five years (2009-2013) were obtained from Florida SHOTS (State Health Online Tracking System). This included both trivalent inactivated influenza vaccine (TIV) and live attenuated influenza vaccine (LAIV). The amount of vaccine administered was compared year to year, and the rate of change was calculated for LAIV, TIV, and total amount of vaccine administered. Results: From 2009-2013, the amount of total vaccine administered in pediatricians office increased by 80.52%, TIV increased by 79.33%, and LAIV increased by 81.69%. **Conclusion**: The SLIV

program did not have a negative impact on vaccine administered in private pediatric practices'. Vaccine uptake actually increased over time. The presence of an SLIV program in a given community may promote awareness of influenza and thus encourage more children to receive influenza vaccine both in school and at their pediatrician's practice.

### 28. DETECTION AND ISOLATION OF AIRBORNE INFLUENZA A H3N2 VIRUS USING A SIOUTAS PERSONAL CASCADE IMPACTOR SAMPLER

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The air we breathe contains microorganisms that can cause infectious respiratory diseases. After two occupants of an apartment were diagnosed with influenza in February of 2013, efforts were made to detect and isolate airborne influenza virus using two different types of active air samplers: a Sioutas Personal Cascade Impactor Sampler (PCIS) and an SKC BioSampler. The PCIS collects size-fractionated particles by impaction on polytetrafluoroethylene filters, whereas the SKC BioSampler collects airborne particles in liquid media. Influenza H3N2 virus was collected by both types of air samplers. The PCIS collected a range of particle sizes containing influenza virus near one of the sick individuals but only ultrafine particles when the samplers were positioned farther away. Viable virus was present in the liquid collection media of the SKC BioSampler and some PCIS filters. These findings suggest that influenza patients produce ultrafine aerosol particles that contain viable virus.

### 29. MODELING SARS OUTBREAKS IN CHINA: COMBINING ANALYSIS OF HOSPITAL AND NATIONAL SCALE ONSET REPORT DATA

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We analyzed SARS (severe acute respiratory syndrome) outbreaks across mainland China, with onset report data from two hospitals in Beijing and Tianjin, as well as patient records on the national scale. To quantify disease transmissibility in hospital setting, and to estimate the effectiveness of control measures, we first applied chain-binomial models with homogeneous mixing within, but heterogeneous mixing across hospital departments. We found that transmission rates with hospital departments were much higher than that across departments, and that hospital workers were nearly as twice susceptible as in-hospital patients. The outbreaks in the two hospitals were contained by quarantine and other protective measures such as wearing gloves and masks, and the scale could have been much smaller if diagnosis of the index cases had not been delayed and intervention had been consistently implemented during early stage. The understandings from hospital SARS outbreaks data, including assumptions on disease natural history, were then applied to modeling national report data. We fitted a Poisson model with temporal-spatial pattern on the county level to estimate the R0 (basic reproduction number) of SARS on the national scale. The estimated R0 was smaller than that from hospital outbreak data, due to different contact rates, various mixing pattern, and less influential superspreading events.

### 30. MULTILOCUS SUPPORT FOR A CENTRAL MEXICO CENTER OF ORIGIN FOR THE POTATO LATE BLIGHT PATHOGEN, PHYTOPHTHORA INFESTANS

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Phytophthora infestans is a destructive plant pathogen best known for the disease that caused the Irish potato famine and continues to be the most costly potato pathogen worldwide. Identification of its elusive center of origin is critical to understanding the mechanisms of repeated global emergence of this pathogen. There are two competing hypotheses on the evolutionary origin of the pathogen. There are two competing theories placing the origin in either South America or in central Mexico. Both regions are centers of diversity of Solanum host plants. Genetic data have been used to support each of these positions. To test these competing hypotheses, we conducted phylogeographic and approximate Bayesian computation analyses, which are approaches suitable to unraveling complex demographic histories. We sequenced four nuclear genes in isolates sampled from populations in the Andes, Mexico, and elsewhere. To infer the ancestral state, we included the closest known relatives P. phaseoli, P. mirabilis, P. ipomoeae, as well as the interspecific hybrid P. andina. We obtained decisive support for a central Mexican origin of P. infestans that is congruent with its natural history. Our findings support the hypothesis that populations found in the Andes are descendants of the Mexican populations and reconcile previous findings of ancestral variation in the Andes. While centers of origin are well documented as centers of evolution and diversity for numerous crop plants, P.

infestans is a rare example of a plant pathogen with a known geographic origin. This work has important implications for our understanding of the coevolution of hosts and pathogens, and harnessing of plant disease resistance to manage late blight.

### 31. PRELIMINARY GENOMIC ANALYSIS OF PYTHIUM INSIDIOSUM, THE ONLY MAMMALIAN PATHOGEN IN A GENUS OF PLANT PATHOGENS

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Pythiosis is a deadly disease of horses and dogs in tropical and subtropical regions, including Florida and the southeast United States. It also affects humans in Southeast Asia and is considered a emerging human pathogen in the United States. This disease is caused by Pythium insidiosum, a fungal-like organism that is the only mammalian pathogen in a genus of plant pathogens. Very few fungi are known to be pathogens of both plants and animals and it is striking that most or all trans-kingdom fungal pathogens are also emerging pathogens of humans. Our research addresses the evolutionary mechanisms of this domestic mammal and emerging human pathogen using genomic data. Our specific aim is to identify genes involved in mammalian pathogenesis and examine their evolutionary origin. We hypothesize that P. insidiosum has acquired a novel set of genes (e.g., through

horizontal transfer) or reprogrammed a subset of ancestrallypresent genes that are responsible for its ability to infect
mammals. As a first step towards testing this hypothesis, we have
generated a draft genome for an isolate of P. insidiosum
originating from lesions on the mouth and eye of a 2-year-old boy.
Genomic DNA was isolated and used to prepare a sequencing
library using Illumina's TruSeq sample preparation reagents that
was run using the MiSeq instrument. The trimmed and quality
filtered reads were assembled using Velvet in conjunction with
the VelvetOptimiser tool. The assembled genome will be
annotated using MAKER. We will conduct comparative genomic
analyses using published data that will include six other Pythium
species as well as distantly related human pathogens to obtain
insight into genes that underlie pathogenicity.

## 32. PORPHYROMONAS EVADES UBIQUITINATION-NDP52/P62 MACHINERY AND INDUCES AUTOPHAGOSOME-LIKE VACUOLES FOR 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, detailed characterization of the intracellular fate of P. gingivalis in GECs has yet to be fully elucidated. Objective: 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. Method: Primary GECs were infected with wild-type-strain or FMN-greenfluorescent-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. Result: Concordant with our recent findings, confocal analyses displayed steady-state level of co-localizations between P. gingivalis and ER (~90%) over 24-hours, whereas less than 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 ubiquitinlysosomal-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.

#### 33. COMPARISON OF AVOCADO, SWAMPBAY, AND CAMPHOR AS HOSTS OF RAFFAELEA LAURICOLA

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Raffaelea lauricola, a fungal symbiont of the Southeast Asian ambrosia beetle Xyleborus glabratus, causes laurel wilt in members of the Lauraceae plant family. North American species such as avocado (Persea americana) and swamp bay (Persea palustris) are particularly susceptible and have experienced widespread mortality. In contrast, camphor (Cinnamomum camphora ), an Asian Lauraceous tree, is relatively tolerant as infected trees develop localized symptoms or remain asymptomatic. After inoculation, R. lauricola systemically colonizes, and multiplies in, host tree xylem. To determine whether disease development is related to the extent and rate of pathogen colonization, a gfp-labelled strain of R. lauricola was generated and used to inoculate avocado, swamp bay and camphor. Trees were harvested 3, 10 and 30 days after inoculation (DAI) and disease severity was rated on a 1-10 scale. Cross sections from 5, 20, 50, and 100 cm above the inoculation point were plated on a semi-selective medium and the rate of colonization was calculated in mm/day. The extent of xylem colonization was determined with fluorescence microscopy as the percentage of xylem lumena that were colonized by the pathogen.

Internal symptoms were observed 3 DAI in avocado and external symptoms appeared 10 DAI in avocado and swamp bay. By 30 DAI, severe disease developed in these hosts, but no wilting and only slight defoliation was observed in camphor. Avocado and swamp bay had higher disease ratings than camphor 30 DAI (6.8) and 5.5 vs. 2.3, p<0.003), but colonization differed only between avocado and camphor (1% vs. 0.1%, p<0.02). Colonization in swamp bay (0.4%) did not differ significantly from either avocado or camphor. Notably, the highest disease ratings occurred in trees with 1.2-1.6% xylem colonization. There were no differences among species in the rates of colonization. In summary, resistance to laurel wilt was associated with lower levels of xylem colonization in camphor vs avocado and swamp bay; however, little colonization was observed in severely affected trees. Although wilting was previously related to tylose formation in laurel wilt-affected avocado, it is not clear how such a minor presence by the pathogen would induce these changes in susceptible hosts or how propagation of R. lauricola is minimized in a tolerant host such as camphor.

## 34. MALARIA INDICATOR SURVEYS DEMONSTRATE A MARKEDLY LOWER PREVALENCE OF MALARIA IN LARGE CITIES OF SUB-SAHARAN AFRICA

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Africa is rapidly urbanizing, with one in eight sub-Saharan Africans now living in a large city (pop. >750, 000). Although malaria transmission is known to be reduced in urban as compared to rural settings, public health and clinical decision makers often take a "one-size-fits-all" approach. Decision makers require additional evidence regarding the burden of malaria in large cities. Applying methods developed in the 2013 Ghana

Urban Malaria Study, we analyzed existing data from nationally representative household surveys which had measured malaria parasitemia by microscopy among children six to 59 months of age in 15 countries of sub-Saharan Africa since 2010. Geocoordinates for each survey cluster were used to determine distance to the center of the large cities. Each survey cluster was then classified as rural, large city, or "other urban." Rural designations were retained from the individual surveys (typically <5000 pop). For urban clusters within 25 km of the center of a large city, Google Earth© satellite imagery was used to determine whether they fell within the city, according to pre-set criteria. Data from all clusters within large city boundaries were pooled and compared to rural cluster within 150 km of the city center. In the case of two countries for which survey geo-coordinates were not available, clusters located in large cities were identified based upon their designated district. In 19 of 20 large cities, from 0 to 7.4% of children were found to be parasitemic. Apart from Antananarivo, Madagascar, where both the large city and the comparison rural communities were parasite-free, the prevalence in each of the large cities was 0 to 40% of that found among children living in rural communities within 150 km of these cities or within the same zone of malaria endemicity. For example, prevalence in Lagos, Nigeria, was 1.5% while outlying rural areas were 50.0%. In 14 of the 20 large cities, all of the children living in 75% or more of the clusters were malaria parasite-free. The positive predictive value of reported fever was consistently lower in urban areas. We concluded that existing data from malaria indicator surveys can be used to document the substantially lower prevalence of malaria in specific large cities. These findings will help policy makers, programmers, and clinical workers to improve management of febrile illness, adjust malaria control priorities, and strengthen monitoring and research, with an eye on the contrasting needs of urban vs rural populations.

### 35. LEVOFLOXACIN DOSE OPTIMIZATION FOR MULTIDRUG RESISTANT TUBERCULOSIS BASED ON LUNG MICRODIALYSIS IN PATIENTS

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The World Health Organization estimated 500, 000 new multidrug-resistant tuberculosis (MDR-TB) cases in 2011. Around 9% of MDR-TB cases also present resistance to fluoroquinolones and one of the injectable agents; the so-called extensively drug-resistant tuberculosis. We hypothesize that the concentrations of levofloxacin (LEVO), a main fluoroquinolone for the treatment of drug resistant TB, are lower inside tuberculous lesions as compared to serum and that the current dosage regimen is not optimal. This favors the development and amplification of resistance, leading to treatment failure. Therefore our project includes 3 parts: (1) developing methods in support of a clinical trial to evaluate LEVO pharmacokinetics in MDR-TB patients, and (2) developing an in vitro system to evaluate the drug effect. Further, (3) the data generated will be evaluated using modeling and simulation for dose optimization. A pilot clinical study was

performed to evaluate the penetration of LEVO inside the tuberculous cavity with 3 patients. The trial was conducted with MDR-TB patients undergoing resectional lung surgery in the Republic of Georgia using microdialysis (MD). Patients received either 750 or 1000 mg of LEVO orally. Samples were collected from the resected pulmonary tissue using MD and serum samples were collected for pharmacokinetics analysis. All patients showed low drug absorption. The Cmax values were 1.1 and  $4.0 \mu g/mL$ for both patients on 750 mg doses, and 5.9 µg/mL for the patient on 1000mg dose, whereas the expected Cmax ranges from 8-12μg/mL. The observed half-lives ranged from 4.6 to 5.9 hours. The free drug concentration in the tissue ranged from 0.46 to 9.59 µg/mL. To evaluate the pharmacodynamics (PD) of LEVO against the microorganism, an in vitro system containing Mycobacterium tuberculosis (ATCC 25177) exposed to LEVO human concentration profile was set. Multiples of LEVO minimum inhibitory concentration (MIC) values were added daily and samples were collected and plated. Time-kill curves were obtained by plotting the change in the colony forming unit per mL (CFU/mL) over the time. Multiples of MIC doses currently are being tested to evaluate the kill behavior. The PD information will be correlated with the PK behavior in order to establish a PK/PD model. Using this model it will be possible to simulate and predict the LEVO doses that optimize treatment. This study may be the foundation for the optimization of the disease treatment, benefiting patients that cannot be cured by the first line treatment and curbing the growth of drug resistance.

#### 36. BIOFILM FORMATION BY MYCOBACTERIUM ABSCESSUS WITHIN A LUNG CAVITY

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Lung infection with Mycobacterium abscessus is an emerging infectious disease that is considered incurable. We present a novel finding that may help explain the poor outcomes with treatment. A 55 year old man with chronic obstructive pulmonary disease was referred to UF for a productive cough, progressive shortness of breath and fatigue. Chest imaging demonstrated a 4.4 cm cavitary lesion in the right upper lobe, emphysematous changes, bronchiectasis and scattered nodules. Sputum cultures had grown M. abscessus. He initially improved after daily treatment with azithromycin orally and two intravenous drugs: amikacin and imipenem-cilastin. However, 2 months later he developed fevers, night sweats, and more shortness of breath. Chest imaging showed changes consistent with a hypersensitivity alveolitis due to a spill of the cavity. He was treated with corticosteroids and the antimycobacterial regimen was intensified by the addition of linezolid. Chest imaging two months later showed resolution of the ground glass opacities and decreased nodules but persistence of the lung cavity. The right upper lobe was surgically resected to prevent further spills and to offer him the best chance of cure. Gross examination of the specimen identified a cavitary lesion, 4 x 3 x 3 cm, containing hemorrhagic and necrotic luminal content. Microscopic examination revealed confluent necrotizing granulomas with central cavity formation and numerous colonies of acid-fast bacilli. Microbiology was notable for growth of M. abscessus within 3 days. Scanning electron microscopy of the cavity wall

demonstrated bacilli embedded in a matrix typical of biofilms. There were no bacilli observed in a remote sample of the lung. Specialized assays detected high levels of total bacteria (7.17x107 CFU) in the lung cavity, of which 7.17x105 CFU were protected in a functional biofilm and included MRSA, streptococcal species, anaerobic bacterial species, and fungi species. In contrast, no biofilm bacteria (0 CFU) and essentially no planktonic bacteria (7 CFU) were detected in the sample from a remote portion of the lung. This is the first report documenting the existence of biofilm within a lung cavity in a human patient. Bacteria in biofilm are usually more resistant to antibacterial therapy than individual 'planktonic' bacteria, as used in drug susceptibility tests. This may explain why M abscessus lung infections are usually incurable with medication alone. The presence of biofilm in this cavity suggests that there may be a role for the use of biofilm dispersants as adjunctive therapy in the future.

# 37. PHARMACOKINETICS OF A SINGLE ORAL OR RECTAL DOSE OF CONCURRENTLY ADMINISTERED ISONIAZID, RIFAMPIN, PYRAZINAMIDE, AND ETHAMBUTOL

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**Background**: Tuberculosis(TB), caused by Mycobacterium tuberculosis, is a concern in captive Asian elephants (Elephas maximus). Treatment of TB in elephants utilizes multidrug protocols including isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and/or ethambutol (EMB). **Methods**: In this study, a single

combined dose of INH 5 mg/kg, RIF 10 mg/kg, PZA30 mg/kg and EMB 30 mg/kg was administered orally to six Asian elephants, and a single combined dose of INH, RIF, and PZA was administered rectally to five elephants. Blood samples were collected at 14time points. PZA and EMB concentrations were determined using validated gas chromatography assays. INH and RIF concentrations were determined using validated high performance liquid chromatography assays. Results: Rectal INH produced an earlier Tmax than expected. Oral INH produced comparatively lower Cmax but higher AUC values. Oral RIF and oral EMB were well-absorbed, with RIF Cmax values approaching the human therapeutic range and EMB Cmax values at the upper end of the range. Rectal RIF was not absorbed. Rectal PZA produced faster but lower median Cmax values, at the low end of the typical human therapeutic range. Oral PZA produced comparatively higher Cmax and higher AUC values. **Conclusion**: Results of this study indicate that currently recommended monitoring times for rectal INH and oral RIF do not provide an accurate assessment of PK parameters for these drugs. This study demonstrates notable individual variability, indicating that dosing of these medications requires individual elephant monitoring and adjustment. This study provides an update to previous pharmacokinetic studies of these medications in Asian elephants.

### **38.** CLINICAL PHARMACOKINETICS OF PYRAZINAMIDE IN PATIENTS WITH TUBERCULOSIS

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**Objectives**: The treatment of active tuberculosis (TB) requires multiple drugs and a treatment duration of at least 6 months. The

standard regimen for drug-susceptible TB consists of rifampin, isoniazid, pyrazinamide (PZA), and ethambutol. The use of PZA during the first two months of TB treatment allows for the shortening of treatment from nine to six months. Recent evidence suggests that PZA activity is dose dependent and that maximum concentration (Cmax) values > 35 ug/ml are associated with better efficacy. Higher doses than those currently recommended in the U.S.A. (25 mg/kg) might be needed to achieve this Cmax target. The goal of this study was to determine the proportion of patients with a Cmax > 35ug/ml and to estimate the pharmacokinetics (PK) of PZA among patients enrolled in Tuberculosis Trial Consortium (TBTC) trials 27 and 28. Methods: The data are from PK sub-studies of TBTC Studies 27 and 28. In both studies, PZA was dosed at approximately 25 mg/kg daily, rounded to the nearest 500 mg. Seventy-two patients participated in the sub-studies. Blood was collected pre-dose then 1, 2, 6, 8, 12 and 24 hours post dose. Noncompartmental analysis (NCA) was performed with WinNonlin Professional Version 4.0. Population PK analysis was analyzed using nonlinear mixed effect modeling software, Monolix version 3.2. Results: NCA: The median dose patients received in the two studies was 1500 mg. The median (interquartile range) for Cmax and time to maximum concentration (Tmax) were 29.77 (9.16) ug/ml and 1 (1) hour respectively. Only 18 patients (25%) had Cmax >35 ug/ml and females in general had a higher Cmax compared to men. The remaining parameters estimated from the NCA analysis are shown below. Population PK Analysis: A one-compartment model with first order absorption and linear elimination best described PZA PK.. Residual variability was described using a combined error model. The median population estimates for total body clearance (Cl/F) and volume of distribution (V/F) were 4.42 L/hr and 47 L respectively. Significant covariates influencing PZA's PK were body weight and sex.. Women had a lower V/F compared to men, and both Cl/F and V/F increased with body weight. Parameters estimated from the Population model are shown

below. **Conclusions**: Higher doses of PZA than those currently will be required to achieve the proposed Cmax target of 35 ug/ml. Sex and weight both significantly affected PZA pharmacokinetics, suggesting that weight-based dosing for this drug is appropriate.

### 39. A COST-EFFECTIVENESS ANALYSIS OF VACCINATION STRATEGIES TO REDUCE THE BURDEN OF JAPANESE ENCEPHALITIS IN BANGLADESH

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Japanese encephalitis virus (JEV) is a zoonotic, mosquito-borne Flavivirus that circulates among Ardeid birds and can be amplified by pigs. Despite the availability of safe and effective vaccines, Japanese encephalitis (JE) causes an estimated 67, 900 human cases each year worldwide and has recently been recognized as an important cause of acute encephalitis and long-term neurological sequelae in Bangladesh. To inform decision-making for future vaccine introduction, we evaluated the cost-effectiveness of human vaccination in three regions of Bangladesh where population-based incidence estimates are available: Rajshahi, Khulna, and Chittagong. We compared the status quo

(no vaccination) to a feasible intervention: routine childhood vaccination through incorporation of the SA-14-14 2 vaccine into the Expanded Programme on Immunization. We also assessed the cost-effectiveness of annual vaccination of domestic pigs to reduce human cases in Rajshahi, where detailed data on pig populations are available. We conducted a 2-way uncertainty analysis of the pig vaccination model because the proportional contribution of pigs to human cases and costs associated with implementation of pig vaccination are unknown. Interventions were considered very cost-effective (VCE) below a threshold equivalent to the per capita GDP (\$818) per disability adjusted life year (DALY) averted and cost effective (CE) below a threshold of 3X the per capita GDP (\$2, 454). Routine childhood vaccination is expected to be VCE, with a cost per DALY averted of \$126 in Rajshahi, \$246 in Khulna, and \$745 in Chittagong. Probabilistic sensitivity analysis indicates that routine childhood vaccination has >99% probability of being CE in Rajshahi and Khulna and 92% probability of being CE in Chittagong. Childhood vaccination is likely to be VCE in Rajshahi (96%) and Khulna (89%). Vaccination of domestic pigs in Rajshahi would be cost-effective relative to both the status quo and childhood vaccination for all scenarios where pigs are responsible for at least 35% of human cases and the cost per pig vaccinated is <\$10. With a higher contribution of pigs to transmission, vaccination of domestic pigs would be cost effective at much higher costs per dose. Our findings suggest that including IEV vaccine into the EPI program would be a cost-effective approach to reduce death and disability from JE in Bangladesh, particularly in Rajshahi and Khulna. The contribution of pigs to human IE in Bangladesh should be quantified to determine if pig vaccination could also be a costeffective public health strategy. This poster was previously presented at the ASTMH annual meeting (November 2013, Washington, DC).

### **40.** PILOT MOSQUITO SURVEILLANCE STUDY WITHIN EVERGLADES NATIONAL PARK

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Everglades National Park (EVER), located in the southwestern tip of Florida, spans more than 1.5 million acres and hosts roughly one million visitors annually. Over the next few years, EVER habitats will be affected by the Comprehensive Everglades Restoration Plan which aims to redirect water flows through two major EVER water bodies: Shark River and Taylor Slough. As water flow and distribution changes, habitats suitable for many mosquito species believed to inhabit the park will change as well. In order to capture baseline data regarding current mosquito species distribution and abundance throughout the park, including the presence of potential vectors for human and animal viruses, we undertook a park-wide mosquito surveillance pilot study. From June 12 to August 20, 2013, we set more than 50 standard CDC light traps baited with carbon dioxide in five distinct habitats (mangrove, cypress, sawgrass, pineland, hardwood hammock) within EVER, as well as two housing sites. A portion of mosquitoes from each trap was identified to species. The trap sample proportions were used to estimate the total number of mosquitoes within each trap. A total of 29 species in 10 genera were collected, with the predominate species collected being Culex nigripalpus and Aedes taeniorhynchus. Statistical analyses were performed to determine the effects of habitat and time on overall mosquito abundance as well as individual species abundance. Preliminary analyses of the mosquito population data show that both time and habitat play a significant role in overall mosquito abundance within the park. For Aedes taeniorhynchus both time and habitat are significantly associated with species abundance. For Culex nigripalpus and Culex cedecei, abundance is significantly associated with habitat, but not with time.

# 41. MOLECULAR DETECTION OF EHRLICHIA SPP. AND RICKETTSIA SP. IN HOST-SEEKING TICKS AND WHITE-TAILED DEER BY 3-DYE MULTIPLEX QPCR

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Amblyomma americanum (the lone star tick, LST) is an aggressive host-seeking tick that is quickly expanding its geographic range in the United States. Despite being considered a nuisance specie until the 1990's, the tick is known to be the vector for a number of human and animal pathogens including Ehrlichia chaffeensis, Ehrlichia ewingii and the putatively nonpathogenic, Rickettsia amblyommii. To determine the prevalence of these organisms in host-seeking A. americanum in Florida, approximately 1, 500 ticks were collected and screened using a multiplex, quantitative 3-dye PCR. Additionally, white-tailed deer (Odocoileus virginianus) from forty-five counties across the state of Florida were surveyed for the presence of

antibodies against Ehrlichia spp., Borrelia burgdorferi and Anaplasma phagocytophilum. When available, whole blood samples from deer were also tested by PCR. In lone star ticks, 14.6%, 15.6% and 57.1% had detectable levels of E. chaffeensis, E. ewingii and Rickettsia spp., respectively. Infecting Rickettsia was 98-100% identical to Candidatus Rickettsia amblyommii in all Rickettsia sp. positive LST's. Approximately 45% of 393 deer surveyed had detectable antibodies to Ehrlichia spp. Three had antibodies to Borrelia burgdorferi (<1%) and none had antibodies to Anaplasma phagocytophilum. In the deer, 7.3% were positive for E. chaffeensis, 6.0% were positive for E. ewingii and 7.9% were positive for Rickettsia spp. (>10 copies detected). In conclusion, the SFG Rickettsia, Rickettsia amblyommii is highly prevalent in host-seeking LST's in central Florida and was detectable in a mammalian host by PCR. White-tailed deer from this area had a seroprevalence of nearly 50% to Ehrlichia spp. The molecular and serological evidence of these organisms in LST's and in deer, underscores the potential of exposure to these agents for people living in central Florida.

### 42. MOLECULAR EVIDENCE OF BORRELIA SPP. IN HOST-SEEKING TICKS AND WHITE-TAILED DEER IN NORTH-CENTRAL FLORIDA

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Lyme borreliosis is the most frequently reported vector-borne disease in the U.S. caused by many species within the Borrelia burgdorferi sensu lato genogroup. These pathogens are maintained in nature through a cycle involving various rodent species and Odocoileus virginianus, or the white-tailed deer (WTD). Those at risk are humans, dogs, horses, cattle, and other animals. Increasing numbers of cases are being reported and diagnosed annually. The geographic distribution includes areas in the southeast U.S., which are not typically considered endemic for Lyme disease. Amblyomma americanum, or the lone star tick (LST), is the most common human biting tick in the South. Tick bites from Ixodes scapularis (deer ticks), the vector for Borrelia burgdorferi, are rarely reported. This raises many questions regarding the causative agent(s) of Lyme-like illness in parts of the U.S. inhabited by the LST. In this study, 740 nymphal LSTs and 310 adult LSTs were collected from three state parks located in north-central FL (NC FL), and analyzed for the presence of Borrelia spp. One hundred banked samples of gDNA from deer ticks and 57 banked samples of gDNA from the whole blood of WTD were also analyzed. All samples were tested using a nested PCR assay. Amplicons were then sequenced and analyzed using BLAST and ClustalW. Sequence analysis was performed on positive samples. Overall, of those confirmed by sequencing, 13/74 nymphal pools (17.6%) and 5/310 individual adult ticks (1.6%), all amplicons were 95-99% identical to Borrelia lonestari. Borrelia spp. could not be determined in an additional 1.6% of the adult tick amplicons submitted for sequencing. No Borrelia spp. were detected in any deer tick or WTD samples. The findings overall indicate that in NC FL, and possibly other areas where the LST is the predominant tick species, the potential for exposure to B. lonestari is significantly greater than the risk of exposure to B. burgdorferi. These results necessitate further investigation and consideration that Lyme-like illness may be caused by infection with different Borrelia spp. that may go undetected using current methods designed to diagnose Lyme disease.

### **43.** ENTOMOLOGICAL INVESTIGATIONS FOR UNDERSTANDING JAPANESE ENCEPHALITIS VIRUS TRANSMISSION DYNAMICS: LESSONS FROM BANGLADESH

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Understanding pathogen transmission dynamics is imperative for identifying and implementing cost effective interventions for optimal impact. One of the first steps toward understanding transmission dynamics of mosquito-borne zoonoses is to identify the host and vector species necessary for maintaining, amplifying and bridging transmission to humans. Such investigations were first undertaken for Japanese encephalitis virus (JEV) in Japan in the 1950's. Since this time, the dominant vector species - Culex tritaeniorhynchus – and reservoir hosts – pigs and ardeid birds – that were identified in these studies have generally been assumed to drive JEV transmission across the whole of Asia. This transmission cycle is likely to be responsible for human risk in areas where pigs are dominant within the community of vertebrate hosts and Cx. tritaeniorhynchus, confirmed in field and experimental settings to feed predominantly on large mammals, is relatively more abundant than other potential vector species. Such ecological contexts are found in Thailand and Malaysia; however, the presumption that this group of species drives transmission in all regions may impede our understanding of spatiotemporal variation in transmission dynamics of JEV. Countries where transmission drivers may differ from that of Japan include India, Indonesia and Bangladesh, where dead-end hosts (cattle) are found in substantially higher density than pigs. We utilize field data obtained during a preliminary entomological survey in Bangladesh to show that the observed dominance of any mosquito species within a community can be dependent on

the sampling method employed. In addition we utilize an equation for the basic reproduction number of a zoonotic mosquito-borne virus, parameterized from field data and literature surveys, to demonstrate that the vector species observed to be most abundant may not necessarily drive transmission. To conclude, we emphasize that multiple, carefully selected mosquito sampling methods should always be considered for estimation of mosquito relative abundance as well as species blood-feeding patterns, when undertaking surveys to implicate vector and host species in new geographic regions.

## 44. FINE-SCALE SPATIAL EPIDEMIOLOGY OF AVIAN MALARIA IN A GRASSLAND ECOSYSTEM: DISEASE HAZARD AND RISK TO GRASSLAND BIRDS

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Infectious disease agents have become a predominate cause the decline of wildlife species due to their increased frequency and geographic distribution in recent years. Mass mortality events in wild bird species have been attributed to vector-borne diseases, threatening the survival of individuals and the population viability of many species. The spatial transmission patterns of vector-borne diseases are not uniform across the landscape and are influence by the distribution of their vector species. Avian malaria is an example of a vector-borne disease endemic to North America that affects native bird populations. To developed an avian malaria risk assessment for a native grassland species, the

Greater Prairie-chicken (Tympanuchus cupido), we (1) modeled the spatial distribution of vector occurrence across the Smoky Hills of Kansas utilizing occurrence data and biotic/ abiotic factors, (2) determined the hazard of parasitism associated with Prairie-chicken nest locations, and (3) evaluated if shared habitat affiliation between vector and host facilitated increased disease risk. Our results indicated that the hazard for vector-borne infections was increased at nest locations; however this increased hazard did not translate into an increased risk of carry avian malarial pathogens. Predictive maps, as we have developed here allow for risk assessments of infectious disease agents important to wildlife species and may be used to guide management decisions to limit their spread.

## 45. ENTOMOLOGICAL CAPACITY STRENGTHENING FOR SURVEILLANCE OF VECTORS OF MALARIA, FILARIASIS AND ARBOVIRUSES IN HAITI

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There is limited infrastructural and human resource capacity for entomological studies on vector borne diseases in Haiti, yet many mosquito-borne diseases cause illness to thousands of locals and visitors to Haiti. This project will build an entomology laboratory and train Haitian entomology technicians to strengthen the vector borne disease surveillance capability in Haiti. The laboratory would support surveillance, research, and intervention work conducted in support of the Haitian people. The entomology lab will be equipped with standard entomology equipment to enable

mosquito rearing, mosquito dissections, and insecticide susceptibility studies of major mosquito disease vectors to generate date that will be critical for implementation of wide scale vector control operations in Haiti. This laboratory would support the Haiti Ministry of Health and other collaborators like U.S. Department of Defense entomologists, CDC entomologists and a platoon of scientists interested in studying/ preventing vector borne diseases in Haiti.

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

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Vector Borne Diseases place a huge burden of infection on the poorest human populations, constituting 17% of parasitic and infectious diseases worldwide. Malaria alone threatens half of the world's population, with 250 million cases and 800, 000–1, 000, 000 deaths per year. In the Caribbean, the improvements in access to vector control, medical care, economic and housing conditions have eliminated malaria from all islands except Hispaniola. By contrast, in Haiti which encompasses the western portion of the Island of Hispaniola, this disease is endemic and becomes epidemic in the rainy seasons. The magnitude 7 earthquake which struck Port au Prince in 2010 left 1.5 million people homeless, destroyed 60% of the government and administrative buildings, and killed 25% of the civil servants in

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 the protozoan parasite, Plasmodium falciparum, and its mosquito vector is Anopheles albimanus. 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. Assessing the genetic diversity and the presence of drug resistance markers in the parasite population would allow developing tailored interventions and over time provide information to further refine them during implementation. 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  $\sim$  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 initial analysis is expected to supply detailed sequence information on the genetic complexity of the Leogane isolate, including allelic variants of genes encoding vaccine antigens and genes responsible for drug resistance. Phylogeographic information will come from comparing our sequence data with published sequence data on isolates from South America, Africa and Asia, which will provide information better defining the origins of the Haitian P. falciparum population. 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, in order to determine the population structure, drug resistance genotypes and phylogeography of the malaria parasite in these regions.

### 47. Previous exposure to malaria in the Ouest and Sud-Est departments of Haiti

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Malaria transmission continues to occur in Haiti, with 32,000 cases confirmed in 2011. As rates of malaria decrease, passive surveillance measures become less sensitive for capturing transmission intensity. Using serological methods relying on key malaria antigens AMA-1 and MSP1, we screened 648 serum samples using indirect ELISA methods, for previous exposure to malaria. Using a cutoff value based on 3 standard deviations above our negative control serum (OD of .301), we found 24.8% (161/648) were positive using AMA-1, and 16.8% (109/648) were positive using MSP-1. Of the 13.4% (87/648) of the samples were positive by both antigens, 3.4% (22/648) were positive by MSP-1 only, and 11.4% (74/648) were positive by AMA-1 only. A grand total of 28.2 % of the population was positive for malaria exposure using either antigen. Not surprisingly, both age and location were significant risk factors for likelihood of being exposed (P-value<.001). More participants were positive using AMA-1 given the sensitivity of the antigen and the long window of detection (~20yrs). By site of location, antibody presence varied with the 46% (58/126) found in the rural community of Chabin

and 55.8% (43/77) at the Jacmel Clinic, with only 17.5% (60/343) and 21.5% (22/102) found in the Christianville and Jacmel schools. This data is suggestive of high transmission foci in Haiti, and that these findings should be taken into consideration when tailoring prevention and elimination strategies.

### 48. SIMULATION STUDIES FOR DENGUE TRANSMISSION AND WITHIN-HOST IMMUNE RESPONSES

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Dengue is a neglected tropical infectious disease transmitted between mosquitoes and human beings. There are four known serotypes cocirculating with similar clinical symptoms. Infection with one serotype provides permanent immunity against itself and partial immunity against other serotypes. Secondary or tertiary infections often cause more severe diseases than primary ones. Detecting dengue infection and determining serotypes can be difficult under current laboratory techniques. For example, ELISA can help the detection of infection but does not tell which serotype. Our goal is to explore how to use incomplete laboratory data to answer interesting questions such as quantification of partial cross-immunity and whether it wanes over time. We simulate dengue epidemics together with immune responses and use a likelihood approach to estimate the epidemic parameters. In this simulation study, infection outcomes are observable only during a hypothetical study period and only on a random sample of symptomatic infections. This simulation study is motivated by a dengue cohort study conducted from 2004 to 2010 in

Nicaragua. We found that missingness in laboratory information can severely bias the estimates. In addition, failing to account for non-naive infection history before study entrance may also severely undermine the quality of the inference.

#### 49. TROPHIC CASCADES AND TICK-BORNE PATHOGENS

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The transmission of vector-borne zoonotic diseases to humans depends on multiple species interactions that influence host and vector abundance and infection prevalence. Recent research has revealed that many pathogens, including those causing Lyme disease, babesiosis, and anaplasmosis, are transmitted most efficiently by hosts, such as rodents, that occupy low trophic levels. Consequently, changes in community structure that increase the abundance or infection prevalence of these "pathogen amplifiers" are likely to increase risk of transmission of infectious diseases. One potentially key driver of community change is the trophic cascade, in which an apex predator suppresses a smaller "mesopredator," allowing small prey to increase in abundance. For example, coyotes have been shown to suppress numerous smaller predators; their suppression of fox populations (Vulpes vulpes, Urocyon cinereoargentus, and Vulpes velox) is the best documented. The resulting reduction in predation by mesopredators has led to increased rodent and songbird diversity and increased nesting success of ducks. The range expansion and population increase of covotes in the US Northeast and Upper Midwest during the mid-20th century may have resulted in a trophic cascade that has allowed pathogenamplifying prey species to increase in abundance. If so, this would at least in part explain the increase in prevalence of tick-borne diseases in this region. Spatial and temporal patterns of human

Lyme disease incidence are consistent with this hypothesis. We field-tested the trophic cascade hypothesis for Lyme disease, babesiosis, and anaplasmosis for in Dutchess County, NY. At each site we collected ticks and tested them for the pathogens that cause these three diseases and assessed patterns of carnivore occupancy with baited camera traps. Bobcats had the strongest disease reducing effect - being associated with significantly reduced tick infection prevalence for all three pathogens. Coyotes and foxes had a more nuanced impact on the probability of tick infection with each pathogen, but generally covotes were associated with a higher probability of infection and foxes with a lower one. If infectious diseases are sensitive to changes in predation, then the continuing widespread extirpation of top predators and the consequent restructuring of predator communities may have important consequences for human health.

#### 50. MODELING FRAMEWORK AND ANALYSIS OF DENGUE COHORT DATA IN NICARAGUA FROM 2004 TO 2010

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The spread of dengue has emerged in different places around the world and has increasingly become a concern in public health. Dengue is a serotype specific infection and there are four known serotypes of the virus co-circulating. It is believed that any infection provides a lifetime protection against the infecting serotype, and relatively short time protection against other serotypes. Also, the level of protection is believed to differ

between first and second infection. We develop a model to estimate the hazards of all serotypes for each study year. A Bayesian Analysis using Markov chain Monte Carlo (MCMC) sampling techniques is implemented to simulate the parameters in our model. We assume competing risks among the four serotypes with in the same year. The model takes in to account for covariate effects. We examine how the number of prior infections, the time since last infection and ELISA titer affect infection and outcome. Many of these serotype-specific infections may be missing in the real data. Infections were confirmed using four diagnostic methods: PCR, reporter virus particles (RVP), plaque reduction neutralization test (PRNT) and ELISA. ELISA provides a single measurement of a non-serotype specific antibody level, whereas PRNT and RVP are serotype specific and hence each provide four measurements of antibody levels for the four serotypes. Since the entire history of infection of an individual is not observed, we impute infection history of individuals before they enroll in to the study and when they are missing during the study period 2004-2010. The model accounts for uncertainty in infection status, missing ELISA values. unknown baseline immunity, and prior information on circulating strains in each year. The data is incomplete, and the missingness makes the analysis difficult.

#### 51. Analysis of the Vaccine Efficacy of the Dengue Vaccine

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**Background**: Dengue is a vector-born disease spreading widely in tropical countries. Four dengue serotypes are co-circulating complex pattern of cross-immunity. Sanofi conducted five Phase II and IIb clinical trials of its dengue vaccine candidate. Our goal is to estimate serotype-specific and overall vaccine efficacy based on these studies. **Design and Data**: In each study, individuals were randomly assigned to either vaccine or placebo. Each individual was given three shots every 6 months and was then followed for a certain period, typically 18 months. Individuals with dengue symptoms were laboratory tested for the infecting serotype, but such test is not always conclusive about the serotype. At baseline, preimmunity level to dengue virus was measured in all or a random sample of subjects, depending on the study. **Method**: We propose to base vaccine efficacy on the ratio of average hazard between the vaccine group and the placebo group. The average hazard is the cumulative hazard divided by the duration of the study. The cumulative hazard is estimable even if the number of infections is small or zero. Vaccine efficacy combining studies and/or serotypes are obtained by weighting study- or serotype-specific average hazard using exposed persontimes. Missing preimmunity level and serotypes are handle using multiple imputation. **Results**: The overall vaccine efficacy combining all serotypes and studies, accounting uncertainty in missing pre-immune status and serotypes, is 0.62 with a 95% CI of [0.42 0.75].

# 52. RESISTANCE OF PUERTO RICAN AEDES AEGYPTI TO PERMETHRIN, ETOFENPROX, AND PROPOXUR BY LD50 ESTIMATION

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Emerging insecticide resistance is a problem for vector control, especially in disease endemic areas. Resistance is not desirable from a control perspective because it is associated with a higher cost in order to achieve a comparable level of control for a population of insects that begins to exhibit resistance. It is important to identify and characterize developing resistance in terms of extent and mechanisms so that future control strategies can be optimized. Pyrethroid resistance has previously been documented in Puerto Rican populations of Aedes aegypti mosquitoes. In this study, resistance to three insecticides was determined for susceptible (Orlando - ORL) and resistant (Puerto Rico - PR) strains of Aedes aegypti. The insecticides used were technical permethrin (95.85%), etofenprox (96.8%), and propoxur (99.9%). Groups of 10 female mosquitoes were topically dosed on the thorax in triplicate using acetone as a solvent. Mortality was assessed for each dose replicate at 24hr to determine the number of dead or obviously impaired mosquitoes, characterized by twitching and erratic or upside-down flight. Dose response curves and regression analysis were generated for each chemical pair of mosquitoes (ORL and PR). Resistance ratios were calculated using the LD50 values. As expected, there was a higher resistance ratio for both permethrin (221.9) and for etofenprox (941.7), as pyrethroid resistance has previously been documented in Puerto Rican mosquito populations. Although the resistance ratio for propoxur was not large (6.4), it is higher than the 2-4 fold increase expected from vigor tolerance found when comparing wild populations of mosquitoes to laboratory strains. Future work will investigate the possible mechanisms that underlie this observed resistance including the examination of insecticide penetration, metabolism and altered target sites in vector mosquitoes.

### 53. A PRELIMINARY ANALYSIS OF BED NET COVERAGE AND PERCEPTIONS OF MALARIA IN THE SOUTHERN DEPARTMENTS OF HAITI.

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As efforts to eliminate malaria increase in Haiti, more information on bed nets, insecticide use, and window screen coverage is needed. This study examines the qualitative responses of a survey to a range of questions focusing on malaria exposure. A total of 376 households surveys were collected in, Gressier, Chabin, and Jacmel Haiti, as part of a larger malaria study in the Ouest and Sud-Est departments of Haiti. Based on the number of bed nets per household and total number of people living in each house, the average household bed-net coverage is 31%. Major variation was observed by sampling location, with Gressier having 48.1% coverage, Jacmel having 25.4%, and the rural community of Chabin having 17.1%. This study provides valuable baseline data on bed net coverage, as well as information on the knowledge, behaviors, and beliefs surrounding malaria exposure and infection. Further research must be conducted examining the barriers and effectiveness of bed nets in Haiti.

### 54. OCCUPATIONAL RISK FOR TICK BORNE DISEASE: PRELIMINARY FINDINGS OF A FLORIDA STATE PARK EMPLOYEE SURVEY

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Friedheim Tourism Institute, College of Health and Human Performance; **Jason Blackburn** - Department of Geography, Emerging Pathogens Institute; **Oghenekaro Omodior** - Eric Friedheim Tourism Institute, College of Health and Human Performance

Tick-borne diseases are an emerging public health crisis that has the potential to affect millions of outdoor workers due to their expanding spatial reach and increasing case rates across the United States. Yet, a significant knowledge gap exists regarding occupational risk factors and no tools are available for assessing the risks across populations, job types and locations, etc. This void is a critical barrier to scientific progress, the informed decision-making process central to occupational risk management, and by extension, the protection of public health. In Florida, tick-borne diseases have increased exponentially in the last decade and they have the potential to threaten the state's health, tourism, and economy (CDC, 2013). In the occupational context, tick-borne diseases have the potential to cause serious illness in individuals working in outdoor environments as well as a financial burden for employers (i.e. lost productivity; disability claims). The purpose of this research was to survey Florida State Park employees with a view to understanding their knowledge. risk perceptions, and personal protective behaviors related to tick-borne diseases. In the winter of 2013/2014, Florida State Park employees were asked to respond to a series of questions regarding their tick-borne disease knowledge, risk perceptions, personal protective behaviors, amount of time spent working outdoors, and personal demographics. 200 employees from 160 state parks in five park management regions in Florida completed the survey. Preliminary results suggest a relationship between 'personal protective behaviors' and four independent variables: (1) knowledge; (2) perceived severity; (3) behavior; and (4) demographics. While a majority of state park employees were knowledgeable about tick-borne diseases and they did perceive their severity to be moderate to high, they did not perceive the

individual risk to be significant and their preventative actions to protect themselves against tick bites varied. The results of this study are congruent with antecedent research on tick-borne disease prevention (Herrington, 2004) and this highlights the need to improve education and to influence the adoption of personal protective behavior in the workplace. Herrington, J. (2004) Risk perceptions regarding ticks and Lyme disease: a national survey. American Journal of Preventive Medicine, 26(2): 135–140. U.S. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases, Division of Vector-Borne Diseases (2013). Ticks. http://www.cdc.gov/ticks [accessed January 29, 2014].

# 55. TOXICITY OF NICOTINIC RECEPTOR AGONISTS AND THEIR EFFECT ON REPELLENCY OF DEET AGAINST AEDES AEGYPTI MOSQUITOES

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We recently demonstrated that some new quinuclidine benzamide compounds acted as agonists of insect nicotinic acetylcholine receptors (nAChR). In the current study, we tried to estimate the toxicity of 8 nAChR agonists, including 7 quinuclindine compounds and one neonicotinoid insecticide, imidacloprid, against female adult yellow fever mosquito, Aedes aegypti. We found that imidacloprid had the highest toxicity among all these nAChR agonists with LD50 of 1.5 ng/mosquito. All other compounds we tested showed mild toxicity against Aedes aegypti, with LD50 values in the low microgram level. The cytochrome P450 inhibitor, piperonyl butoxide (PBO), decreased 3-fold the toxicity of one of those quinuclidine benzamide

compounds, LMA 10224, indicating that oxidative metabolites of LMA 10224 may have higher toxicity than the parent compound. In contrast, the R-isomer of LMA 10224 was 8-fold less toxic and its toxicity was increased by piperonyl butoxide, indicating stereochemical specificity for both receptor fit and metabolism. We also tested whether imidacloprid could impair the avoidance behavior of Aedes aegypti mosquitoes. Laboratory investigations using a T-maze apparatus revealed that Aedes aegypti females present long term avoidance behavior when they are exposed to repetitive applications of lemon oil or DEET. We took advantage of this avoidance behavior to test the effect of a sublethal dose of imidacloprid on the avoidance behavior of mosquitoes A. aegypti. Our data reveal that 0.5 ng/mosquito imidacloprid reduces the avoidance behavior of mosquitoes exposed to lemon oil, on the first day of exposure, after the second trial; whereas imidacloprid affects DEET repellency only the first day of exposure, after the second trial. The present data were consistent with the finding that A. aegypti mosquitoes possess long term avoidance behavior and treatment of mosquitoes with sublethal dose of imidacloprid can alter repellency, presumably via central cholinergic pathways.

# 56. COMPETENCE IN CONTEXT: UNITING EMPIRICAL AND THEORETICAL PERSPECTIVES ON VECTOR AND HOST COMPETENCE FOR MOSQUITO-BORNE ZOONOSES

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Competence – the key measure of an individuals' ability to transmit an infection – is a prime example of the complexity inherent in vector-borne zoonotic disease systems. Although currently modeled as separate, constant parameters, new experimental research demonstrates that host and vector competence are intimately related and dependent on

temperature. Methods and insights from empirical competence studies are unified with mathematical modeling of transmission dynamics and risk for this group of pathogens. We present a quantitative framework that would allow exploration of context dependent competence as a determinant of spatiotemporal variation in epidemiological patterns of mosquito-borne zoonoses.

# 57. MUSCLE GLUTAMATE RECEPTOR AGONIST-INDUCED PARALYSIS IN AEDES AEGYPTI BY FEEDING AND INTRA-THORACIC INJECTION ASSAYS

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Glutamic acid is the primary neurotransmitter capable of activating and desensitizing glutamate receptors in insect muscle. It works via the depolarizing subtype of glutamate receptor (GluRd), coupled to an intrinsic cation channel. Activation of the receptor underlies generation of the excitatory junction potential and triggers muscle contraction, whereas desensitization is transition to a non-conducting, persistent ligand-bound state of the channel. Rapid and brief activation coupled with prolonged desensitization of muscle excitatory GluRd receptors could underlie paralysis if mimicked by an insecticide. By using headless larvae (to enhance compound penetration) and electrophysiological methods, previously we showed that the agonists of muscle GluRd induce paralysis of the yellow fever mosquito Aedes aegypti larvae by blocking synaptic transmission. In the past year, we expanded this study by conducting additional toxicity assays, as well as including phenylpiracetam, pramiracetam, and rolipram to our experiments. Phenylpiracetam and pramiracetam are derivatives of piracetam,

a GluRd-directed compound that proved to be a strong paralytic agent in our earlier headless larvae assay (PC50=8 ppm). Rolipram is a phosphodiesterase inhibitor. We performed a feeding assay and intra-thoracic injection of adult female Aedes aegypti. In the feeding assay, we suspended the agents in 5% sugar water to feed the mosquitoes for 48 hours. Our results suggest that the toxicity occurred in the following rank order of effectiveness: feeding < headless larvae < injection assays, which suggests that the agents might be readily metabolized in mosquito gut or not be able to penetrate into the hemocoel. The following compounds had mortality of < 50% at 1 mg/ml by sugar water treatment: AMPA, aniracetam, l-aspartic acid, cyclothiazide, domoic acid, d-glutamic acid, l-glutamic acid. NMDA, phenylpiracetam, piracetam, quisqualic acid, tetrazoleglycine, and willardiine. We obtained PC50 values (previously not done) for phenylpiracetam, pramiracetam and rolipram of 136, 44 and 49 ppm in headless larvae, respectively. l-BMAA was the most potent oral toxicant, with lower but significant activity shown by pramiracetam and rolipram. In the injection assay, compounds were introduced into the thorax of adult mosquitoes in 200 nanoliter volume which showed potent toxicity, with most LD50s between 5-20 ng/insect. Exceptions were aniracetam, lglutamic acid, NMDA, tetrazole-glycine, and willardiine, which had LD50s in the range of 147 (NMDA) to 534 (l-glutamate) ng/insect, while the value for propoxur was only 0.14 ng/mosquito. To see if other insects were sensitive to these compounds, we performed a sugar water feeding assay with adult female Drosophila melanogaster. Interestingly, there was little or no toxicity to adult Drosophila, indicating selectivity for mosquitoes. Our results suggest that glutamate agonists might be good candidates for further development as new mosquitocides.

#### 58. LASTING IMPACTS OF LARVAL COMPETITION AND PESTICIDES ON ADULT SURVIVAL IN THE DENGUE VECTOR AEDES AEGYPTI

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Mosquito control efforts rely heavily on reducing the adult population size through application of pesticides which should lower risk of disease transmission. Reductions of the adult population size are usually assumed to act additively with other sources of mortality in nature. However, alternative effects on adult population size are possible. Additionally, there may be effects on the surviving adult mosquitoes beyond the effect of population size such as alterations in life history traits and competence for pathogens. We exposed Aedes aegypti larvae to a factorial combination of larval competition (varying density) and pesticide Bacillus thuringiensis israelensis (Bti) and measured their development, survivorship to adulthood, fecundity, and survival of adults. The presence of Bti consistently shortened development time and reduced survivorship to adulthood, relative to controls. We found significant negative effects of competition on development to adulthood and survival of A. aegypti adults, but survival also depended on the concentration of Bti. Fecundity of surviving females was significantly affected by the presence of Bti with consistent increases in fecundity with increasing concentrations of Bti. The lasting impact of larval competition and the presence of pesticides in the environment influence parameters that contribute to vectorial capacity which is used as an indicator of risk of disease transmission. Our results have general application to further improving our understanding of mosquito control and finding methods to most effectively apply pesticides to minimize risk of disease transmission.

#### 59. VULNERABILITY ASSESSMENT FOR TICK-BORNE DISEASE RISK IN FLORIDA PARKS – A MIXED METHODS APPROACH

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There is evidence in the scientific literature suggesting a geographic redistribution of ticks and tick-borne pathogens across North America. With particular reference to natural environments and park places in Florida, where ticks have been collected in high numbers, there is little, if any information documenting tick hotspots, environmental correlates of ticks, tick density maps, park visitor and employee knowledge, attitude, perceptions and behavior as regards tick exposure. We present a mixed methods research design study proposal to assess human vulnerability to tick-borne pathogens in Florida parks. We propose to use the following mixed methods for this study: Spatial statistics to identify hotspots for ticks and tick-borne pathogens in a Florida state park, molecular biology techniques (PCR and DNA sequencing) to identify the genotypes of selected disease agents in ticks, look for similarities/differences between the variants isolated and similar species in the Northern United States, use remotely sensed data (Normalized Difference Vegetation Index) to associate land cover types with tick hotspots, use surveys and direct observation to identify areas of high human activity and social risk-factors in the park, correlate tick/pathogen hotspots with areas of high human activity, use GIS to create maps of all relevant associations detected, and develop a vulnerability assessment matrix. When completed, the linkage of tick, environmental and human data, as evidenced from this study can be used to develop models for extrapolating and predicting risk of tick-borne disease over broad geographic areas, especially where data is not available. A vulnerability assessment is necessary for identifying areas of urgent need, increasing the

effectiveness of control measures, preventing outbreaks and epidemics as the case may be, and has implications for policy development.

#### 60. LARGE-SCALE PATTERNS OF MALARIA INCIDENCE IN THE BRAZILIAN AMAZON

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Despite extensive research on malaria in the Brazilian Amazon, there has been relatively little published on large-scale patterns of malaria incidence in the Brazilian Amazon. Here, we report on these large-scale patterns based on 2.4 million malaria cases registered by the Brazilian surveillance system between 2004 and 2008 over 4.5 million square km2. Using a Bayesian geospatial model, we find that there is an overall lower malaria risk in the southeastern part of the Brazilian Amazon (i.e., Mato Grosso state) while hotspots are present in northern Rondonia, Amapa, and Roraima states and central Para state. Comparing results using 2004 versus 2008 data further reveals that malaria risk has been decreasing in most of the Brazilian Amazon region, except in Amazonas state and the western part of Acre state. We also contrast seasonal patterns in malaria incidence for three types of sites: sites that can only be accessed by river (riverine sites), by road (road sites) or by both (mixed sites). The peak season across all sites tended to be in the months of July and August; yet, there were also striking seasonal differences across the three types of sites. In the riverine sites, December was the month with the least amount of malaria cases whereas for road sites the month with the lowest number of malaria cases was April. Finally, the mixed sites tended to exhibit a multimodal seasonal pattern, with a minor peak during January. Both mixed and road sites had smaller amplitude in seasonal pattern than the riverine site. The observed large-scale patterns can help optimize the temporal and

geographical allocation of resources to prevent and control malaria in the region.

## 61. GEO-SPATIAL RISK MODELING FOR WEST NILE VIRUS IN TARRANT COUNTY, TX USING ENVIRONMENTAL AND DEMOGRAPHIC DATA

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The prevalence of West Nile Virus (WNV) reached its highest levels in 2012 and has led to one of the largest outbreaks since its introduction to the United States in 1999. The study area of Tarrant County, TX had experienced one of the largest amounts of human cases across the U.S in 2012. For WNV control, the local health agency needed a model to estimate the spatial distribution of WNV risks. Using the spatial empirical Bayesian rate smoothing and multivariate logistic regression approaches, a geo-spatial risk model is constructed to identify key environmental and demographic factors at the census tract level that govern the average mean rate of WNV infected mosquitoes. The validated risk model is then used to predict areas with the rates of WNV infection higher than the average. Of the 35 environmental and demographic variables used, the residence vacancy, race, income, amount of vegetation and SNAP benefits are found to be statistically significant. The results also indicate an increased amount of risk in the NE section of Tarrant County. This study informs the Tarrant County to diversify their mosquito control efforts by 1) concentrating on specific areas that show an increased risk of disease transmission and 2) focusing on novel factors which lead to a significant decrease in disease transmission and cases.

### **62.** SEROLOGICAL ANTHRAX SURVEILLANCE IN WILD BOAR (SUS SCROFA) IN UKRAINE

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Anthrax, caused by Bacillus anthracis, is an acute disease affecting wildlife, livestock, and humans worldwide, though its impact on these populations is under appreciated. In Ukraine, surveillance is passive, and anthrax is often detected in livestock. However, wildlife are not subject to surveillance, although anthrax deaths (such as in wild boar, Sus scrofa) have been documented. Wild boar is a plentiful and widespread species in Ukraine, and frequently hunted. We initiated a screening study testing Ukrainian wild boar blood samples for antibodies to B. anthracis. We mapped results relative to known livestock anthrax hotspots. We discovered evidence of exposure in wild boar up to 35 km from livestock anthrax hotspots and over 400 km from previous anthrax reports in boars. We make recommendations about using wildlife species as biosentinels for anthrax in Ukraine.

#### 63. ASYMPTOMATIC SPREAD OF HUANGLONGBING AND IMPLICATIONS FOR DISEASE CONTROL

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Huanglongbing (HLB) is a bacterial infection of citrus trees transmitted by the Asian citrus psyllid Diaphorina citri. Mitigation of HLB has focused on spraying of insecticides to reduce the psyllid population and removal of trees when they first show symptoms of the disease. These interventions have been only marginally effective, since symptoms of HLB do not appear on leaves for months to years after initial infection. Lack of knowledge about disease spread during the asymptomatic phase is exemplified by the heretofore unknown length of time from initial infection of flush by adult psyllids until the flush becomes infectious. We present experimental evidence showing that young flush become infectious within 10 -15 days after receiving an inoculum of C. Liberabacter (bacteria). Using this critical fact, we specify a microsimulation model of disease spread in a grove of citrus trees. We apply a range of psyllid introduction scenarios to show that entire groves can become infected with up to 12,000 psyllids per tree in less than one year, before most of the trees show any symptoms. We also show that intervention strategies that reduce the psyllid population by 90% during the flushing

periods can delay infection of a full grove, and thereby reduce the amount of insecticide used throughout a year. This implies that psyllid surveillance and control, using a variety of recently available technologies, should be utilized from the initial detection of invasion and throughout the asymptomatic period.

## 64. MODELING THE POTENTIATION OF IN VITRO AZTREONAM ACTIVITIES BY AVIBACTAM AGAINST FOUR B-LACTAM-RESISTANT BACTERIAL STRAINS

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The efficacy for aztreonam (ATM) plus avibactam (AVI), a βlactamase inhibitor, was evaluated against four Gram-negative pathogens producing metallo-beta-lactamases: E. coli (n=2), K. pneumoniae (n=1) and P. aeruginosa (n=1). Methods: ATM MIC was determined with increasing AVI doses using broth macrodilution method. The subsequent kill kinetic studies for each strain using a checkerboard design with AVI and ATM concentration ranges of 1- 16 µg/mL and 0.015 – 8192 µg/mL, respectively, were modeled by a 2-stage (primary and secondary persistence) pharmacodynamic model. The effect of AVI was modeled as a reduction in the ATM EC50 of the death rate. The ATM degradation rate was dependent on the dormant bacterial load. Results: Table 1 shows significant decrease in ATM MIC with increasing AVI dose. The potency increase was evidenced by shift in ATM EC50, which was consistent with the fold reduction on MIC due to AVI. Conclusion: AVI significantly decreases the ATM dose required to reach maximum response in vitro.

#### 65. Animal factors influencing cefotaxime resistance in cattle

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The third-generation cephalosporins are used extensively in human medicine and to some extent in veterinary medicine therapeutically. Enterobacteriaceae members are the potential carriers of the resistance genes against the cephalosporins, and these bacteria have the highest capacity to transmit the resistance to other animals or humans. In this study, we studied cefotaxime resistant microorganisms from 300 new born claves by screening cefotaxime (a third-generation cephalosporin) resistant microorganisms, which have never been exposed to antibiotics across their entire life cycle. Fecal samples from calves were collected every 3 months over a period of one year. Samples were collected in March, June, August and December 2013. The samples were diluted in Luria Bertani broth and plated on MacConkey plates containing cefotaxime 4µg/ml. The cefotaxime resistant colonies were purified on the media containing cefotaxime and stored in 10% glycerol stock for future use. The prevalence of cefotaxime resistance in calves for four samplings was 61%, 50%, 68% and 6%. There was an initial decrease in the prevalence of resistance which was reversed at the time of weaning of calves. The prevalence of cefotaxime resistance

decreased in the December, suggesting that seasonality may effect on the shedding of resistant bacteria. There was statistically significant association between breed group and the prevalence of cefotaxime resistance in calves. However, no preference in the prevalence of cefotaxime resistance between male or female calves was observed. In addition, we also found that five calves didn't shed resistant bacteria in any of the four samplings and five others were positive in all of the samplings. The findings of this study suggest that antibiotic resistance develops in nature, but physiological state of animal and environmental factors influence the prevalence of cefotaxime resistance.

## 66. Animal risk factors that modulate the prevalence and persistence of Shiga toxin-producing Escherichia coli in cattle

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Shiga toxin-producing Escherichia coli (STEC) are serious public health problem and cause more than 110, 000 illnesses annually in the US. Depending on the strain, STEC can produce one or both of the Shiga toxins Stx1 and Stx2. Cattle are the primary reservoirs for STEC, and both dairy and beef cattle have been shown to shed STEC. This study represents an extensive on-farm

investigation of STEC prevalence within a cattle herd at North Florida Research and Education Center at the University of Florida for three years. Fecal samples were collected from rectoanal junction of animals. The samples were collected in August 2011 (92 animals), July 2012 (264 animals), and October 2013 (animals samples). The prevalence of STEC in the cattle was 47%, 50%, and 37%, respectively. We found that heifers shed significantly less STEC than cows in the first year. Cows had 16.1 times the odds of harboring any strain of Shiga-toxin producing E. coli than heifers. In the second year, multiparous cows were approximately 49% less likely to carry stx2 than primiparous cows, suggesting that the parity of the cows affect on STEC shedding. Studying the 74 overlapping animals between 2011 and 2012 showed that the prevalence of total STEC (47.1%), stx2 (44.3%), stx1/2 (67.3%) increased in 2012 sampling to 55.7%, 54.3% and 79.6% respectively. The prevalence of STEC reduced to 37% in the third year from 100 heifers and 215 cows. This study provides insight into the animal factors that modulate the STEC prevalence in farm animals that will be needed to develop mitigation strategies to reduce the STEC prevalence and the numbers at the pre-harvest level.

## 67. IN VITRO ACTIVITY AND EFFICACY OF A NOVEL ANTIBACTERIAL GSK2140944 AGAINST YERSINIA PESTIS (YP) IN A MURINE-AEROSOL CHALLENGE MO

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**Background**: GSK2140944 (GSK944), a novel bacterial topoisomerase inhibitor in clinical development, is currently being evaluated for use against biothreat and conventional pathogens. GSK944 selectively inhibits bacterial DNA replication

through a unique binding mode that is different from that of fluoroquinolones, thereby allowing GSK944 to be active against isolates carrying resistance determinants to established antibacterials. GSK944 was evaluated both in vitro against a YP 30 strain diversity panel, and in murine post-exposure prophylaxis (PEP) and treatment models of inhalational plague. Methods: Minimum inhibitory concentrations (MICs) were determined according to CLSI microdilution methods. Female BALB/c mice were exposed to aerosolized YP, and antimicrobials were administered for 5 or 10-days starting at 24h or 42h post exposure. GSK944 was dosed orally q12h. Ciprofloxacin and phosphate buffered saline (PBS) controls were dosed IP q12h. Survival was monitored up to 42 days post-exposure, at which point lungs and spleens were cultured to confirm bacterial eradication. In the treatment study evidence for clinical and positive bacteriology were established at 42h. Results: The MIC50/90 for the YP diversity set was 0.5 and 1 ug/mL and ranged from 0.03 to 1 ug/ml. Efficacy results are shown below. Lung and spleen cultures from surviving mice were all negative at termination of the experiments, indicating that all surviving mice in each of the cipro and GSK944 treatment cohorts were cleared of infection. Conclusions: GSK944 was efficacious in both the PEP and active infection treatment murine Y, pestis aerosol models studied. These results indicate that GSK944 warrants further study for biothreat and conventional pathogens.

### 68. EFFICACY OF ACHN-975 IN A MURINE PNEUMONIC PLAGUE (YERSINIA PESTIS) MODEL

<u>Henry Heine</u> - Department of Medicine, College of Medicine, UFL; <u>Lara Chuvala</u> - Department of Medicine, College of Medicine, University of Florida; **Renaldo Riggins** - Department of Medicine, College of Medicine, University of Florida; **George Drusano** -Department of Medicine, College of Medicine, University of Florida **Background** ACHN-975 is a novel LPS biosynthesis inhibitor antibiotic being developed for gram-negative infections. Because of its novel mode of action, ACHN-975 should be unaffected by pre-existing resistance to other antibiotic classes and therefore represents an attractive countermeasure for drug-resistance threat agents. Due to its potent in vitro activity against Yersinia pestis, it was evaluated in an aerosolized Y. pestis (the causative agent of plague) murine infection model. Methods Female BALB/c mice were administered antimicrobial 24h post exposure to Y. pestis for 5-days and were monitored an additional 21 days post-treatment, at which point surviving animals were euthanized and spleens cultured to confirm there was no residual infection. ACHN-975 was dosed subcutaneously at 5, 10, 20, or 40 mg/kg q6h for 5 days. Ciprofloxacin 15 mg/kg dosed IP q12h was the positive control and the ACHN-975 vehicle, q6h was the negative control. **Results** All ACHN-975 doses tested provided significant survival benefit over the PBS control (p<0.05). Survival was 100% for the 20 and 40 mg/kg (80 and 120 mg/kg/day), 90% for the 10mg/kg (40 mg/kg/day) and 70% for the 5mg/kg (20 mg/kg/day) ACHN-975 doses. The ciprofloxacin 15 mg/kg (30 mg/kg/day) control also had 100% survival. Spleens removed from surviving animals at the termination of the experiment were free of Y. pestis. **Conclusion** Based on the efficacy data in this murine model, ACHN-975 shows great potential as a therapeutic for treating inhalational Y. pestis and warrants further testing. Given its novel mode of action, ACHN-975 could be particularly important as a treatment option against Y. pestis strains that are resistant to current antibiotics.

## 69. THE APPLICATION OF CHITOSAN MICROPARTICLES TO CONTROL UTERINE DISEASE IN COWS REVEALED BY METAGENOMIC ANALYSIS

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Uterine disease is a major concern in the dairy industry. After parturition, about 40% of dairy cows suffer from uterine disease, which is mainly caused by multiple strain infections. Control of these bacteria using antibiotics is limited due to availability of treatment methods, and thus development of a new therapeutic alternative for uterine disease is necessary. In an earlier study, we found that CM exert a broad spectrum of antimicrobial activity at neutral pH, indicating that CM can be used for uterine disease treatment. Here, we present the antimicrobial activity of CM by showing reduction of pathogenic bacteria in the uterus of dairy cows with uterine disease. Metagenomic analysis was conducted to determine the antimicrobial effect of CM in the uterus. Metagenomic analysis samples were selected after CM infusion into cow uterus on the basis of shedding of intrauterine pathogenic E. coli (IUPEC). Pyrosequencing of the 16S RNA gene of microbiota revealed that total microflora of uterus was significantly altered by CM or antibiotic (ceftiofur) treatment. F. necrophorum was the most abundant pathogenic species in the uterus and was significantly decreased by the treatment of CM, whereas F. necrophorum remained abundant in the no treatment group. A. pyogenes was also reduced by CM but not by ceftiofur treatment. Surprisingly, non-pathogenic bacteria such as Porphyromonas endodontalis were less influenced by CM. These results provide promising use of CM as an alternative treatment for uterine disease.

## 70. SPATIOTEMPORAL PATTERN ANALYSIS OF THE SPREAD OF BANANA XANTHOMONAS WILT (BXW) IN KAGERA REGION, TANZANIA

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Banana is an important food and cash crop for the majority of households in Kagera region of Tanzania. In January 2006 a new disease, banana Xanthomonas wilt (BXW) was reported from the Muleba district of Kagera region. All banana cultivars are susceptible to this disease. In poorly managed fields the yield can reduced to zero. BXW is caused by the gram-negative bacterium Xanthomonas campestris pv musacearum (Xcm). The spread of the disease is primarily by farm tools, infected planting materials and pollinating insect vectors. Currently, there is no effective control method for the disease. Due to its rapid spread there is a growing concern for the disease to spread to other major banana producing regions. Therefore, there is a need to understand spatiotemporal patterns of BXW spread which might help in identifying disease risk factors, designing efficient surveillance methods and improving management of the disease. The overall objective is to examine and describe the spatiotemporal patterns of the spread of BXW. Disease survey data collected between 2008 and 2010 from stratified random sampling were used in this study. Presence or absence of disease was recorded in each visited farm, regarded as case for this study. Spatial point pattern analysis by Ripley's K, and average nearest neighbors index (ANNI) were used to explore clustering of BXW cases. Ripley's K was performed using two distances 1000 and 5,000 m at 20 steps (0 - 100) with 99 permutations, for a p< 0.01 confidence interval.

ANNI was calculated using Euclidian distances, resulting in the average distance between each feature to its nearest neighbors compared to a random distribution of neighbors. All analyses were performed in ArcGIS 10.1. K function analyses showed that BXW cases were significantly clustered across the entire Kagera region. Very strong clustering of BXW cases was observed at 1000m. Cluster occurrence did not differ between 2008 and 2010. The ANNI confirmed that BXW cases were highly spatially clustered across the entire Kagera region (P < 0.0001). Broadly, these results suggest that BXW spread rapidly over short distances within this region and there was high cross infection between neighboring farms. This short distance spread may have been due to the movement of vectors, exchange of infected planting materials between neighboring farmers or the use of neighbors' farm tools without disinfection.

### 71. DECEITFUL KILLERS: C. ELEGANS MUTATION ACCUMULATION LINES SUSCEPTIBILITY TO P. AERUGINOSA

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Understanding the relationship between an individual's genotype and its susceptibility to pathogens is an important goal of medical genetics and evolutionary biology. Of fundamental importance is the nature of genetic variation in susceptibility to pathogens introduced into a population of (potential) hosts by mutation. Characterizing the "mutational variance" (VM) provides a first step toward understanding the evolutionary factors underlying genetic variation within and between populations: all else equal, the genetic variation present in a population is expected to be proportional to the input of genetic variation by mutation. Here we quantify the cumulative effects of spontaneous mutations on

susceptibility to the pathogenic bacterium Pseudomonas aeruginosa in a population of the nematode Caenorhabditis elegans. Perhaps surprisingly, spontaneous mutations do not affect the expected time to death ("LT50") of a population of worms exposed to P. aeruginosa. However, VM for susceptibility to P. aeruginosa is on the order of VM for many life history traits in the same population, which implies that the trait "pathogen susceptibility" provides a large mutational target. The previous finding of high genetic variance for the same trait among natural isolates of C. elegans is straightforwardly explained by the high VM and weak purifying selection on deleterious mutations; maintenance of genetic variation for pathogen susceptibility by balancing selection need not be invoked.

#### 72. METAL ION CHELATION BY TIGECYCLINE EXPLAINS ATYPICAL NONLINEAR PLASMA PROTEIN BINDING BEHAVIOR

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BACKGROUND: Free fractions of the drugs are responsible for efficacy and thus understanding plasma protein binding (PPB) is vital in the dosing regimen design. Unlike typical nonlinear PPB where the free fraction increases nonlinearly with increase in the total concentration, the free fraction decreases with the increase in total concentration in atypical nonlinear PPB. Tigecycline, a glycylcyline antibiotic, shows atypical nonlinear PPB; however, the mechanism of such behavior is unknown. Being structurally similar to tetracyclines, tigecycline may chelate metallic ions. This work investigates the influence of metallic ion chelation as mechanism of tigecycline's atypical nonlinear PPB behavior.

METHODS: The PPB was determined in pooled human plasma (N≥6) in the presence (1.5 mg/mL) and absence of EDTA at 0.1, 1

and 10 µg/mL of tigecycline by ultrafiltration. Metal ion chelation by EDTA was saturated by addition of magnesium and calcium ions. All the samples were analyzed using a validated LC-MS/MS method. The mechanistic model was simulated to fit the free fraction determined at different concentrations in the range of 0.1 to 200 µg/mL. **RESULTS**: The percent free fractions of tigecycline at 0.1, 1 and 10 µg/mL in the absence of EDTA were 13.8±2.2, 7.4±6.7, 2.2±2.2, respectively, while in the presence of EDTA these were 39.9±9.2, 35.4±8.9 and 33.1±8.4. Presence of EDTA significantly increases the free fraction and nullifies (p=0.4) the concentration dependent protein binding but addition of calcium and magnesium ions decreased the free fraction of tigecycline. **CONCLUSION**: The metal chelated tigecycline crosses the filtration membrane and determined as unbound fraction, thus shows atypical nonlinear PPB behavior. Simulation of the mechanistic model also supported this conclusion.

### 73. CEFTOBIPROLE MEDOCARIL (BAL-5788) FOR THE TREATMENT OF COMPLICATED SKIN AND SOFT STRUCTURE INFECTIONS

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Ceftobiprole medocaril (BAL-5788) is a parenteral prodrug of ceftobiprole, a novel bactericidal cephalosporin with broad activity against Gram-positive and Gram-negative bacteria, including methicillin resistant Staphylococcus aureus (MRSA). With S. aureus resistance on the rise, including some cases of resistance to vancomycin, and MRSA as the most prevalent pathogen in complicated skin and soft structure infections (cSSSIs), the need for safe, effective, and well-tolerated antibiotics is crucial. Ceftobiprole has been shown to be noninferior to vancomycin and vancomycin plus ceftazidime in the treatment of MRSA cSSSIs and was well tolerated in clinical trials. In addition,

it has exhibited in-vitro activity against Gram-negative pathogens similar to cefepime and ceftazidime, making it a potential candidate for cSSSI empiric broad-spectrum monotherapy. Our group performed in vivo microdialysis in healthy subjects to determine unbound, active extracellular concentrations in muscle and subcutaneous adipose tissue. Models were developed to fit pharmacokinetic data from the microdialysis study and pharmacodynamic data from time-kill curves of two MRSA strains. With these models, the probability of target attainment (PTA) of T>MIC above 30% or 40%, and 1- and 2-log kill were simulated. PTA for T>MIC >40% was >90% in both tissues and plasma, while for PTA for 1-log kill was >90% for plasma and muscle tissue, and ~88% for subcutaneous adipose. Ceftobiprole's volume of distribution at steady state is comparable to extracellular fluid volume and it is rapidly eliminated, renally, with a terminal half-life of approximately 3 hours. Protein binding is consistent across a range of concentrations and relatively low (~16%). Ceftobiprole is currently approved in Europe, but still under review in Switzerland, for treatment of adults with community- and hospital-acquired pneumonia, with the exception of ventilatorassociated pneumonia.

## 74. MONTE CARLO SIMULATION TO DETERMINE THE EFFECT OF ATYPICAL NONLINEAR PLASMA PROTEIN BINDING ON CLINICAL BREAKPOINT OF TIGECYCLIN

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**BACKGROUND**: Tigecycline is recommended for the treatment of mild-to-moderately severe complicated intra-abdominal infections but excess deaths associated with tigecycline treatment led to a black box warning. The clinical breakpoint established using target total AUC/MIC ratio of 6.96; however, free

concentration is responsible for efficacy and PPB was not considered during clinical breakpoint determination. Tigecycline shows atypical nonlinear plasma protein binding (PPB) in human within therapeutic range. Current work assesses the effect of atypical nonlinear PPB on clinical breakpoint of tigecycline. **METHODS**: The PPB was determined using ultrafiltration. An empirical model (fu= $\beta$  (Ct)- $\alpha$ ) was fitted between unbound fraction and total concentration. The inter-individual variability was determined by population fitting of this model to the PPB in 6 males & 6 females at 0.1, 1 and 10 ug/mL. Ten thousand individuals with 100 mg/day dosing at steady state were simulated, the fAUC/MIC were determined and the probability of cure was predicted using a logistic regression model. The target (fAUC/MIC) ratio was calculated at the 90% probability of cure. The probability of target attainment was calculated at different MICs. **RESULTS**: The empirical model described the data reasonably well. The population estimates (95% CI) of  $\alpha$  and  $\beta$ were 0.373 (0.341-0.405) and 97.2 (76.2-117.8), respectively. The inter-individual variability of  $\beta$  was 28.7%. Male showed higher free fraction than female and the difference was statistically significant at  $0.1 \mu g/mL$  (p=0.012) but had no significant covariate effect on the model parameters. The fAUC/MIC target for tigecycline was determined as 2.1. CONCLUSION: The tigecycline may have lower clinical breakpoint (0.5-1 mg/mL) than current breakpoint (2 mg/mL).

### 75. HPV POPULATIONS IN ASYMPTOMATIC MALE CARRIERS, A STUDY OF NATURAL HISTORY BY DEEP SEQUENCING

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Medicine, University of Florida; **Brent Gardner** - Department of Pathology, Immunology, and Laboratory Medicine, College of Medicine, University of Florida; **Amanda Lowe** - Department of Pathology, Immunology, and Laboratory Medicine, College of Veterinary Medicine, University of Florida; **Fahong Yu** - Mineral Resources Research Center, University of Florida; **Xinrui Zhang** - Department of Pathology, Immunology, and Laboratory Medicine, College of Medicine, University of Florida; **Anna Giuliano** - H. Lee Moffitt Cancer Center; **Maureen Goodenow** - Department of Pathology, Immunology, and Laboratory Medicine, College of Medicine, University of Florida

Infection by multiple HPV types, either high risk types alone or in combination with low risk types, is a risk factor in development of cancers in both men and women. We applied pyrosequencing coupling with our novel custom HPV-QUEST genotyping tool and a bioinformatics pipeline to evaluate diversity and persistence of mucosal HPV types and variants in a subset of 18 asymptomatic men from the HPV in Men [HIM] Study. Twenty-one genotypes [11 high-risk and 10 low-risk] were detected in a cross-sectional analysis. Six of 18 individuals were infected by a single HPV type, either HPV16 [n = 4] or HPV6b [n = 2]. In contrast, infection by multiple [2 to 6] different HPV genotypes was detected in 12 of 18 individuals. Frequency of infections by oncogenic type(s) was as high as 78% and included co-infection by non-oncogenic HPV type(s) in 70% of these samples. While sequencing revealed high frequency of identity between samples and reference genomes. variants of reference HPV genotypes were often seen in low frequency as well as predominant HPV genotypes. In comparison to Linear Array [LA], pyrosequencing/HPV-QUEST provides sensitive detection and accurate genotyping of a broader spectrum of HPV types, including low frequency types, genetic variants of reference types, and types not included in hybridization assays.

# 76. SPATIOTEMPORAL DYNAMICS OF SIV INFECTION AND NEUROTROPISM IN CD8-DEPLETED AND NATURALLY PROGRESSING RHESUS MACAQUES

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Despite the success of combined antiretroviral therapy (cART) in controlling viral replication and prolonging life in HIV-infected individuals, HIV-associated neurocognitive disorders (HAND) remain a frequent complication of infection. While SIV infection of rhesus macaques is a well-studied model for HIV infection in humans and the progression to AIDS, SIV-associated encephalitis (SIVE) in this model also provides critical insight into the pathology of HAND. The goal of the present study was to characterize SIV intra-host viral evolution over the course of infection and its relation to the development of SIVE in both CD8-depleted and naturally progressing rhesus macaques. A total of eighteen macaques (six CD8-depleted and twelve naturally progressing) were intravenously infected with a SIVmac251 viral

swarm and monitored longitudinally in terms of lymphocyte counts, viral load, and neurological impairment prior to the onset of AIDS, with the exception of four macagues euthanized at 21 days post infection (dpi). Viral sequences were also obtained longitudinally throughout infection from several lymphoid and non-lymphoid tissues, including plasma, lymph node, bone marrow, and lung epithelial lining fluid, as well as meninges and various tissue compartments within the brain at necropsy. Bayesian phylogeographic analysis was used to ascertain viral gene flow for all of the macaques and spatiotemporal distribution of neurotropic virus for the brain-infected macaques. Results for the CD8-depleted macaques indicated that the brain was continually seeded with SIV throughout infection; however, a significant increase in brain viral effective population size occurred 50-80 dpi in all animals. The virus seeding of the brain at the time of viral expansion frequently originated from CD14+ cells in the bone marrow. These results indicated not only a possible mechanism for SIV neuropathogenesis, but also a potential new target for the development of therapies that may slow or prevent the onset of HAND.

#### 77. EARLY ADAPTATION AND SELECTION OF ENVELOPE AND NEF GENES IN 12 RHESUS MACAQUES INFECTED WITH SIV

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Background. In this study twelve rhesus macaques were intravenously inoculated with the SIVmac251 viral swarm to study how immunodeficiency viruses evolve immediately postinfection and when encountering diverse immune systems. Methods. At three weeks post-infection, SIV envelope (env) sequences were generated from tissues (plasma, CD3, CD14, and bronchial lavage) using a single genome sequencing approach. Linked nef sequences were generated for four of the macaques. Two hundred linked viral swarm env and nef sequences were also generated for comparison to macaque data. Pairwise distance, including synonymous (dS) and nonsymonymous (dN) rates, were calculated for each tissue. Recombination breakpoints and individual recombinant sequences were identified. Clustering analysis based on each particular sequence divergence level, fixed-effects-likelihood selection analysis and signature analysis were performed and compared among macaques and viral swarm sequences. Phylogenetic analysis was performed to view evolutionary similarities and differences in virus adapting under varied immune systems and tissues. Results. Macague sequences significantly varied from viral swarm sequences (p<0.005). This difference was due mostly to significant differences in synonymous changes (p<0.0005). Clustering analysis grouped most macaque sequences independently from viral sequences. No significant evolutionary differences were observed in different macaque tissue types. Recombination breakpoints were frequently shared among sequence populations generated from different macaques. Positively selected codons in env were also similar among macagues and overlapped only moderately with viral swarm sequences. In nef, positively selected codons were again similar among macaques and occurred in structurally important nef domains; however, no positively selected sites were detected in the viral swarm sequences. Moreover, nef sequences were more diverse than envelope sequences. Phylogenetic analyses were comparable among macaques, often with like viral swarm sequences segregating within different

macaque sequence clades. Conclusions. At three weeks, the virus was moderately evolved from the viral swarm sequences and was adapting and evolving similarly in the animals. This suggests an advantage to the emergence of specific genetic motifs even when the virus is challenging different immune systems. Thus, specific alterations in the virus may play a role in driving early infection. This is the first study to examine how immunodeficiency viruses adapt and evolve in diverse immune systems. A longitudinal study using the macaque model for HIV infection is underway.

#### 78. MALE REPRODUCTIVE SUCCESS VS. FEMALE AVOIDANCE: SIGMA VIRUS PERSISTENCE IN DROSOPHILA MELANOGASTER

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Host and pathogen fitness are always intertwined, as the pathogen's fitness depends on availability of hosts. However, pathogens by definition do not benefit their hosts, but rather harm them. Virulence, the harm inflicted by a pathogen on its host, is potentially an evolvable property and for parasites to persist, virulence must be kept within certain bounds. However, parasites are short-sighted, so selection sometimes drives virulence to the point that hosts (and therefore pathogens) go extinct, at least locally. However, virulence may be offset by increasing host fitness. Sigma virus (DMelSV; family Rhabdoviridae) is a naturally occurring disease agent that is maintained in Drosophila through vertical transmission in the germ cells of both males and females. Infection by this non-lethal virus has shown to increase fitness of male Drosophila, thereby increasing viral fitness. Infected males have higher reproductive success than uninfected males by siring both more offspring and a higher proportion of offspring in multiply mated females, at least when the infected male is the first male. Females are actively involved in choice of sperm for fertilization, both in terms of their willingness to remate, and by selective usage of sperm following remating (cryptic female choice). It is unclear if the fitness advantage gained by sigma virus infection is by infected sperm outcompeting the sperm of uninfected individuals, possibly by displacement, or by preferential use of sperm of infected males by females. Here we show that female flies are more likely to remate after copulating with an infected male than an uninfected male. In addition, a female whose second mate is uninfected produces more progeny than one whose second mate was infected. These data suggest female resistance to manipulation by infected males, both because females create an opportunity for cryptic female choice by remating, and because infected males do less well as second mates. Interestingly, there is no evidence that females can determine the infection status of males prior to mating. Thus far, we have studied only the response of uninfected females to male infection status; infected females may behave differently. Additional modeling, as well as additional data contrasting response of infected vs uninfected females, is required to understand whether or not increased male fertility trumps cryptic female choice to increase persistence of the biparentally transmitted sigma virus.

### 79. Do Sponges Facilitate Transmission of Panulirus argus Virus 1 among Juvenile Spiny Lobsters?

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Florida Bay is characterized by a mosaic of habitat types but is dominated by seagrass beds and hard-bottom substrates. Spongedominated hard-bottom habitat exhibits greater Panulirus argus Virus 1 (PaV1) prevalence than seagrass beds, demonstrating a potential relationship between habitat structure and PaV1 transmission. PaV1, the first naturally occurring virus known to infect lobsters, is in most life history stages of the Caribbean spiny lobster Panulirus argus including the early benthic juvenile (EBJ) stage. EBJs were used in this sentinel study to determine if sponges act as PaV1 vectors. We hypothesized that sponges, around which healthy and infected lobsters reside, may facilitate transmission of PaV1 via their water filtration activity. Prior to deploying EBJs in the Florida Bay, we used a PCR assay to test for PaV1 infection. Healthy EBJs were then suspended in cages above vase sponges Ircinia campana, and diseased (or healthy control) lobsters were tethered to the base of each sponge. After three days, EBJs were removed and re-assayed for PaV1 infection. Preliminary trials showed that EBIs in two of the three disease groups contracted PaV1. However, several lobsters in the control groups also contracted the virus, indicating that sponges may act as vectors for PaV1 regardless of the proximity of an infected lobster. These results are clearly equivocal, but additional replication and a no-sponge treatment are needed to confirm these findings.

### 80. NOVEL CYCLIC PEPTOIDS TO TARGET HIV RNA FOR AIDS THERAPY

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In the three decades since the isolation of HIV, the AIDS pandemic remains one of the most devastating diseases in human history. Millions of people continue to be infected every year, and much still needs to be done in the global fight against HIV and AIDS. The development of a highly efficient drug with less toxicity and low cost is in urgent need. This proposal will focus on developing

innovative cyclic peptoids to target HIV TAR RNA as antiviral drugs. An efficient and facile synthetic method to generate a library of compounds for quick structure-activity relationships (SAR) study is proposed here for comparative screening of libraries of synthetic molecules against HIV RNA for their inhibitory effects. By integrating chemical synthesis with the studies on the mechanism of functions of cyclic peptoids in HIV development, our approach will significantly decrease the amount of time and effort required to screen chemical libraries to target HIV RNA. Our studies will have a high impact on developing novel and specific methods for the treatment of HIV.

# 81. New introductions of Colletotrichum spp. causing anthracnose on lucky bamboo (Dracaena sanderiana) and Cordyline (Cordyline frut

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Increasing international trade in ornamental plants has resulted in the inadvertent introduction of new plant pathogens and pests into the United States. For example, the Asian tiger mosquito (Aedes albopictus), a vector of several serious human virus diseases, entered the U.S. on lucky bamboo imported from China in 2001. Similarly, imported lucky bamboo and other ornamental plants could carry various plant pathogens without being noticed at the port of entrance into the U.S. In 2012 and 2013, imported lucky bamboo (Dracaena sanderiana) and Cordyline plants (Cordyline fruticosa) 'Hawaiian Ti' that developed anthracnose symptoms were found in Gainesville, Florida. Colletotrichum spp. was isolated and Koch's postulates were fulfilled. PCR was performed on the ITS and 28S rDNA regions of the isolates, and the sequences were compared with those of Colletotrichum spp. in GenBank. Sequence analysis indicated that the Colletotrichum isolates from D. sanderiana and C. cordylinicola belonged to C. dracaenophilum or C. gloeosporioides species complex, and C. cordylinicola, respectively. C. dracaenophilum and C. cordylinicola were new to Florida and the U.S. Latent infections on both plant species developed into anthracnose lesions within 2-3 months, and 25-43 % of healthy-looking lucky bamboo and 34 - 44% of asymptomatic 'Hawaiian Ti' plants became diseased in 3 months. Colonies of C. dracaenophilum were white and became pale pink in the center, C. gloeosporioides was grey with scattered yellowish pigments, and C. cordylinicola was orange, with slight shades of pink and light mouse grey aerial mycelium on PDA. Sclerotia and setae were present on C. dracaenophilum and C. gloeosporioides, but were absent on C. cordylinicola. Application of the systemic fungicide Azoxystrobin at 0.075 g a.i. L-1 significantly reduced anthracnose development on both latently infected and inoculated lucky bamboo plants. Thus, it is recommended that lucky bamboo cuttings are treated preventively with this or similar fungicides.

### 82. COMMUNITY AND ACADEMIC PARTNERSHIPS TO SUPPORT SEAFOOD SAFETY IN THE GULF OF MEXICO AFTER THE DEEPWATER HORIZON OIL SPILL.

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This grouping of poster presentations describes a range of interactions between our Healthy Gulf Healthy Communities research team, and our community partners along the Northern Gulf of Mexico, together focusing on seafood safety following the Deepwater Horizon oil spill. Community engagement is a critical aspect of this research effort so that we can functionally implement risk assessment and risk communication relative

potential exposure to Gulf oil spill-related contamination of seafood amidst the complexity of issues and diversity of target audiences. Analytical toxicology of inshore-caught seafood species and community-specific food frequency data from different coastal Gulf regions are used for risk assessment. Community participation in Cedar Key, Steinhatchee, Eastpoint, Apalachicola, Pensacola and Mobile Bay has been vital in supporting the design and implementation of field sampling and data collection, and understanding similarities and differences between the different communities in addressing a range of environmental and public health concerns relative to the oil spill. Multiple elements of communication are used to share technical content in ways that are relevant to a variety of audiences including seafood workers and fishers who depend on Gulf seafood harvest for their livelihood. Community communications provide scientific context and explanation regarding communications from BP, and federal and state agencies, processes applied to regulate fishery closures and re-openings, the confounding effects of climate change, disease and a challenging economy. Longer-term community engagement has led to a level of trust in these different communities and supports the ability to meaningfully review and translate for lay audiences analytical toxicology data relevant to regional seafood. Thanks to support from the National Institute of Environmental Health Sciences this project incorporates expertise from UF in the areas of aquatic pathobiology, analytical toxicology, risk assessment, risk communication, food science and human nutrition, biostatistics, environmental education and community engagement. We also thank our community partners, the many Gulf coast citizens, and academic research colleagues (including Amber Barnes, Joe Bisesi, Babette Brumback, Helena Chapman, Nicole Darrow, Georgia Hinkley, Marianne Kozuch, Sam Mathews, Mary Leigh Morris, Nnanyelugo (Gerald) Odezulu, Candice Lavelle, Kaitlyn Summerfield, Cullen Roberts, Jessica Rowland,

Luke-Trimmer Smith and Marissa Valentine) for their most able laboratory and field contributions.

# 83. SPLENIC MACROPHAGE AGGREGATES IN RED SNAPPER: POTENTIAL BIOMARKER OF PAH EXPOSURE POST-DWH OIL SPILL

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Macrophage aggregates (M $\Phi$ ) are distinctive groupings of lymphocytes and macrophages that can be histologically observed in the spleen, liver, kidney and other organ systems of many fishes. Macrophage aggregate numbers and volume may increase in response to certain environmental contaminant exposures, and have been applied as reliable biomarkers of exposure. The purpose of this study was to discern the utility of altered splenic M $\Phi$  severity in red snapper (Lutjanus campechanus ) as a biomarker of exposure to polyaromatic hydrocarbons (PAHs) after the Deepwater Horizon oil spill. Red snapper were collected in 2011 and 2012 from the Northern GoM and fresh tissues were preserved for histology. Stained spleen tissues were microscopically observed for M $\Phi$  aggregate area,

frequency, and size distribution, and scored for severity on a scale of 0-5. These data were evaluated relative to location of capture, fish condition index and hepatic polyaromatic hydrocarbons (PAHs). No differences in MΦ aggregate severity ranks were observed between the various locations of capture (F=0.668, p=0.57). A positive correlation between splenic M $\Phi$  severity and fish total length (p=0.05) was observed, but not for fish weight (p=0.15) indicating that larger fish have a lower condition index. Splenic MΦ aggregate severity appeared predictive of hepatic PAH burden (F=4.71, p=0.004), indicating the utility of M $\Phi$ aggregate evaluation to discern levels of PAH exposure and uptake in red snapper. This study provides important precursor knowledge for longer term assessments of chronic effects of PAHs on fish communities in the Northern GoM. Results also bridge environmental and public health concerns associated with the health of Gulf of Mexico fisheries, its seafood, and our perception of Gulf ecosystem resources. Portions of this study were supported by the Florida Fish and Wildlife Conservation Commission, and the UF Aquatic Pathobiology Laboratories

### 84. 3D PRINTING OF BIOLOGICAL CONSTRUCT FOR PATHOGEN EVALUATION

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Pathogen evaluation involves a series of in vitro and in vivo studies to evaluate the effects of various known as well as unknown pathogens in their hosts. By combing unique properties of microfabricated three-dimensional (3D) cellular constructs and cell-based biological assays, we aim to develop 3D in-vitro organon-a-chip micro-devices to simulate the relevant human organ systems for pathogen detection and evaluation. Vascularization is often identified as a main technological barrier for building 3D living organs. As such, the fabrication of 3D biological vascular trees is of great importance for the overall feasibility of the envisioned organ-on-a-chip micro-device. Using drop-on-demand inkjet technology, we have successfully free-form fabricated 3D zig-zag NIH 3T3 fibroblast-based alginate tubular structures which mimic the simple anatomy of blood vessels. The postfabrication cell viability was above 85% even after a 24-h incubation period. As a proof-of-concept study, the resulting fabrication knowledge helps fabricate organ-on-a-chip microdevices with complex geometries for pathogen-related studies.

### 85. PRIMARY ENVIRONMENTAL DRIVERS AND TEMPORAL VARIABILITY OF HARMFUL ALGAL BLOOMS (HABS) IN WESTERN LAKE ERIE

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Understanding primary environmental drivers and temporal variability of harmful algal blooms (HABs) is important for guiding HABs impacts mitigation plans in Lake Erie. The objective of this study is to explore the primary environmental driving factors and characterize temporal variability of chlorophyll a (Chl) and Phycocyanin (PC), which are determinants of HABs, in western Lake Erie. Ten years' biweekly chlorophyll a (Chl) and Phycocyanin (PC) at 15 sampling locations near coast of western Lake Erie were derived from the MERIS data. 14 environmental variables, covering the same period, were also collected. A Multivariate Adaptive Regression Spline (MARS) approach was used to predict Chl and PC and to identify significant environmental factors influencing Chl and PC at different seasons and locations. Wavelet analysis was employed to describe and identify temporal patterns of bi-weekly Chl and PC concentrations over the entire western Lake Erie. Estimates of Chl and PC showed different predictabilities and differences in importance of selected environmental drivers at different locations and seasons using MARS. For Chl, the most frequently selected environmental drivers are soluble reactive phosphorus concentration in spring, streamflow in summer, silica concentration in fall, and water temperature in winter. For PC, the most frequently selected environmental factors are solar radiation in spring, water temperature in summer, silica concentration and wind speed in fall, and water temperature in winter. Wavelet analysis suggested that Chl and PC showed strong seasonal and inter-annual pattern - the 3-year mode dominates the observations for Chl and PC, respectively.

#### 86. DEFINING PROACTIVE DISEASE SURVEILLANCE IN FLORIDA

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Proactive surveillance is defined as a program that can detect unanticipated or unknown pathogens in addition to those that are anticipated. For zoonotic disease surveillance, this type of approach is especially important because of the diversity of hosts and vectors that can contribute to disease emergence. Florida, in particular, is a state that holds a high diversity of native faunal species as well as a nearly unprecedented diversity of exotic invasive species, all of which can contribute to the risk of disease emergence. To test the utility of this approach to detect unanticipated threats to public health, we employed a broad spectrum, commercially available microarray capable of detecting >6500 pathogens whose sequences are accessioned in Genbank. We extracted total nucleic acid from the blood and feces of 35 feral swine collected from throughout the southeastern United States and used the microarray to detect possible pathogens in those samples. We had > 149 positive hits for a diversity of bacterial, viral and fungal microbes. We are currently in the process of confirming the positive detections with conventional PCR. We have confirmed the presence of a tick-borne Flavivirus within the state of Florida. Neither deer tick virus nor Powassan virus have previously been reported in Florida, but are public health concerns in the northeastern United States. Confirmation of positive detection of Leishmania spp. and several viruses of interest to the livestock industry remain to be completed. While the gold standard technique of proactive pathogen surveillance is next generation sequencing, bioinformatics pipelines capable of handling millions of sequences from hundreds or thousands of

samples remain elusive. Nonetheless, our broad spectrum microarray approach confirms the utility of developing proactive surveillance programs for the protection of public health in Florida and elsewhere.

# 87. FOOD FREQUENCY QUESTIONNAIRE TO DISCERN SEAFOOD CONSUMPTION PATTERNS IN NORTHERN GULF OF MEXICO COMMUNITIES

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The Deepwater Horizon oil spill had notable direct and indirect outcomes on coastal Gulf of Mexico communities. To estimate impacts on commercially harvested Gulf seafood, the US FDA conducted a risk assessment based on standard assumptions and methods. Regulatory agencies concluded that Gulf-harvested seafood was safe to consume, yet varying degrees of distrust by Gulf coast residents persisted. This distrust, in part, was associated with conclusions and policy decisions based on assessments that may not adequately reflect risk in individual

coastal communities. Use of national average statistics and lack of toxicity adjustment for early-life susceptibility have detached the risk assessment from the coastal communities most impacted by the oil spill. In an effort to optimize risk assessment and include important regional differences, we developed a food frequency questionnaire (FFQ) to analyze community-specific body weight and seafood consumption patterns, including noncommercial inshore species. A pictorial seafood portion guide for shrimp, fish and crab was validated and used as a resource for implementing the FFQ. A graphic guide to inshore gulf fishes was also produced to aid in survey implementation and optimize accurate identification of finfish harvest and consumption data for the study. In the first phase of implementation we observed a significant difference in the body weight and seafood consumption rates within Gulf communities as compared to the national average. Results show that Gulf coastal residents weigh as much as 5.4% less and consume as much as 492% more than the national average, based on NHANES data. Household data amassed from this FFQ will then be used to perform risk assessment for multiple Gulf communities on a regional scale. Additional questionnaire components address the perception of Gulf seafood safety and may reflect difference in resiliency between different coastal communities. These efforts were supported by a grant from the National Institute of Environmental Health Sciences, the UF Center for Environmental and Human Toxicology, and the UF Aquatic Pathobiology Laboratories.

### 88. ASIAN FOOD SAFETY TRENDS IN THE UNITED STATES: EXAMINING CDC DATA FROM 1990-2008

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Engineering Department; Wei-Yea Hsu - Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida, Department of Family, Youth and Community Science Department; Maurice Marshall - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida, Food and Environmental Toxicology Lab; Amarat Simonne - Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida, Department of Family, Youth and Community Science Department

Foodborne illness outbreak data reported by the CDC were cross checked, analyzed, and ranked based on number of outbreaks, cases per outbreak, etiology, outbreak location, and food vehicles to examine the food safety trends associated with one of the top three ethnic foods in the United States, Asian foods. From 1990 to 2008, 8.7 % of the total foodborne disease outbreaks in the U.S. (17, 640 outbreaks) were associated with the top three ethnic food categories (Italian, Mexican, and Asian). Asian foods represented approximately 20.6% of outbreaks (315 outbreaks) and 9.6% of cases (3529 cases) among the total foodborne disease outbreaks associated with the top three ethnic foods. During the 18-year period, fewer cases per outbreak occurred for Asian cuisine than for Italian and Mexican cuisines. The majority of outbreaks from Asian foods occurred in restaurants and delicatessens (60%), and was caused by unknown etiologies (62.2%). Bacterial agents were the most prevalent of the known etiologies (77%), followed by viruses (18%) and chemicals and toxins (5%). The bacterial etiologies identified included Bacillus cereus/Bacillus (45%), Salmonella spp. (33%), Staphylococcus spp. (16%), Clostridium spp. (3%), Campylobacter spp. (1%), Vibrio parahaemolyticus (1%), and Plesiomonas shigelloides (1%). Asian foods most frequently associated with illness were Asian-style cooked and fried rice (40%), sushi (15%), noodle and lo mein dishes (8%), and eggrolls and spring rolls (7%). This

epidemiological analysis suggests the need for further examination of special issues concerning ingredients, preparation, cooking, serving, and handling of Asian foods in the United States.

# 89. DEVELOPING, CONNECTING AND PROMOTING RESEARCHERS AND THEIR WORK IN SEX AND GENDER DIFFERENCES IN HEALTH

Nancy Schaefer - University of Florida, (UF) Health Science Center Libraries; Hannah F. Norton - University of Florida, (UF) Health Science Center Libraries; Michele R. Tennant - University of Florida, Health Science Center Libraries; Rolando Garcia-Milian - University of Florida, (UF) Health Science Center Libraries; Mary E. Edwards - University of Florida, (UF) Health Science Center Libraries

**Objectives**: This poster presents accomplishments and projected goals from two rounds of funding for the UF Health Science Center Libraries from the National Library of Medicine (NLM) and the Office for Research on Women's Health (ORWH). The aims of the project are to facilitate the growth of basic research in sex and gender differences in health and to help develop a diverse workforce with the ability to recognize these differences and appropriately apply them to healthcare. **2013** 

Accomplishments: To increase access to research in the areas of sex and gender differences, librarians added \$5,000 in new journals and books to the UF Health Science Center Libraries' collection, presented specific resources of the NLM and ORWH in classes, and created a resource guide that highlights sources, search tips, and CE opportunities in this area. They also increased access to current research and assisted UF researchers with dissemination of their work in this area by financially supporting seven open access publications. Collaboration workshops held in the spring and fall of 2013 enabled researchers from across campus who are interested in this area to meet and share ideas,

news and resources. **2014 Goals**: The librarians will add two new journals on sex and gender differences in health, host collaborative workshops in the spring and fall of 2014 and offer funding to support publication of open access articles in this subject that are authored by UF faculty, students, residents or fellows. New activities will include the hosting of a day-long workshop in the fall of 2014, enhancing researcher records in VIVO and depositing research product into the UF Institutional Repository. Librarians will collaborate with the Center for Pre-Collegiate Education and Training and with genetics and public health faculty to present in their classes and will present resources in departments with a strong interest in the topic. **Conclusions**: By creating comprehensive collection and access plans (collection building, open access funding, depositing research product into UF's Institutional Repository), the library is partnering with both external agencies and internal campus collaborators to facilitate access to and dissemination of past and current research. Hosting additional CoLABs and a day-long workshop and enhancing researcher records in VIVO can spur research collaboration on campus and lay the foundation for researcher and student engagement in this area of inquiry.

#### 90. USING GENOTYPE DATA TO DETECT FREQUENCY OF THE A-VARIANT OF THE GLUCOSE-6-PHOSPHATE DEHYDROGENASE GENE IN HAITI

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Florida; **Connie Mulligan** - Department of Anthropology, UF Genetics Institute, College of Liberal Arts and Sciences

Haiti is a country on the island of Hispaniola that is a candidate for the eradication of malaria. This disease affects 30, 000 to 200, 000 individuals in Haiti every year. As a preventative measure a single dose of primaguine has been added to the Haitian malaria treatment policy. Primaquine is used to treat the gametocyte stage of the parasites life cycle in order to block transmission of the malaria. A complication with primaguine may arise in individuals with glucose-6-phosphate dehydrogenase deficiency (G6PDd), a genetic disorder caused by mutations in the G6PD gene. Individuals with G6PDd can suffer from acute hemolytic anemia when administered primaquine. The Haitian population has high West African ancestry, and the most prevalent G6PD genetic variant in West African populations is the A- variant. Thus, we are investigating the frequency of the A- variant in Haitian G6PDd individuals. As part of a larger investigation into rapid diagnosis of G6PD deficiency in Haiti, we used DNA genotyping to detect the G6PD deficiency A- variant in a sample of G6PDd individuals (as determined by standard spectrophotometric assay) seeking treatment. A total of 124 G6PDd individuals were included from four health center sites in the Southeast and West Departments of Haiti. Our research demonstrates that 69.3% of the samples carry the A- variant. Interestingly, only 43.8% of the individuals who are categorized as class I or II deficient (e.g. very severely deficient or severely deficient according to World Health Organization classifications) carry the A- variant. Although the A- variant is the primary cause for the increased risk of hemolytic anemia in G6PDd individuals, it is also useful to look at other variants. In our study we found that 9.6% of G6PDd individuals carried the G6PD A+ allele, which lowers G6PD levels less that the A- variant. In sum, these results suggest that there are other mutations within the Haitian population that cause G6PDd. Further investigation is needed to determine the other G6PDd variants that exist in Haitian

populations in order to identify all individuals with G6PDd-mediated risk of acute hemolysis upon primaquine treatment for malaria.

# 91. WATER-RELATED INFRASTRUCTURE IN POST-EARTHQUAKE HAITI: HIGH LEVELS OF FECAL CONTAMINATION, AND NEED FOR ONGOING MONI

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Background: The Leogane and Gressier Districts of Haiti were at the epicentre of the January 2010 earthquake. With the identification of cholera in October, 2010, much of the international aid coming to the region was redirected toward provision of potable water. Methods: Using community health workers, we inventoried non-surface water sources in the region (ca. 270 sq. km.) in 2012/2013, and screened water from 359 sites for fecal coliforms and Vibrio cholerae. Findings: Most international (IO) and non-governmental organizations (NGO) have now left the region. An IO/NGO responsible for construction could be identified for only 56% of 377 water points evaluated; 16% of water points were non-functional at any given time, and 37% had evidence of fecal contamination, with spatial clustering

of contaminated sites. Twenty-five percent had evidence of an entity responsible for management; however, having such an entity did not change the risk of fecal contamination. Among improved water sources (82% of sites), 24.6% had fecal coliforms, versus 80.9% of unimproved sources. In a subset of 46 water sources sampled at multiple time points, fecal contamination levels increased significantly from 36% to 51% immediately after passage of tropical storm Sandy in October 2012, with a return to 34% contamination in March 2013. While no toxigenic strains of cholera were identified, non-toxigenic V. cholerae non-01/non-0139 was isolated from 6 fecallycontaminated water sources. **Interpretation**: Provision of safe water requires ongoing monitoring/maintenance, including assessment of changing patterns of fecal contamination associated with weather events. While drilling wells in emergency situations is of potential value, long-term sustainability of potable water delivery at a regional scale depends on planning mechanisms that provide a better understanding of the quality of water drawn at these sources, functionality, and community-based management schemes supported by a national plan for the management of potable water.

# 92. Investigating the Influence of Oral Exposure to Silver Nanoparticles on Intestinal Microbiota in Non-Human Primates

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Due to the growing use of nanoparticles in foods, pharmaceuticals and personal care products, it is important to consider possible effects following oral exposure to these materials. Silver nanoparticles (AgNPs) are being utilized in various applications due to their antibacterial properties, including food packaging, agua culture and drinking water treatment. The antibacterial properties of AgNPs have been demonstrated by several groups, leading our group to investigate their affect on commensal intestinal microbiota following oral administration. In this study, non-human primates (n=5) were given 20 nm AgNPs in 10mL of Gatorade (62.5 µg/mL) once daily for 8 days. Feces were collected on the days before and after the treatment regime, as well as 1 week after treatment stopped; feces were collected for similar time points during a vehicle-control treatment (Gatorade only). High throughput 16S rRNA sequencing was used to determine if AgNP treatment had an influence on the overall microbiota diversity and prevalence of specific operational taxonomical units (OTUs). When investigating overall diversity at the phylum level no significant differences were observed. However, when analyzing specific OTUs at the 98% similarity level, we observed more than 30 in which the number of sequences increased upon nanoparticle exposure but only three in which sequence numbers decreased. In contrast the number of OTUs with significant increases/decreases was similar for the vehicle-control treatment. This observation suggests that AgNPs select for, rather than against particular gut bacteria. Corresponding health impacts may be difficult to predict but the impact of AgNPs ingestion on intestinal microbiota should be considered in future applications.

### 93. In-vitro Uses of Microdialysis to Measure Unbound Drug Concentrations

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**Objectives**: Microdialysis is a minimally-invasive sampling technique which enables measurement of unbound drug concentrations and this technique has been extensively used in humans and animals including some novel usages such as plasma protein binding determinations, cytokine/biomarker sampling from tissues and delivery of drugs. Methods: The in vitro microdialysis may be used for the determination of unbound concentrations in isolated tissues, plasma, cell culture systems and bacterial culture without affecting the test system. This technique is a relatively new technique employed for the determination of plasma protein binding, which was largely determined by ultrafiltration technique due to its high throughput. However, ultrafiltration carries several disadvantages and may not be used in circumstances, e.g. determination of unbound concentration in cell culture or bacterial cultures. While centrifugal force used in ultrafiltration may rupture cells, determination of unbound concentration in highly pathogenic bacterial cultures may be practically challenging using ultrafiltration. Additionally, the sample volume required by ultrafiltration is a major limitation in many situations. Results: In our lab, we have used microdialysis technique in different situations where use of ultrafiltration was difficult. In our current work we present use of microdialysis in different circumstances and compare it with the ultrafiltration technique, if possible. In-vitro microdialysis technique was applied to measure the unbound concentration of a selected antibiotic agent. The experiments were performed using a microdialysis probes CMA63 and the infusion rate was optimized

to exhibit satisfactory probe recovery. Probe recovery was estimated using extraction efficiency technique. The entire set up was maintained at 37±1 °C during the sampling and recovery experiment. **Conclusion**: We have successfully used the microdialysis technique in different situations where ultrafiltration was not a viable technique. One of the major limitations of microdialysis technique is that the drug should be dialyzable.

### 94. THE EFFECT OF PEA HULL FIBER ON MICROBIOTA COMPOSITION

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Gut microbiota has been associated with proper digestive function, immunity, and other metabolic functions. Microbiota differs among individuals and over time, and it is influenced by environmental factors, including diet. Distortions in microbiota composition have been linked to inflammatory disorders, metabolic diseases, and conditions such as obesity. Dietary fiber is one of the main fuels utilized by gut microbes that results in the generation of various fermentation products such as short chain fatty acids. In our study, gut microbiota was analyzed to determine if added pea hull fiber influenced the composition of bacteria. Five healthy male participants completed a 45 day, fully controlled feeding, weight maintenance study during which they were provided a high protein diet. The study was separated into

three intervention periods of 15 days, and an additional sample was collected approximately 30 days after the last intervention. In the second period only, participants received 20 g of pea hull fiber in addition to the high protein diet. The addition of pea hull did not affect fecal microbiota composition in a consistent pattern. This may be due to small sample size. However, an alternative explanation may be that pea hull fiber is resistant to fermentation and therefore does not modulate microbiota. The proportion of bifidobacteria present after the washout period was significantly higher than during the study, suggesting that the high protein diet suppressed numbers of bifidobacteria. Supplementation with bifidobacteria may help to maintain a healthy microbiota composition when consuming high protein diets. Further research is required to confirm the findings of this pilot study.

#### 95. DIURNAL OSCILLATIONS MODERATE TEMPERATURE EFFECTS ON SOME EPIDEMIC COMPONENTS OF PHYTOPHTHORA INFESTANS

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Temperature has profound effects on potato late blight (Phytopthora infestans) development under constant temperatures but epidemic studies have failed to incorporate daily temperature oscillations. Besides increases in global average temperatures, changes in temperature amplitudes are predicted. We hypothesized that optimum temperature curves for various growth and development rates of P. infestans would be

flatter at increasing daily temperature amplitudes, and investigated effects of seven constant (10, 12, 15, 17, 20, 23, 27°C) and diurnally oscillating temperatures (±5°C and ±10°C) around the same means on epidemic components: number of lesions, incubation period, latent period, lesion growth rate and sporulation intensity for two P. infestans clonal lineages, US-8 and US-23. A four parameter thermodynamic model was used to describe relationships between temperature and disease development rates. We confirm the hypothesis that there are accelerated development rates at low mean temperatures with increasing oscillations, but slower development rates at high mean temperatures with increasing oscillations compared to constant temperatures (P<0.005). There were no significant differences between the clonal lineages for incubation development rate and latency progression rate (P>0.10). However, there were significant interactions (P<0.005) between clonal lineage and temperature amplitude for number of lesions formed, lesion growth rate and sporulation intensity. The 5°C amplitude effect on number of lesions compared to constant temperatures was greater for US-8 than for US-23, while the 5°C amplitude effect on sporulation intensity was greater for US-23 than for US-8. For lesion growth rate, the 10°C amplitude shifted the optimum temperature to a lower range for US-8 than for US-23. In conclusion, the oscillating temperatures flattened the optimum curves for incubation and latency progression rates compared to constant temperatures similarly for US-8 and US-23, while the optimum curves of other epidemic components became more pronounced (less flat) under oscillating compared to constant temperatures depending on the clonal lineage used.

### 96. EVIDENCE OF GEOGRAPHIC AND TEMPORAL VARIATIONS IN COMMUNITY ANTIBIOTIC USE, FLORIDA, 2010-2012

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Antimicrobial resistance is a worldwide problem posing a serious threat to human health. In 2013, the CDC conservatively estimated that in the US alone two million illnesses and at least 23, 000 deaths were directly the result of antibiotic resistant infections. Each year many more die from complications due to antibiotic resistant organisms and are sickened by infections such as Clostridium difficile in which antibiotic use is a contributing factor. In addition to the cost in human life, annual financial impact has been estimated at \$20 billion in direct healthcare costs and \$35 billion in loss productivity. Antibiotic use is the most important contributing factor to the development of antibiotic resistance. It has been demonstrated that half of all antibiotics prescribed are unwarranted or improperly prescribed. In recent years, antibiotic stewardship has effectively reduced unwarranted antibiotic use in US hospitals. Subsequently, we have observed a reduction in the incidence of some antibioticresistant pathogens (e.g. Methicillin-resistant Staphylococcus aureus) in that setting. However, best practices for antibiotic stewardship have not carried over to the community, where rates of antibiotic resistant infections have remained largely unchanged. Largely, limitations in knowledge of trends in community antibiotic use have resulted in the absence of targeted public health interventions. To address this gap, we obtained 24 months of community antibiotic prescription data from IMS Healthcare Solutions Xponent data system. We investigated regional and seasonal variations in antibiotic prescription patterns for major drug classes. In total, there were 15, 196, 983 antibiotic prescriptions for 2011 equating to a prescribing rate of 0.81 prescriptions per person. We observed the highest

prescription rates for Δβ-lactam antibiotics; however, prescribing patterns varied across Florida counties by healthcare provider composition and demographics. Children aged 1-4 years old received the greatest proportion of antibiotics. Additionally, several antibiotic classes demonstrated seasonality with prescriptions peaking in winter months, coinciding with seasonal peaks in the incidence of viral respiratory infections. Overall, these data demonstrate variations in healthcare provider prescribing practices and highlight communities as well as provider groups in which direct public health messaging should be aimed.

# 97. MOSQUITO TOXICITY AND PHARMACOLOGICAL EFFECTS OF FLUORINATED COMPOUNDS ON GAMMA-AMINOBUTYRIC ACID-ACTIVATED CHLORIDE CHANNEL

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It is well established that the chloride current mediated by gamma-aminobutyric acid (GABA) receptors plays an important role in the physiological activities of insects, where it serves as the principal inhibitory neurotransmitter in the nerve and muscle systems. For this reason, it has been exploited as a target in the research and development of insecticidal compounds. Here we studied the insect toxicity and GABA receptor blocking activity of fipronil, a commercial insecticide, and three new compounds with related structure (fluorinated phenylpirimidinones named SGA-A and SGA-B, and RPA 98283, which is deaminated fipronil). Toxicological tests on Aedes aegypti and Drosophila

melanogaster included surface contact exposures in glass tubes, as well as additional topical treatments to adult female mosquitoes. The surface contact glass assay showed that the toxicity of the SGA compounds were 10 times greater than fipronil on both Drosophila (LC50: fipronil: 6, SGA-A: 0.6, SGA-B: 0.7, RPA 98283: 0.017, ng/cm2), and Aedes aegypti (LC50: fipronil: 9, SGA-A: 0.6, SGA-B: 1.1, RPA 98283: 0.02, ng/cm2). However, in topical treatments of Aedes aegypti fipronil was more toxic than the SGA compounds (LD50: fipronil: 0.31, SGA-A: 4.4, SGA-B: 31, RPA 98283: 0.43, ng/mosquito). Thus, fipronil seems to bind avidly to glass, reducing its effectiveness on this surface. Electrophysiological assays tested these compounds on the GABAA receptors in rat cortical neurons using whole-cell patch-clamp. Our results showed that co-perfusion with the compounds could significantly decreased the time constant of the descending phase of GABA-induced current (IC50: fipronil: 5.4, SGA-A: 1.3, SGA-B: 27, µM), as well as the persistent current amplitude (IC50: fipronil: 4.3, SGA-A: 2.6, SGA-B: 19, μM). They also generated these IC50s (fipronil: 6.8, SGA-A: 3.1, SGA-B: 22, μM), when effect was measured as reduction in total current (area under the curve). Because potencies on rat neurons were much lower than that reported for fipronil on insects, it suggests these compounds will have lower mammalian toxicity.

# 98. OOCYTE EXPRESSION OF NATIVE RECEPTORS FROM INSECT MEMBRANES AS AN EXPERIMENTAL PLATFORM FOR NEW INSECTICIDE DISCOVERY

Roopali Yadav - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; Steeve Thany - Université d'Angers, 2Laboratoire Récepteurs et Canaux Ioniques Membranaires (RCIM); Jeffrey Bloomquist - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida We recently initiated studies of isolated membranes injected into Xenopus laevis frog oocytes as a method for expressing native insect receptors in a convenient experimental platform without genetic engineering. The impetus for this project is that it has proven impossible to express functional insect nicotinic acetylcholine receptors in cells or oocytes without using a mammalian subunit as part of the receptor. Thus, "pure" insect receptors suitable for pharmacological study have proven elusive. For these experiments, nerve membranes either from cow brain or from cockroach nerve cord were prepared using a standard protocol and injected into Xenopus oocytes. The injection volume was kept at 50 nl, and 2-6 hr after injection two electrode voltage clamp recordings were done and currents recorded. Controls involved Xenopus oocytes injected with an equal volume of the final suspension buffer alone or with DNAse and RNAse free distilled water. Initial results from the expression of cow brain membranes in Xenopus oocytes found the presence of GABA currents upon application of 100 µM GABA to the bath. These currents disappeared upon washing with drug-free ND96 solution. We further tested for the presence of nicotinic and muscarinic currents and observed small currents upon application of 100 µM acetylcholine to the bath. These results demonstrate the expression of GABA and muscarinic acetylcholine receptors (no response to nicotine) from frozen cow brain synaptosomes. We have also tested the expression of insect membranes from cockroach nerve cord in Xenopus oocytes, and found the expression of acetylcholine receptors (presumably nicotinic subtype) and GABA receptors. We saw small inward currents upon application of 350 µM acetylcholine and 100 µM GABA. We plan to conduct similar studies with membranes prepared from mosquitoes.

### 99. FABRICATION AND APPLICATIONS OF LAMINATED PAPER-BASED ANALYTICAL DEVICES (LPAD)

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Inexpensive, portable, disposable devices are highly desirable in both clinical settings as well as resource-poor regions. A great deal of attention has been devoted to the development of microfluidic platforms to fill the needs of low-cost disposable devices, however, traditional microfluidic devices have several drawbacks that make truly field-usable-devices difficult to produce. More recently, interest in paper-based microfluidics has increased with myriad devices constructed to conduct various assays, because paper has several benefits over traditional microfluidic materials that make it of particular interest within this context. First, using paper as a substrate simplifies a microfluidic system by using capillary action for fluid transport, thus eliminating the need for accessories such as pumps. Second, paper has a long history of use as an analytical platform, and therefore a wide repertoire of compatible assays already exist. Finally, paper is inexpensive, widely available, and derived from renewable resources. By and large these devices have been produced by patterning hydrophobic zones in hydrophilic paper via photoresist or wax, or simply by cutting paper using a laser. Here we present a fabrication method for producing devices by simple craft-cutting and lamination that makes it possible to rapid-prototype laminated paper-based analytical devices (LPAD). Devices constructed using this method have been exploited for simultaneous colorimetric detection of bovine serum albumin (BSA) and glucose in synthetic urine as well as chemiluminescent detection of cotinine in mouse serum. The colorimetric assays are capable of detecting BSA and glucose at

clinically relevant concentrations, with the detection limit of 2.5  $\mu\text{M}$  for BSA and 0.5 mM for glucose, while the chemiluminescent assay was able to detect concentrations as low as 5 ng/mL. The devices have a potential to be used for detecting pathogens such as dengue virus.

### 100. ESTIMATING THE IMPACTS OF ROTAVIRUS VACCINATION ON GENDER DISPARITIES

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This study aims to assess the gender disparities in rates of vaccination coverage amongst girls and boys in India over time, and estimate their potential impacts on the introduction of rotavirus vaccination, across various geographic and household economic settings. A Microsoft Excel based spreadsheet model is used to model the expected health and economic outcomes disaggregated by gender of the child, for one annual birth cohort of children during the first five years of life. Three data sets have been used in the model: National Family Health Survey-3 2005-6. District Level Health Survey-3 2007-8 and Coverage Evaluation Survey 2009 to estimate the changes over time, in 3 highest mortality states and 6 regions of India. There is an overall increase in vaccination coverage in India. The gap between boys and girls in vaccination coverage over time is estimated to have reduced. Increase in gender parity is estimated to increase in benefits and decrease in cost effectiveness ratio per DALY. It is of immense importance to sustain the efforts of bridging the gender gap in health care utilization and creating enabling conditions to introduce the proposed universal rotavirus vaccination.

Preferential treatment to boys increases the risk of mortality amongst girls along with increasing the cost of implementation of vaccination programs.

### 101. CHOLERA CONUNDRUM: TEACHING HIGH SCHOOL STUDENTS ABOUT CHOLERA

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During the summer of 2011, the University of Florida's Center for Precollegiate Education and Training's (CPET) ICORE: Emerging Pathogens program for secondary science teachers modified an existing activity on cholera to incorporate key elements of the cholera outbreak in Haiti. For the past 2 years of programs, this activity has been revised to make it more realistic, bringing in UF research on the outbreak, as well as expanding the scope of the lesson. The activity begins with a broad introduction to pathogens through CPET's Menacing Microbes lesson, utilizing plush microbes to educate students on a wide variety of microbial organisms. Students then learn more about cholera through a webquest on the Center for Disease Control website, or through a talk from a UF cholera researcher. Following this introduction, all students are asked to act as aid workers testing simulated water samples for the presence of cholera. These samples are from different areas all over Port-au-Prince, and each student group looks for cholera detection through a dipstick rapid test, simulated in this activity with pH buffers and 4-pad pH indicator strips, the results of which students mark on a map of the region. Following a brief discussion of these results, students learn about antigens and antibodies through the different cholera biotypes. Again using simulated samples – this time two colorless solutions which form a precipitate when combined, students look for the

O1 type cholera in the same areas, mapping their results with the rest of their class. Both this part of the activity and the previous utilize results conducted by UF EPI researchers in Haiti. Students and teachers have additional opportunities through lesson extensions to discuss realistic methods of cholera control and barriers to managing the epidemic. CPET has conducted this activity with numerous teacher and student groups over the past 3 years, each commenting on the relevance of the topic, particularly with high numbers of Haitian students in Florida schools.

## 102. A PHARMACOKINETIC COMPARISON OF INTRAVENOUS MOXIFLOXACIN IN CYNOMOLGUS MACAQUE AND AFRICAN GREEN MONKEYS USING MONTE CARLO SIMUL

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Moxifloxacin, a fourth generation fluoroquinolone developed by Bayer AG was approved for human use by the FDA in 1999 for the treatment of serious and life threatening bacterial infections to include community acquired pneumonia and complicated intraabdominal infections. The majority of new generation antibiotics, such as moxifloxacin, do not have a Food and Drug Administration (FDA) indication for use as therapy against the bacterial biothreat agents. Prior to efficacy studies against these agents, the pharmacokinetic (PK) properties of moxifloxacin must be determined in perspective animal models. Four African green (AGM) and four cynomolgus macaque (CMM) monkeys were administered intravenous moxifloxacin using equivalent dosing regimens and blood samples were taken over 24 h for determination of drug levels for PK analysis. Plasma samples were analyzed for moxifloxacin concentrations using reverse phase high performance liquid chromatography (HPLC) with fluorescence detection. The NHP PK data was compared to a

human Monte Carlo simulation in order to devlope a humanized dosing regimen matching the lower 10% of the human population Area under the curve(AUC) value to meet FDA animal rule efficacy studies. Comparison of the predicted dosing data from CMMs to AGMs indicated that there were minimal differences in the dosing concentrations and no differences in the dosing schedule required to simulate the human AUC0-24. Based on these data both species are viable choices to use and are both acceptable predictors of humanized moxifloxacin dosing.

#### 103. DEVELOPMENT OF A METHOD TO ANALYZE FLUCONAZOLE AND BAICALEIN SIMULTANEOUSLY

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Fluconazole, a triazole antifungal, is much known in clinical practice. Baicalein is a flavonoid, generally ingested from daily dietary. Recently some authors related a supposal synergic effect between both compounds to kill fungi in an in vitro culture. In order to investigate the interaction between them in a Caco-2 cells model, has been necessary to develop a chromatographic system to quantify the fluconazole and baicalein. The method was established using a C18 column (125 mm x 4 mm), the mobile phase (a mixed of water and methanol containing 0.05 % trifluoacetic acid in each one) was a gradient elution mode. Flow rate was set at 1.0 mL/min. Detection wavelengths of fluconazole and baicalein were 205 nm and 275 nm, respectively. In conclusion, this study demonstrated that this gradient elution method is adequate to separate both compounds and for each one could be seen a linear behavior between area and concentration in a high range tested (1 to  $100 \mu g/mL$ ).

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