

# EPI RESEARCH DAY 2012

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# EPI RESEARCH DAY 2012

## Letter from the Director

Welcome to the fifth annual EPI Research Day! As you peruse the research at today's event, it is my hope that it will give you a feel for the wide range of emerging pathogens-related research conducted by EPI members and collaborators. I would say a particular word of thanks to UF investigators from outside of Gainesville, and collaborators with the Florida Department of Health.

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

Dr. Scott Halstead is recognized as one of the world's outstanding experts on dengue fever, with a rich lifetime of contributions to the epidemiology, pathogenesis, and immunology of this infection. He currently serves as Scientific Advisor, Dengue Vaccine Initiative, International Vaccine Institute, Seoul. Dr. Roger Glass, Director, Fogarty International Center and Associate Director of NIH for International Programs, is internationally recognized as an expert on viral enteric infections, and has been a leader in the development and implementation of global vaccine strategies. I greatly appreciate their contribution to today's activities.

Please visit our website, [www.epi.ufl.edu](http://www.epi.ufl.edu), to join our list-serves, and to keep up with our news, events and seminars throughout the year.

**J. Glenn Morris, Jr. M.D., M.P.H. & T.M.**

*EPI Director and Professor of Medicine*

# EPI RESEARCH DAY 2012

## Schedule of Events

9:00 AM – 10:00 AM	Breakfast (EPI Room 150) and Poster Set up (EPI Front Lawn)
10:00 AM – 1:00 PM	Poster Session (Presenters, please stand by your posters)
12:00 PM – 12:45 PM	Lunch (EPI Room 150)
12:45 PM – 1:00 PM	Keynote Assembly (Cancer Genetics Research Complex Auditorium 101)
1:00 PM – 1:15 PM	Welcome by Dr. David Norton, VP of Research and  Introductions by Dr. J. Glenn Morris, Director, EPI
1:15 PM – 3:15 PM	Keynote Speeches
3:30 PM – 4:00 PM	Remove Posters

# EPI RESEARCH DAY 2012

## Keynote Speakers

### **Dr. Scott Halstead, M.D.** **(1:15-2:15)**

Scientific Advisor, Dengue Vaccine Initiative  
International Vaccine Institute  
Seoul, Korea

**“Dengue is Winning: How Do We Turn the Tables?”**

### **Dr. Roger Glass, M.D., Ph.D.** **(2:15-3:15)**

Director, Fogarty International Center and  
Associate Director of NIH for International Programs  
Bethesda, MD

**“The Control of Rotavirus in the United States and Beyond: Current Global Challenges to Vaccine Introduction”**

## 01. DEVELOPMENT OF BEAD-BASED SUSPENSION ARRAY FOR DETECTION AND CHARACTERIZATION OF SALMONELLA

Soohyoun Ahn, Food Science and Human Nutrition, Agricultural and Life Sciences (Institute of Food and Agricultural Sciences), University of Florida; Muhsin Aydin, Molecular Biosciences Program, Arkansas State University

Salmonella is the leading cause of foodborne illnesses in the United States. Recent outbreaks associated with Salmonella-contaminated foods and related economic loss show the importance of timely control of this harmful pathogen. Because of deleterious effects of Salmonella on public health and economy, it is highly desirable to develop a detection method that can identify Salmonella in food before they reach the consumers. While conventional detection methods using culture or biochemical tests can identify pathogens with good sensitivity, they are laborious and time-consuming. Alternative methods (e.g. ELISA, PCR) can provide great sensitivity and rapidity, but often they suffer from a lack of specificity and limited multiplexing ability.

The goal of this study is to develop a sensitive and rapid identification system for Salmonella using bead-based suspension array. In this study, high multiplexing ability of bead-based suspension array was combined with PCR amplification to detect Salmonella with high sensitivity and specificity. In this study, mixture of 7 types of beads, each functionalized with different oligonucleotide probes designed from Salmonella-specific and serovar-specific genes were loaded into 96-well microplate and used as a bead-suspension array platform. The presence of Salmonella and their characteristics were confirmed by reading fluorescent signal from hybridization between oligonucleotide probes and DNA samples, using Bio-Plex suspension Array system. The developed DNA array was tested with both synthetic targets of complementary sequence and PCR products obtained with DNA isolated from Salmonella. This assay could detect target DNA at the concentration of 1 to 10 pM without any amplification, and when combined with PCR, it could detect 1 CFU/mL within 6 hrs. Our results show the developed bead-suspension array can be a rapid and reliable method for simultaneous detection and identification of multiple Salmonella serotypes. The developed array also shows a great potential to be adapted for detection of multiple foodborne pathogens in foods with high multiplexing ability. Currently the developed array is being tested with poultry and poultry feed samples artificially contaminated with Salmonella.

## 02. AN OVERVIEW OF PROPOSED NIH-FUNDED STUDY FOR CHOLERA IN HAITI

Afsar Ali, Department of Environmental & Global Health, College of Public Health & Health Professions, University of Florida; Edsel Redden, Department of Environmental & Global Health, College of Public Health & Health Professions, University of Florida; Ira Longini, Department of Statistics, College of Public Health & Health Professions, University of Florida; Judith Johnson, Department of Pathology, College of Medicine, University of Florida; Mohammad Jubair, Department of

Environmental & Global Health, College of Public Health & Health Professions, University of Florida, Emerging Pathogens Institute; Glenn Morris, Emerging Pathogens Institute

Cholera is an ancient disease that continues to be a global health threat, particularly in countries where sanitary and hygienic conditions are impaired. The causative agents of cholera are toxigenic *Vibrio cholerae* which is ubiquitous to a variety of aquatic environments, including fresh, estuarine and marine waters. Humans acquire *V. cholerae* upon consumption of water and food contaminated with the bacterium. The available recent case report data from the WHO indicates that there were a total of 221,226 cholera cases in 45 countries during 2009, which certainly represents a small fraction of the total number of cases due to vast underreporting of cases worldwide.

Cholera was confirmed in Haiti on October 21, 2010, following the catastrophic earthquake that hit the island on January 12, 2010. The initial cases were clustered along a 20 mile stretch of the Artibonite River and patients reported consuming untreated water from the river or canals just prior to the onset of disease. It is widely believed that Artibonite River promoted the index case of cholera that ultimately spread to the rest of the districts in Haiti. It is worth noting here that before this current wave of cholera, Haiti never witnessed a cholera epidemic for the last 60 years, even during the recent (1990s) cholera epidemics in South America. To date cholera is still continuing in Haiti with more than 400,000 cases and greater than 6,000 reported deaths since the start of the epidemic.

To understand the origin and evolution (if any) of *V. cholerae* strains causing cholera in Haiti, our group collected stool samples from cholera patients (19 patients) early in the epidemic. Based on the isolates (n=187) from 13 patients (who were positive for *V. cholerae*), we demonstrated minimal variability among the isolates using variable number tandem repeat (VNTR) analysis, indicating a single clonal introduction of *V. cholerae* in Haiti that was closely related to South Asian strains. Based on our findings, we submitted a proposal to National Institute of Health (NIH) for a multifaceted study involving: (a) dynamics of cholera transmission via person to person contact or environment to person contact, (b) detection of *V. cholerae* from aquatic reservoirs in Haiti, (c) case control study, and disease modeling, prediction and prevention.

Unlike other cholera endemic countries, Haiti's cholera epidemic is new and thus will provide us with a wealth of knowledge on the epidemiology, ecology, disease transmission and evolution and allow us to design improved cholera intervention strategies, including vaccination, to combat cholera in Haiti and potentially at the global level.

## 03. SALMONELLA TRANSFER POTENTIAL FROM GLOVES TO MULTIPLE TOMATOES DURING HAND HARVESTING UNDER LABORATORY CONDITIONS

Pardeepinder Brar, Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; Dr. Michelle Danyluk, Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida

Tomatoes have associated with numerous outbreaks of Salmonella. Harvesting of tomatoes is suspected as one of the potential stages of cross contamination. The objective of the research was to evaluate Salmonella transfer from contaminated gloves to tomatoes, upon numerous touches. Experiments were performed using mature green, round tomatoes with two types of gloves (reusable and single-use) and two hygienic conditions of reusable glove (clean and dirty). Gloves were made dirty by rubbing with a tomato leaf for 20 s. Tomatoes were touched with inoculated gloves (6 log CFU/surface) for 5 s following drying for 0, 1 and 24 h for clean gloves and 0 and 1 h for dirty gloves. Tomatoes, 25 for clean and 10 for dirty gloves, were touched subsequently with gloves. All the clean glove samples were placed in sterile sampling bags with 20 ml of Butterfield's Phosphate Buffer; 0.1% Tween-20 was added to the dirty reusable glove samples. Following stomaching (gloves) or rubbing (tomatoes), samples were enumerated on non-selective and selective agar, supplemented with rifampicin. Enrichments were performed when populations fell below the detection limit. Clean reusable gloves transfer more Salmonella to fewer tomatoes in comparison to single-use and dirty reusable gloves. Salmonella can transfer from gloves to tomatoes during harvesting. Gloves that may become dirty over the course of a harvesting shift do not increase the risk of Salmonella cross-contamination.

#### 04. DISPERSAL OF SALMONELLA ON TOMATO PLANTS BY RAIN SPLASH

Juan Cevallos, Plant Pathology, College of Agricultural and Life Sciences, University of Florida; Ganyu Gu, Department of Plant Pathology, College of Agricultural and Life Sciences, University of Florida; Michelle Danyluk, Citrus Research and Education Center, College of Agricultural and Life Sciences, University of Florida; Ariena van Bruggen, Plant Pathology, College of Agricultural and Life Sciences, University of Florida

Salmonella enterica outbreaks have increasingly been associated with tomatoes during the past decade. However, the mechanisms of Salmonella dispersal in agricultural fields are poorly understood. The effect of Salmonella rdar morphotype and tomato trichome density on pathogen dispersal by rain splash or aerosol was investigated. Trichome density on tomato leaves was increased or decreased by applying jasmonic or salicylic acid, respectively. Artificial rain matching intensity and drop-size distribution of Florida rain was generated in a BSL3 greenhouse facility. Rains of 50 mm/h or 100 mm/h were applied to  $10^8$  CFU/mL suspensions of GFP-labeled kanamycin-resistant Salmonella Typhimurium with and without the rdar morphotype. Cells dispersed by rain splash were recovered by making leaf imprints on LB agar with kanamycin. Aerosolized cells were recovered by sampling the air in lactose broth by using an impinger or Petri dishes containing the culture media. Cells lacking rdar morphotype (MAE 119) showed a significantly higher dispersion than cells expressing rdar morphotype (MAE 110) when the trichome density was below 200 per  $\text{cm}^2$ . Conversely, MAE 110 cells showed significantly higher dispersal at trichome densities above 300 per  $\text{cm}^2$  when compared to MAE 119. Salmonella aerosol formation after rain was only observed in MAE 110 cells in air samples and when a resuscitation step in lactose broth followed by enrichment in tetrathionate broth was used. Salmonella cells were detected on tomato fruits but

not on leaves after aerosol formation. Results suggest that Salmonella rdar morphotype favors the pathogen dispersal at high trichome densities on tomato leaves and increases Salmonella's ability to form and survive in post-rain aerosols.

#### 05. VACCINATION STRATEGIES FOR EPIDEMIC CHOLERA IN HAITI WITH IMPLICATIONS FOR THE DEVELOPING WORLD

Dennis Chao, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center; M. Elizabeth Halloran, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center; Ira Longini, Department of Biostatistics, Colleges of Public Health and Health Professions and Medicine, University of Florida

In October 2010, a virulent South Asian strain of El Tor cholera began to spread in Haiti. Because Haiti had not seen cholera for decades, the population was highly susceptible to infection and illness from the disease. By October 2011, nearly half a million cases of cholera and a quarter million hospitalizations due to the disease were reported in this country of ten million.

There was a general reluctance to use vaccine when cholera first appeared in Haiti. Cholera vaccine was in short supply, and the most affordable vaccine, Shanchol produced by Shantha Biotechnics, had not yet been prequalified by the World Health Organization. It was believed that a mass vaccination campaign could not be effective after the start of a cholera outbreak and that such efforts would take resources away from the treatment of cases and efforts to improve sanitation and hygiene.

To evaluate the efficacy of a cholera vaccination campaign in Haiti, we used a computer simulation model of cholera transmission in Haiti.

#### 06. ROLE OF THE E. COLI COMMON PILUS IN ADHERENCE AND INVASION OF BLADDER EPITHELIAL CELLS BY UROPATHOGENIC E. COLI

Jorge Giron, MGM, College of Medicine, University of Florida; Zeus Saldana, MGM, College of Medicine, University of Florida; Yushan Zhang, Urology, College of Medicine, University of Florida; Yehia Daaka, Urology, College of Medicine, University of Florida; Jose Puente, Molecular Microbiology, Instituto de Biotechnologia, Universidad Nacional Autonoma de Mexico

A role for the type 1 and P pili in colonization of the urinary tract by uropathogenic Escherichia coli (UPEC) is well established. E. coli strains that cause intestinal and extra-intestinal infections produce a new pilus called E. coli common pilus (ECP, however the contribution of ECP to the adherence of UPEC to epithelial cells and pathogenesis remains to be elucidated. We report that a significant number of UPEC strains produce ECP when interacting in vitro with epithelial cells and that UPEC strain CFT073 mutated in the major pilin structural gene *ecpA* are significantly ( $p < 0.05$ ) deficient in adherence to cultured epithelial cells in vitro as compared to their parental strains. Residual adherence and biofilm formation in these mutants is likely due to the presence of additional

adhesins such as T1P and P pilus. Complementation of *ecpA* in the mutant restored adherence and biofilms architecture to wild-type levels. Ex vivo infections of infant mice bladders revealed that the *ecpA* mutant is deficient in adherence and invasion of bladder epithelial cells. These data suggest that ECP must contribute to the adhesive and invasive capabilities of UPEC strains and that they must play an accessory role in adherence during the multi-step infection of the urinary tract, contributing to the complex interaction of UPEC with host uroepithelial cells.

## 07. INGRESS AND MOVEMENT OF SALMONELLA IN TOMATO PLANTS GROWN IN DIFFERENT SOILS

**Ganyu Gu**, Emerging Pathogens Institute and Plant Pathology Department, University of Florida; **Juan Cevallos-Cevallos**, Emerging Pathogens Institute and Plant Pathology Department, University of Florida; **Ariena van Bruggen**, Emerging Pathogens Institute and Plant Pathology Department, University of Florida

Several *Salmonella enterica* outbreaks have been traced back to contaminated tomatoes. A two-phase experiment was conducted twice to investigate the movement of *S. enterica* Typhimurium in tomato plants via leaf inoculation as affected by soil management and endophytic microbial diversity. In the first phase, 84 tomato plants grown in conventional or organic soils were inoculated 2-4 times before fruiting with two GFP-labeled *S. enterica* Typhimurium strains. Microbial communities in plants were investigated two weeks before and after inoculation. Inoculated and adjacent leaflets were tested for *Salmonella* survival from day 1 to day 30 after each inoculation. Fruits and seeds were also examined for *Salmonella*. In phase 2, extracted seeds including internally contaminated ones were planted in conventional soil, and infection of leaves and fruits of the second generation was checked. After inoculation, significantly more *Salmonella* CFU survived inside plants grown in conventional than in organic soil ( $P < 0.05$ ). All contaminated fruits were from tomato plants grown in conventional soil; the chance to detect contaminated fruits was about 3%. The contamination rate of the seeds from infected fruits was about 5%. There was a negative correlation between bacterial diversity in plants and the rate of internalization. In the second generation, no infection of leaves and fruits was detected. However, one plant from the 12 internally contaminated seeds died after germination in year 2; none of the control seedlings died. Thus, *Salmonella* can contaminate fruits and seeds through leaf inoculation, but the chance of seed transmission of *Salmonella* is very low.

## 08. PRIORITIZING INVESTIGATION OF REPORTED CASES OF SELECTED ENTERIC INFECTIONS, FLORIDA 2011

**Richard Hopkins**, Epidemiology, College of Public Health and Health Professions, University of Florida; **Leah Eisenstein**, Bureau of Epidemiology, Florida Department of Health

**Background:** Florida has encouraged county health departments (CHDs) to interview every reported case of enteric infection, including salmonellosis (~6,000 cases annually), shigellosis (1,000 to 3,000), campylobacteriosis (~1,100), giardiasis (~2,000), and cryptosporidiosis (400 to 700); 83% were interviewed in 2011. Reductions in funding now make this less feasible. We proposed prioritizing cases for interview, assuming that people are much less infectious once symptoms resolve: (1) case report indicates the person is in a sensitive situation (healthcare worker, childcare worker or attendee, food-handler) or is part of an outbreak; (2) case report received early enough that the person is likely still symptomatic; (3) all others. Group 1 case reports are rare as most initial case reports are laboratory results. We analyzed the likely effect of interviewing only those in group 2 on workload and detection of people in sensitive situations.

**Methods:** Median intervals from onset to specimen collection and to lab report were calculated for all confirmed reported Florida 2011 cases. Usual durations of each illness from textbooks were compared to these median intervals to assess when a case was no longer likely to be symptomatic. This disease-specific timeframe was applied to reported 2011 cases to determine whether they were in Group 2. We estimated the number of interviews saved, how many of those interviewed would have been symptomatic at the time of interview, and the number of people in sensitive situations who would have been missed.

**Results:** Duration of symptoms was assumed to be 6, 14, 14, 6, and 9 days for campylobacteriosis, cryptosporidiosis, giardiasis, salmonellosis, and shigellosis. Median intervals from onset to specimen collection were 3, 6, 10, 3 and 2 days respectively, and from onset to lab result were 7, 9, 13, 7 and 6 days. Applying the proposed policy for one year would reduce interviews by 954 (campylobacteriosis), 52 (cryptosporidiosis), 736 (giardiasis), 3,830 (salmonellosis), and 191 (shigellosis), for a total of 5,763, or 64%, fewer interviews. In a four-month period, out of 34 foodhandlers identified, 10 were symptomatic at time of interview and 7 of these were not in group 2. Of 53 healthcare workers, 14 were symptomatic at interview and 11 of these were not in group 2. Of 469 daycare-associated cases, 107 were symptomatic at interview and 59 were not in group 2.

**Conclusion:** Applying this policy results in substantial savings of CHD staff time. After applying the recommended policy, 120 additional interviews of adults would be needed to detect one additional symptomatic foodhandler, 78 to detect one additional symptomatic healthcare worker, and 32 children and adults to identify one additional symptomatic case associated with childcare.

## 09. THE CORRELATION BETWEEN ANIMAL POPULATIONS AND THE PREVALENCE OF ESCHERICHIA COLI O157:H7 IN CATTLE

**Soojin Jeon**, Department of Animal Sciences and Emerging Pathogens Institute, Institute of Food and Agricultural Sciences, University of Florida; **Mara Brueck**, Department of Animal Sciences and Emerging Pathogens Institute, Institute of Food and Agricultural Sciences, University of Florida; **Nicolas DiLorenzo**, Department of Animal Sciences and North Florida Research and Education Center, Institute of Food and

Agricultural Sciences, University of Florida; **Cliff Lamb**, Department of Animal Sciences and North Florida Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida; **K. C. Jeong**, Department of Animal Sciences and North Florida Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida

Enterohemorrhagic *Escherichia coli* O157:H7 is a significant human pathogen that can cause serious illness including hemorrhagic uremic syndrome (HUS). Cattle are the main reservoir of *E. coli* O157:H7 and the prevalence of cattle shedding this pathogen prior to processing correlates with the frequency of carcass contamination. Cattle that shed more *E. coli* O157:H7 than others are referred to as super-shedders (>104 CFU/g of feces). Super-shedders can cause negative effects, such as increasing the risk of food contamination and subsequent human infection. We evaluated 91 cattle to see if there are i) super-shedding animals at the farm ii) population dynamics of *E. coli* O157:H7 between steers and bulls. In 40% of animals, *E. coli* O157:H7 was detected by multiplex PCR targeting *stx1*, *stx2*, *hlyA*, and O157 after direct plating of rectal anal swab samples. We identified 16 super-shedding animals, and approximately 25% of bulls excreted at levels exceeding 105 CFU/swab while only 4% of steers shed at that level, implicating there are animal factors that contribute to the prevalence of *E. coli* O157:H7. Therefore, understanding the factors that play a role in super-shedding will provide insights into reducing or eliminating *E. coli* O157:H7 at the pre-harvest level, resulting in decreased contamination in the food supply. Further analyses of the correlation between genetic background of animals and prevalence of *E. coli* O157:H7 is currently under investigation.

## 10. ISOLATION AND IDENTIFICATION OF *V. CHOLERAE* NON-O1 STRAINS FROM THE AQUATIC RESERVOIRS IN HAITI

**Mohammad Jubair**, Department of Environmental & Global Health, School of Public Health & Health Professions, University of Florida; **Andrew Kane**, Department of Environmental & Global Health, College of Public Health & Health Professions, University of Florida, Emerging Pathogens Institute; **Glenn Morris**, Emerging Pathogens Institute; **Judith Johnson**, Department of Pathology, College of Medicine, University of Florida, Emerging Pathogens Institute, University of Florida; **Afsar Ali**, Department of Environmental & Global Health, School of Public Health & Health Professions, University of Florida, Emerging Pathogens Institute

Cholera continues to be a major public health threat globally, particularly in countries where sanitary and hygienic conditions are not optimal. Wars, and natural disasters, including tsunamis, Hurricanes and Earthquakes, can be major contributing factors for cholera outbreaks due to the displacement of people caused by such incidences. On January 12, 2010, a major Earthquake in Haiti destroyed much of Haiti resulting in the deaths of more than 200,000 people with millions more lost their homes and took shelters in tents mostly set up by the UN relief agencies. On October 21, 2010, cholera was first reported from patients in the Artibonite province of Haiti which later spread to all 12 districts of Haiti. To date more than 500,000 thousand people have been affected by cholera with close to 7,000 deaths. Before this outbreak, Haiti had not witnessed cholera

outbreak for more than 60 years. The long-term question that needs to be answered is that whether toxigenic *V. cholerae* can establish itself as an endemic pathogen in Haiti. In this context, in majority of cholera endemic countries, toxigenic *V. cholerae* co-exists with non-toxigenic *V. cholerae*. In this study we examined whether non-O1 *V. cholerae* can be isolated from the aquatic reservoirs in Haiti. To this end, we have collected 16 water samples from 16 distinct sites in Haiti. The water samples were microbiologically and genetically examined in the emerging pathogens laboratory. Three out of 16 samples (18.8%) were positive for culturable non-O1 *V. cholerae* strains. The remaining samples were subjected to direct PCR analysis. Interestingly, ten out of 16 (63%) water samples yielded the presence of *toxR* and *ompW* genes of *V. cholerae* non-O1 strains. We did not detect PCR product for *ctxB* or *tcpA* gene associated exclusively with toxigenic *V. cholerae*. We are currently sequencing *toxR* and *ompW* genes of putative non-O1 *V. cholerae* strains isolated from Haitian aquatic reservoirs. Our data indicate that Haitian water provides ideal conditions for the persistence of non-O1 *V. cholerae* strains, and, thus we suggest that newly introduced toxigenic *V. cholerae* strains have excellent probability established as endemic pathogens causing epidemics regularly in Haiti in the foreseeable future.

## 11. EVALUATION OF REGIONAL RISKS FOR SALMONELLA CONTAMINATION OF IRRIGATION WATER FROM MIXED PRODUCE FARMING

**Zhiyao Luo**, Food Science and Human Nutrition, University of Florida; **Ganyu Gu**, Plant Pathology, University of Florida; **Paige Adams**, University of Georgia; **George Vellidis**, Biological and Agricultural Engineering, University of Georgia; **Ariena van Bruggen**, Plant Pathology, University of Florida; **Anita Wright**, Department of Food Science and Human Nutrition, University of Florida

This project examined factors that may contribute to the contamination of produce by irrigation water contaminated with *Salmonella enterica*. Most probable number (MPN) for *Salmonella* and fecal coliforms were determined monthly from irrigation ponds (n=10) at large and small farms with various vegetable and fruit crops in the upper Suwannee River watershed. *Salmonella* detection methods were compared to maximize recovery, and genotypes were determined by rep-PCR (DiversiLab). Parameters included physical/biochemical conditions and the microbial diversity of irrigation water. To date, all 10 ponds were *Salmonella* positive for both water and sediment samples at some time point, and levels ranged from non-detectable to 4.6 MPN/100ml for water and non-detectable to >110.0 MPN/100g for sediment. The % of positive samples ranged from 11.1 to 50% for each pond with some ponds showing significantly higher frequency than others. Concentration by modified Morse swab filtration or by immunomagnetic separation (Dynabead) did not increase detection for *Salmonella*. Isolation of presumptive positive colonies on two selective media (XLT4 and ChromAgar) yielded < 50% agreement with *invA* PCR for each agar, but isolates that were positive on both agars were confirmed to be 100% positive by PCR. Temperature showed positive correlation with *Salmonella*, while negative correlations were observed for dissolved oxygen concentration,

oxidation-reduction potential, and total nitrogen ( $P < 0.05$ ). No correlations with *Salmonella* were observed for fecal indicator bacteria or for microbial diversity. Over 1000 confirmed *Salmonella* isolates have been recovered, and DiversiLab rep-PCR typing for representative strains showed that isolates were distributed among at least 8 phylogroups (based on  $< 85\%$  similarity). Isolates from different ponds occasionally clustered by phylogroup and/or appeared to be clonal ( $> 95\%$  similarity). Strains from irrigation ponds mostly clustered with strains from other environmental aquatic sources, but similarity of pond strains to strains from clinical infections was also observed. These data highlight the diversity of *Salmonella* in irrigation sources, and the results do not support the use of fecal indicators as a predictor for *Salmonella*.

## 12. UNDERSTANDING CHOLERA IN HAITI: REPRODUCTIVE NUMBERS AND VACCINATION COVERAGE ESTIMATES

**Zindoga Mukandavire**, Emerging Pathogens Institute, University of Florida; **David L. Smith**, Emerging Pathogens Institute, University of Florida; **J. Glenn Morris, Jr.**, Emerging Pathogens Institute, University of Florida

Cholera remains an important global cause of morbidity and mortality, capable of causing periodic epidemic disease. In October 2010, cholera appeared in Haiti and cases were first detected in the Artibonite region, and have been followed in the ensuing months by spread of the disease through every department in the country. Implementation of appropriate disease control efforts, including vaccination, requires an understanding of transmission dynamics, which may be best quantified by mathematical models. Using a mathematical model developed to analyze cumulative cases in Zimbabwe, we fitted data on the cumulative number of reported hospitalized cholera cases in Haiti. Basic reproductive numbers ( $R_0$ ) varied by department, ranging from 1.16 to 2.73. At a national level, 45% vaccination coverage would result in an ( $R_0$ )  $< 1$ , which should suppress transmission. In the current debate on the use of cholera vaccines in endemic and non-endemic regions, our results suggest that moderate cholera vaccine coverage would be an important element of disease control in Haiti.

## 13. A WHOLE GENOME DNA SEQUENCING REVEALS GENETIC TRAITS THAT AFFECT SURVIVAL AND PERSISTENCE OF ESCHERICHIA COLI O157:H7 IN CATTLE

**M. Oh**, Department of Animal Sciences and Emerging Pathogens Institute, University of Florida; **M. Y. Kang**, Department of Animal Sciences and Emerging Pathogens Institute, University of Florida; **D. Park**, Department of Bacteriology and Food Research Institute, University of Wisconsin; **C. W. Kaspar**, Department of Bacteriology and Food Research Institute, University of Wisconsin; **K. Han**, Department of Nanobiomedical Science and WCU Research Center, Dankook University; **Mattia Proserpi**, Department of Pathology, Immunology and Laboratory Medicine, College of Medicine, University of Florida; **Marco Salemi**, Department of Pathology, Immunology and Laboratory Medicine, College of Medicine, University of Florida; **K. C. Jeong**, Department of Animal Sciences and Emerging Pathogens Institute, University of Florida

*Escherichia coli* O157:H7 remains a significant concern of food recalls and causes tremendous negative effect on public health. Cattle are a reservoir and considered a primary source of this foodborne pathogen. Previous studies have revealed that an O157 subtype strain (FR1K2455) was predominant on a farm while 4 other clonal variants were rarely isolated. Animal factors are presumed to contribute to the predominance of the strains. However, bacterial genetic traits responsible for the predominance of FR1K2455 have not been addressed. In this study, we have conducted whole genome DNA sequencing using one predominant and four clonal variants to identify genetic variations, which affect the persistence of *E. coli* O157:H7 in cattle. A library was prepared from chromosomal DNA using the Covaris E210, and quality of the library was verified on a Caliper LabChip GX. Sequencing (75bp paired end) was performed on the HiSeq with v3 chemistry, and cluster was generated on the cBOT. Fastq files were aligned using bwa (<http://bio-bwa.sourceforge.net/bwa.shtml>) to the reference *E. coli* genome O157:H7 EDL933. In addition, de novo assembly was performed using Abyss. Bioinformatic comparison analysis using de novo assembly sequences was conducted by Macrogen (Korea). Surprisingly, the predominant strain contains significantly fewer genes. It carries ~500 less genes compared to the clonal variants. Although missing genes fell into several functional categories, a majority number of genes are related to phage, suggesting phages may play critical roles in the persistence of *E. coli* O157:H7 in cattle. In addition, FR1K2455 imported ~100 new genes from other organism including a type IV secretion system, therefore the newly added genes may contribute to the survival of *E. coli* O157:H7. We are currently confirming these bioinformatic comparison data by evaluation of individual genes. Further studies will reveal insights regarding survival of *E. coli* O157:H7 in cattle.

## 14. UNDERSTANDING THE MOLECULAR MECHANISM OF CHITOSAN MICROPARTICLES TO REDUCE ESCHERICHIA COLI O157:H7 SHEDDING IN CATTLE

**M. Oh**, Department of Animal Sciences and Emerging Pathogens Institute, University of Florida; **M. Y. Kang**, Department of Animal Sciences and Emerging Pathogens Institute, University of Florida; **C. W. Kaspar**, Department of Bacteriology and Food Research Institute, University of Wisconsin; **K. C. Jeong**, Department of Animal Sciences and Emerging Pathogens Institute, University of Florida

Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 is an important human pathogen, and cattle are considered as a major reservoir of this pathogen. Many intervention technologies, mainly at the post-harvest levels, are implemented to prevent outbreaks of diseases caused by STEC-O157. However, it is believed that a reduction in the prevalence and numbers will reduce the number of contamination in foods, resulting in enhanced public health. Chitosan has been used to make chitosan microparticles (CM) and is an effective oral delivery agent of drugs and vaccines to the intestinal tract. It has been shown that CM has a huge effect to reduce colonized *E. coli* O157:H7 in cattle. CM significantly shortened the duration of *E. coli* O157:H7 shedding from 13.8 days to 3.8 days. In addition, CM feeding significantly reduced the total number of *E. coli* O157:H7

in cattle, and the pathogen was completely removed from the intestinal tracts of 60% of the cattle. Here, we report the mode of action of CM on reducing E. coli O157:H7 shedding in cattle. Direct ionic interactions between CM and STEC-O157 are probably a key mechanism by which CM may interfere colonization of STEC-O157 and scrubbing off colonized bacteria on the colonization sites. Currently, identification of target molecule(s) in which CM binds to reduce STEC-O157 is under investigation.

## 15. CIGUATERA FISH POISONING IN ST. THOMAS, U.S. VIRGIN ISLANDS: TRENDS OVER 30 YEARS

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**Introduction:** Ciguatera fish poisoning is caused by the consumption of reef fish containing toxins produced by marine microalgae and is the most commonly reported marine food poisoning globally. Our objective was to estimate the incidence of ciguatera in St. Thomas, USVI and assess explanations for temporal trends.

**Methods:** We performed a random digit dial telephone survey in 2010 and 2011. Incidence rates were weighted for age, sex, and education. A second estimate was based on emergency department (ED) data and the percent visiting the ED for their most recent ciguatera episode. Data on seawater temperature, fish landings, and other environmental variables in St. Thomas were obtained from external sources to explore their impact on incidence rates over time.

**Results:** The incidence of ciguatera in adults was 12 per 1000 (95% confidence interval (CI)=10-21). The estimate based on ED visits was 6 per 1000 (95% CI=5-8). We found a significant negative correlation between seawater temperature and annual ciguatera incidence ( $R^2=0.53$ ,  $p=0.0015$ ) for 16 years between 1971 and 2011. Fish landings remained stable over the study period.

**Conclusion:** Our evidence indicates that ciguatera incidence in St. Thomas has declined since 1980. Past studies have suggested that ciguatera would increase with higher seawater temperatures associated with climate change, but this is not consistent with our findings. It is unclear whether St. Thomas has reached an upper temperature threshold limiting the amount of ciguatera or if another factor is causing the decline. Further study is needed to characterize factors that influence ciguatera.

## 16. DISTRIBUTIONAL IMPACT OF ROTAVIRUS VACCINATION IN 25 GAVI ELIGIBLE COUNTRIES: ESTIMATING DISPARITIES IN BENEFITS AND COST-EFFEC

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Other studies have demonstrated that the impact and cost effectiveness of rotavirus vaccination differs among countries, with greater mortality reduction benefits and lower cost-effectiveness ratios in low-income and high-mortality countries. This analysis combines the results of a country level model of rotavirus vaccination published elsewhere with data from Demographic and Health Surveys on within-country patterns of vaccine coverage and diarrhea mortality risk factors to estimate within-country distributional effects of rotavirus vaccination. The study examined 25 countries eligible for funding through the GAVI Alliance. For each country we estimate the benefits and cost-effectiveness of vaccination for each wealth quintile assuming current vaccination patterns and for a scenario where vaccine coverage is equalized to the highest quintile coverage. In the case of India, variations in coverage and risk proxies by state were modeled to estimate geographic distributional effects. In all countries, rates of vaccination were highest and risks of mortality were lowest in the top two wealth quintiles. However countries differ greatly in the relative inequities in these two underlying variables. Similarly, in all countries examined, the cost-effectiveness ratio for vaccination (\$/Disability-Adjusted Life Year averted, DALY) is substantially greater in the higher quintiles (ranging from 2 to 10 times higher). In all countries, the greatest potential benefit of vaccination was in the poorest quintiles. However, due to reduced vaccination coverage, projected benefits for these quintiles were often lower. Equitable coverage was estimated to result in an 89% increase in mortality reduction for the poorest quintile and a 38% increase overall. Rotavirus vaccination is most cost-effective in low-income groups and regions. However in many countries, simply adding new vaccines to existing systems targets investments to higher income children, due to disparities in vaccination coverage. Maximizing health benefits for the poorest children and value for money require increased attention to these distributional effects.

## 17. DISPARITIES IN ROTAVIRUS RISK AND VACCINATION IMPACTS IN NIGERIA: RESULTS FROM AN INDIVIDUAL-BASED MODEL

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Diarrheal disease is a leading cause of child mortality in low-income countries. The development and introduction of a new rotavirus vaccine holds the promise



of reducing child mortality. In order to realize those benefits, the vaccine must reach those who need it the most. However, in Nigeria, efforts to achieve universal utilization of EPI vaccines have not eliminated the gap between rich and poor children. In this study we developed a individual-based model of rotavirus vaccine impacts for Nigeria, where mortality from rotavirus diarrhea is high, and there are large disparities in vaccine access. Our research examined 1) the current co-distribution of disparities in risk and vaccine access, 2) how they would affect rotavirus vaccine impact, and 3) how a reduction in disparities could improve health gains in Nigeria. We used data from the 2008-9 Nigeria Demographic and Health Surveys (DHS) for 3,437 children to develop an individual-based risk model that estimates the distribution of diarrheal mortality risk factors and rotavirus vaccination effectiveness. Child-level effectiveness was estimated based on 1) whether a child received DPT 1 and 2 vaccinations, 2) when they received it, 3) the age distribution of rotavirus illness, and 4) rotavirus vaccine efficacy. Using a constructed asset index to assess economic status, we developed wealth index values for each household. A susceptibility index was developed from estimates of 1) nutritional vulnerability measured by weight for age Z-scores, 2) vitamin A doses and 3) treating diarrhea with oral rehydration solution and used to assess individual risk for each child. Results from the model indicate that a disproportionate fraction of rotavirus mortality is concentrated in children in the poorest households. The poorest children also receive about one-fifth of the estimated vaccine effectiveness, compared to children in the wealthiest households. When children are considered across individual risk quintiles, children in the highest risk quintile have about 20 times higher risk than children in the lowest risk quintile. Geospatial analyses display concentrations of risk and low vaccine effectiveness hotspots, located especially in the northeast and northwest regions of Nigeria. We conclude that disparities in estimates of vaccine coverage and effectiveness will reduce rotavirus vaccine impact, especially for the most vulnerable children. Reducing these disparities in vaccine introduction could provide an estimated two-fold increase in impact. Improving our understanding of the mechanisms behind disparities will inform and influence programmatic and policy decisions and strategies, thereby improving health gains of rotavirus vaccine introduction.

## 18. PERSON-TO-PERSON TRANSMISSION OF VIBRIO CHOLERAЕ IN BANGLADESH

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**Background.** *Vibrio cholerae* infections cluster in households. For cholera transmission in the endemic setting, the objective of this study was to quantify the relative contribution of person-to-person exposure through the contamination of household food, water, or surfaces. Quantifying the relative contribution of person-to-person exposure is important for planning effective prevention and control measures.

**Methods.** Symptom histories and multiple blood and fecal specimens were prospectively collected from household members of hospital-ascertained cholera cases in Bangladesh. We estimated the probabilities of cholera transmission through 1) person-to-person exposure within the household and 2) contact with community-based sources of infection. We estimated the proportion of symptomatic cholera cases attributable to person-to-person exposure. Aspects of the natural history of cholera infection and the effects of covariates on transmission were considered.

**Results.** Significant person-to-person transmission ( $p$ -value<0.0001) occurred among 1414 members of 364 households. Symptomatic cholera cases infected, on average, 4.4% (95% confidence interval [CI]: 2.6%-7.3%) of their household contacts through person-to-person exposure. Overall, 46.6% (95% CI: 37.4%-55.6%) of the symptomatic cholera cases were attributable to person-to-person transmission within the household. Cases of symptomatic cholera were significantly more infectious during the first two days of their infectious period ( $p$ <0.001).

**Conclusions.** Person-to-person exposure contributes substantially to the endemic transmission of symptomatic cholera in an urban setting. We provide the first estimate of the transmissibility of endemic cholera within households. The role of person-to-person transmission must be considered when planning cholera control activities.

## 19. THE RELATIONSHIP OF THE VIUB HYDROLASE TO THE IRON-LIMITED GROWTH AND VIRULENCE OF VIBRIO VULNIFICUS

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*Vibrio vulnificus* is the most common cause of fatal seafood-related bacterial infections in the U.S., but severe disease is limited to individuals with iron-related disorders, such as hemochromatosis, that saturate host iron-binding proteins. Both catechol and hydroxamate siderophore systems are available for high-affinity iron acquisition, and genes related to the vulnibactin catechol

synthesis (venB) and receptor (vuuA) contribute to virulence in mice. The research herein examined the viuB gene encoding a putative hydrolase responsible for removing iron from vulnibactin. DNA sequence analysis segregated two alleles for viuB, and a deduced ViuB Type 2 was associated with clinical origin, increased virulence in mice, and other genetic markers for virulence that were not correlated with ViuB Type 1. Therefore, mutational analysis was used to determine the role of viuB in iron-limited growth and virulence in mice. Although the viuB deletion mutant was significantly deficient for iron-limited growth compared to the wild-type strain, virulence in mouse infections did not differ significantly between mutant and the wild-type. The ViuB-deficient mutant did show significant increases compared to wild-type in the expression of genes related to the activity of the hydroxamate siderophore, suggesting common regulatory pathways. Prior studies had shown that GacS/GacA two-component signal transducers regulated iron-limited growth in *V. vulnificus*. Interestingly, GacS/GacA differentially regulated the two siderophore systems, as expression of the catechol-related genes was significantly decreased in a gacA deletion mutant but was essentially unchanged with respect to the hydroxamate gene expression. These results demonstrate the function of viuB in iron-limited growth of *V. vulnificus* and illustrate the complexity of the response of multiple siderophore systems to iron limitation. However, these data do not support the inclusion of viuB as a virulence determinant for *V. vulnificus*.

## 20. A SIMULATION MODEL FOR THE OSCILLATORY DYNAMICS OF SALMONELLA ENTERICA IN MANURE

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A simulation model was developed to investigate the relative effects of temperature, oxygen concentration, substrate content and competition by copiotrophic bacteria on the oscillatory behavior and survival of *Salmonella enterica* in manure. The model is an extension of the model COLIWAVE for survival of *E. coli* O157:H7 in manure by Semenov et al. (2010). In that model, three distinct oxygen states (anoxic, low oxygen, and aerobic) were distinguished, while in the *Salmonella* model oxygen concentrations in the water phase are calculated taking the water solubility of O<sub>2</sub> and CO<sub>2</sub> released by bacteria into account. At lower oxygen concentrations, *Salmonella* metabolism switches from oxidative to fermentative, becoming more efficient, while that of the competing microbial community becomes less efficient. As a result, *Salmonella* competes better with the copiotrophic community at reduced oxygen concentrations. Parameter values for growth and death of *S. enterica* were first obtained using data sets with population dynamics in sterile manure at different temperatures. Dead bacteria are lysed and their cytoplasm returns to the substrate pool. Oscillations in bacterial populations are attained by the relationships between relative growth and death rates with readily utilizable substrate content and

oxygen concentration. The model contains a logistic and exponential relation of relative growth and death rates, respectively, of *S. enterica* with temperature, resulting in optimum curves for net growth rates similar to the optimum curves reported in the literature. After parameter estimation for growth and death of *S. enterica* in sterile manure, parameters were estimated for the dynamics of copiotrophic bacteria, calibrated with data sets for survival of *S. enterica* in untreated manure. Copiotrophic bacteria oscillated in a similar manner as *S. enterica*, but with a phase shift that was only attained when oxygen concentrations were taken into account. The model has been calibrated and validated using different experimental data. Sensitivity analysis of model parameters is currently carried out. Scenario analysis for manure storage at different temperature, moisture and oxygen conditions will be carried out in the near future.

Semenov, A.V., Franz, E., and van Bruggen, A.H.C. 2010. COLIWAVE: a simulation model for survival of *E. coli* O157:H7 in dairy manure and manure-amended soil. *Ecological Modelling* 221: 599-609.

## 21. A STUDY OF EFFECTOR PROTEINS SECRETED BY ENTEROHEMORRHAGIC ESCHERICHIA COLI O157:H7

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Bacterial pathogens interact with host cells to disrupt a wide range of cellular processes during infection. Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 is a foodborne pathogen that can cause diseases in humans ranging from mild diarrhea to the potentially fatal hemolytic uremic syndrome (HUS). EHEC utilizes a type III secretion system (T3SS) to deliver/translocate the bacterial effector proteins into the host cell, which subvert cellular processes. The T3SS is essential for EHEC to colonize and survive inside host cells. This pathogen encodes many effectors identified by bioinformatics and biochemical analyses, which are located in the locus of enterocyte effacement (LEE) pathogenic island and outside the LEE locus. However, the roles of the majority of the effectors and/or other virulence factors encoded by the LEE and non-LEE loci remain yet to be understood. Currently, we are investigating the functionality of 72 effector-like proteins by using biochemical and cellular biology studies to ascribe cellular functions in virulence. These extensive and comprehensive approaches will provide insights into the molecular mechanisms of EHEC underlying the cellular process that produces virulence in the host cell.

## 22. A COMPARISON OF VIRAL FITNESS AND VIRULENCE BETWEEN EMERGENT ADENOVIRUS 14P1 AND PROTOTYPE ADENOVIRUS 14P STRAINS

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Epidemiological studies from the last decade have suggested that the morbidity and mortality associated with a newly emergent strain of human adenovirus (HAdV-14p1) is greater than other, more prevalent, adenovirus strains. Recent molecular analysis identified very minor genetic differences in HAdV-14p1 compared to prototype HAdV-14p. No studies have evaluated how these differences may affect competitive fitness and virulence. In this study, *in vitro* and molecular assays were performed to evaluate growth kinetics, cellular infectivity, cytotoxicity, and plaque morphology of HAdV-14p1 and HAdV-14p. Growth kinetic data showed no viral replication at 30°C and minimal differences at 37°C for both strains. Cellular infectivity data showed propagation capabilities for both strains in a diverse array of cell lines, with human lung and kidney cells having the highest propagation potential. Cytotoxicity data indicated cellular distress differences induced by both strains of virus in the first 12 hours, but similar distress levels between 12 and 48 hours. Plaque morphology assays showed minimal differences in average plaque diameter. Collectively, the data from these assays indicated that the morbidity and mortality observed in HAdV-14p1 infections is likely not due to advantages in viral growth, cytotoxicity, cellular infectivity, or plaque morphology. Other factors, such as environmental stressors, co-infections, or individual host response are likely contributing to an increase in morbidity and additional studies are necessary to further explore these areas.

### 23. SEROLOGICAL EVIDENCE OF HUMAN INFECTION WITH PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

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**Background:** Porcine reproductive and respiratory syndrome virus (PRRSV) is economically one of the most important pathogens for the swine industry and one of the major components of the multi-factorial porcine respiratory disease complex. It is an enveloped positive-sense single-strand RNA virus classified in the order Nidovirales, family Arteriviridae. PRRSV, enzootic in the United States, can cause a wide range of symptoms in swine of different ages (e.g. abortions and reproductive failure in sows, respiratory symptoms in piglets). It may also have an asymptomatic course or be masked by concurrent infections with other pathogens. The goal of this study is to investigate whether swine workers and people exposed to swine may have evidence of previous infection with PRRSV.

**Methods:** We examined sera collected in the period 2004-2006 from asymptomatic healthy swine workers from Iowa, USA. Currently 612 of the sera, collected in 2006, were tested in a swine immunoperoxidase monolayer assay (IPMA) that was adapted for screening of human sera. Sera from PRRSV hyper-immunized and sero-negative pigs were used as positive and negative control respectively.

**Results:** Twenty-two of 612 tested human sera demonstrated reactivity against PRRSV in the assay (antibody titers ranging from 1:16 to 1:6400). All sera were validated by at least 2 tests with good agreement. The staining pattern closely resembled that seen in the positive control serum. Currently we are performing virus neutralization assays, competitive IPMA, and western blot assays on the positive sera in attempt to confirm the specificity of the antibodies.

**Conclusions:** As far as the authors know this is the first time that human serum has been shown to have elevated antibodies against an Arterivirus, as there is no current knowledge of a human Arterivirus. These data suggest that PRRSV (or another Arterivirus) may be able to infect humans, although the virus has not been associated with human disease. While it is possible that the sero-reactivity in this study may be evidence of cross-reactivity from other viral infections, these data suggest that further studies of possible human infection with PRRSV are indicated.

### 24. EVIDENCE FOR AVIAN H9N2 INFLUENZA VIRUS INFECTIONS AMONG RURAL VILLAGERS IN CAMBODIA

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**Background.** Southeast Asia remains a critical region for the emergence of novel and/or zoonotic influenza underscoring the importance of extensive sampling in rural areas where early transmission is most likely to occur.

**Objectives.** We sought to prospectively study rural Cambodians with intense exposure to poultry for evidence of avian influenza (AI) virus infections.

**Methods.** In 2008, eight hundred adult participants in a prospective population-based study of AI transmission were enrolled from 8 villages in a province where detections of highly pathogenic (HPAI) H5N1 virus had been reported in humans and poultry between 2006 and 2008. From their enrollment sera and

questionnaires, we report risk factor findings for serologic evidence (by microneutralization assays) of previous infection with 18 AI virus strains. Results. The participants had a mean age of 39.6 years, 66.6% were female, and 93.2% reported previous poultry exposure. Serologic assays showed no evidence of previous infection with 13 different low pathogenic AI viruses or with HPAI avian-like A/Cambodia/R0404050/2007(H5N1). However, 21 participants had elevated antibodies against avian-like A/Hong Kong/1073/1999(H9N2). This reactivity was validated with a monoclonal antibody assay specific for avian H9. Multivariate modeling suggested that H9N2 seroreactivity was likely due to yet unidentified environmental exposures.

Conclusions. Study data suggested that a number of participants were previously infected with the avian-like A/Hong Kong/1073/1999(H9N2) virus. Prospective data from this cohort will help us better understand the serology of zoonotic influenza infection in a rural cohort in SE Asia.

## 25. EVIDENCE FOR SUBCLINICAL SWINE AND AVIAN INFLUENZA VIRUS INFECTIONS AMONG ROMANIAN AGRICULTURAL WORKERS

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Background. In recent years, highly pathogenic avian influenza (HPAI) H5N1 and novel swine-like H1N1 epidemics have caused morbidity and deaths among man in many areas of the world. Romania has had multiple incursions of HPAI since 2005.

Objectives. To examine evidence of zoonotic influenza transmission to man, we sought to prospectively study Romanians exposed to swine and poultry.

Study Design. Between 2009-2010, 363 adult participants were enrolled in a prospective, population-based study of influenza transmission in Romania. Both large, modern confined animal feeding operation (CAFO) swine workers in Tulcea and small, traditional backyard farmers in Cluj-Napoca were targeted for

enrollment, as well as a non-animal exposed control group from Cluj-Napoca. Enrollment sera was examined for serological evidence of previous infection with 9 avian and 3 swine influenza virus strains.

Results. Serologic assays showed no evidence of previous infection with 7 low pathogenic avian influenza (LPAI) viruses or with HPAI H5N1. However, 33 participants (9.1%) had elevated antibody titers against avian-like A/Hong Kong/1073/1999(H9N2). Serological reactivity was also detected against SwH3N2 (9.9%), SwH1N1 (11.1%), and SwH1N2 (35.3%) swine influenza viruses (SIVs). Compared to CAFO workers, both small farmers and controls were significantly more likely to have elevated antibody titers against A/Sw/Lutol/3/00(H1N1), even after controlling for cross-reactivity with human influenza infections or vaccinations.

Conclusions. These data suggested that a number of participants were previously infected with the avian-like H9N2 influenza virus or a SIV virus. Prospective data from this cohort will help us to better understand the serology of zoonotic influenza infection in Romania.

## 26. EQUINE INFLUENZA A EPIZOOTIC IN MONGOLIA, 2011: ISOLATION AND CHARACTERIZATION OF A H3N8 VIRUS

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Background: In the early spring of 2011, a limited outbreak of influenza-like-illness occurred among horses near the Mongolian capital Ulaanbataar. Within a few weeks of the onset of the outbreak, horse enthusiasts converged upon Ulaanbaatar from every corner of the country to take part in horse races as part of the annual national celebration of Nadaam. As owners and their horses returned to their homes the viruses traveled with them to each of the 21 aimags (provinces) in Mongolia, thus far causing an 74,608 illnesses and 40 deaths among Mongolia's 1.92 million horses. In the work presented here, we sought to isolate and partially sequence the genome of the virus associated with this epizootic to determine the nature of its origins.

Methods: Early in the outbreak swabs from the nares affected animals were obtained for laboratory analysis. Real-time RT-PCR was used to confirm the presence of influenza A in a subset of the collected specimens. Influenza A positive specimens were cultured in eggs resulting in the amplification of the virus. Viral RNA was isolated and HA NA and Matrix gene segments were amplified from three isolates cultured from samples collected early in the outbreak and sequenced by Sanger sequencing

**Results:** Three viral isolates were obtained from the outbreak. Nearly full length Sanger sequence data for the HA and M gene segments and partial NA segment sequence reveals that virus associated with the outbreak was most similar to viruses that caused outbreaks in the region in 2007-08 with approximately 96-98% identity for the three gene segments.

**Conclusions:** While further genomic analyses are in progress, the viruses associated with the 2011 Mongolian Equine influenza epizootic appear to be similar to a H3N8 equine influenza virus that widely circulated in Central Asia and Europe in 2007. OIE has since also reported this as a reoccurrence of a 2008 outbreak of a same virus.

## **27. THE RATIO OF EMERGENCY DEPARTMENT VISITS FOR ILI AND SELF-REPORTED ILLNESS ON A SURVEY TO SEROPREVALENCE OF 2009 H1N1 INFLUENZA**

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**The Ratio of Emergency Department Visits for ILI and Self-reported Illness on a Survey to Seroprevalence of 2009 H1N1 Influenza A Infection, Florida, 2009**  
**Background:** In an evolving influenza epidemic, existing real-time surveillance systems do not provide needed information about cumulative incidence of infection in the population. A seroprevalence survey carried out in Tampa Bay, Florida in late 2009 yielded such an estimate for the 2009 H1N1 influenza pandemic. We calculated the ratio of influenza-like illnesses detected in 2 existing surveillance systems to actual infections.

**Methods:** Total emergency department (ED) visits for influenza-like illness (ILI) during the epidemic period were estimated using the ESSENCE-FL syndromic surveillance system, by age group, for Florida and for Tampa Bay. Cumulative statewide reports of ILI using 3 different definitions were derived from the Behavioral Risk Factor Surveillance System (BRFSS), for interviews conducted Oct 1-- Dec 27, 2009, using the BRFSS weights to make statewide estimates for persons aged 18 and older.

**Results:** For all ages combined, for the whole state there were an estimated 27.5 infections for every ILI ED visit. Age-specific ratios rose with age, from 8.5 to 1 for children aged under 5 years to 66 to 1 for persons aged 65 and over.

Corresponding ratios for just Tampa Bay were 37 to 1, 11.3 to 1, and 98 to 1. For persons aged 18 and over, there were 3.0 infections for every 1 person reporting ILI (fever and cough and/or sore throat) on BRFSS interview. Ratios ranged from 1.4 to 1 for persons aged 50 to 64 years, to 8.4 to 1 for persons 65 and over. For more stringent BRFSS ILI definitions, ratios are much higher.

**Conclusions.** For jurisdictions using syndromic surveillance systems or ongoing BRFSS-like interviews, these ratios provide a way to estimate weekly and cumulative incidence, which can be used together with estimates of the reproductive number (RO) for planning and forecasting. Syndromic surveillance

data allow more detailed age and geographic breakdowns and a full age range, and are more timely than BRFSS data.

## **28. DETECTION AND GENETIC CHARACTERIZATION OF AN AIRBORNE NON-CULTURABLE TYPE C HUMAN RHINOVIRUS COLLECTED IN AIR SAMPLERS**

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Mainly because of their small size and low concentration, human respiratory viruses are notoriously difficult to collect from the airborne environment. Moreover, a majority of the bioaerosol samplers available are not suitable for the collection of viruses. A further complication is that some of the viruses known to cause respiratory infections in humans have not been cultured in-vitro. We are evaluating air sampling methodologies for studies of airborne virus transmission. By chance, we were able to evaluate the collection performance of an SKC Biosampler, a compact cascade impactor (CCI), Teflon filters, agarose, gelatin filters, and miniature impingers in and within the vicinity of the office of an adult male with a mild URT infection. Rhinovirus C was detected in nasal swab specimens taken from the office worker using RT-PCR and assumed to be the most probable cause of the URT infection. The same virus was also detected in the samplers, and on the computer keyboard and other proximal work surfaces of the office. Moreover, virus was detected in samplers positioned one to three meters away from the subject, indicating some of the virus was aerosolized. The miniature impingers and SKC Biosampler, which collect fine particles in liquid media, recovered the rhinovirus more effectively than the other samplers, followed by gelatin filters. Evidence of reaerosolization of virus particles from the liquid media was apparent. Viruses collected from the human subject and from air samplers were not culturable using standard methods, consistent with the known biology of Rhinovirus C. As a first step towards a reverse-genetics system that will produce virus particles that will enable us to refine our air-sampling methods, we determined the complete genomic sequence of the virus, and present its genetic profile.

## **29. EXPANDED REPERTOIRE OF CELLS THAT OVER-EXPRESS SIAT1- OR SIAT4- FOR INFLUENZA VIRUS ISOLATION**

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The ability to isolate and propagate influenza viruses is necessary for the yearly surveillance of circulating virus strains, for clinical diagnostics, for antiviral susceptibility tests, and vaccine manufacture. Various laboratories have reported

superior results using MDCK-human sialyl-transferase (SIAT)1 cells instead of conventional MDCK cells for the isolation of human influenza viruses. MDCK-SIAT1 cells are engineered to produce increased levels of the  $\pm$  -2,6-linked sialic acid receptors that are bound by human influenza viruses. Similar to the findings of a World Health Organization Center for Influenza Virus Research, we observed that influenza A (H1N1 and H3N2) and B viruses isolated from years 2005 and 2010 were propagated more effectively following inoculation onto MDCK-SIAT1 cells than in MDCK cells. As research and surveillance occurs with viruses arising from both humans and animals, it was hypothesized that cells expressing SIAT4 with increased levels of the  $\pm$  -2,3-linked sialic acid receptors, would allow avian influenza viruses to be propagated more efficiently, and serve as a better platform for isolating wild viruses from a range of species. Here we report the engineering of human A549, simian Vero, and mink Mv1 Lu cells, and of 5 different canine MDCK strains that produce increased levels of either  $\pm$  -2,3- or  $\pm$  -2,6-receptors. These cells express either human SIAT1 or SIAT4 from genetic constructs driven by a eukaryotic promoter that is more active and stronger than the CMV promoter typically utilized to generate recombinant cells, which allowed increase susceptibility of these cells to influenza infection. These new cell types are also resistant to the peptidyl nucleoside antibiotic blasticidin S, due to a resistant marker for blasticidin S being used for selection of the constructs. As blasticidin S is very toxic to both eukaryotic and prokaryotic organisms, an additional benefit of our modified cell lines is that the isolation of influenza viruses can be performed from clinical specimens contaminated with normal respiratory tract flora that would otherwise contaminate the cellular culture.

### 30. NEW CANINE SLAM-EXPRESSING CELL LINES FOR THE ISOLATION AND PROPAGATION OF CANINE DISTEMPER VIRUS

John Lednicky, Environmental and Global Health, Public Health and Health Professions, University of Florida; Julia Loeb, Environmental and Global Health, College of Public Health & Health Professions, University of Florida; Kari Puricelli, MRIGlobal, Kansas City, Missouri

The paramyxovirus Canine distemper virus (CDV) (genus Morbillivirus) is a single-stranded (negative-sense) enveloped RNA virus that is highly contagious and transmitted predominantly through airborne routes. Long known to cause potentially lethal disease among members of the Canidae, Mustelidae, and Procyonidae, CDV has more recently caused mortality in large felids, fresh- and salt-water seals, and various other animals. The virus seems to be evolving quickly; once thought to consist of a single clade, CDV lineages are now classified as American, African, Asian, Arctic, European, and vaccine virus clades. In the continental USA, American type-2 strains predominate, though a new outbreak in Arizona is caused by introduced European strains. CDV is considered difficult to isolate in-vitro, wherein many CDV strains are easiest to isolate using susceptible cells that express canine signalling lymphocyte activating molecule (SLAM). This is because SLAM serves as a virus receptor for CDV and other morbilliviruses. Otherwise, up to one month is required before CDV-induced

cytopathic effects are seen in-vitro. However, only Vero (African green monkey kidney) cells that express SLAM are available to a few laboratories, and some CDV strains replicate poorly in Vero cells. We reasoned that other cell-lines permissive for CDV might be improved if they were also engineered to express canine SLAM. Here, we report the engineering of Vero, MDCK (canine kidney), and Mv1-Lu (mink lung) cells that express canine SLAM. The three SLAM-expressing cell-lines are highly effective for the isolation of CDV strains that utilize canine SLAM as a viral receptor, with dramatic cytopathic effects often noted by 48 hrs post-infection relative to those seen in non-SLAM cells. In contrast, we find some American-type 2 CDV strains that are neurotropic do not require SLAM receptors. This is not surprising, as brain cells do not express much SLAM. Also, the neurotropic CDV strains often have unique virus-encoded fusion (F)-protein sequences, and it is the F-protein that binds to SLAM. The newly engineered cells described here will be useful for diagnostic virology, for in-vitro CDV assays, and might be useful as a quick test for virus receptor specificity.

### 31. THIRD COMPLETE GENOMIC SEQUENCE OF HUMAN PARAINFLUENZAVIRUS 4B

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Due to the difficulties in isolating the virus and the lack of routine surveillance, the clinical significance of human parainfluenza virus 4 (hPIV-4) is less understood than that of the other human parainfluenza viruses. However, mounting evidence indicates that respiratory infections caused by hPIV-4 of infants, young children, and elderly patients are more common than previously thought. There are two subtypes of hPIV-4, termed subtypes 4A and 4B. Following traditional clinical virology practices, hPIV-4B was detected by immunochemistry in primary rhesus monkey kidney cells that had been inoculated with nasopharyngeal swab material obtained from a child with a mild upper respiratory tract illness. Attempts to isolate this paramyxovirus (designated 04-13) in pure culture were not possible due to the presence of a contaminating fast-growing Group VI simian spumavirus (foamy retrovirus) that quickly proliferated in human and simian cell lines. Reverse transcription polymerase chain reaction (RT-PCR) followed by sequencing of a subgenomic section of the viral fusion protein gene indicated the virus was hPIV-4B. Only two complete genomic sequences of hPIV-4B are currently available at GenBank, those of strains SKPIV-4 and 68-333. Paradoxically, the genome of strain SKPIV-4 does not conform to the paramyxovirus rule of six (the length of a paramyxovirus genome should be a factor of the number 6), whereas the genome of 68-333 does. Since we are preparing reverse-genetics clones of various paramyxoviruses, we sought independent insights on how we might structure a reverse-genetics system for hPIV-4B. We thus obtained the complete genomic

sequence of hPIV-4B 04-13 and determined it is highly homologous to the sequence of hPIV-4B 68-333, and importantly, that it conforms to the paramyxovirus rule of six. Here, we present a preliminary genetic analysis of hPIV-4B 04-13.

### 32. PILOT PROJECT: INCREASING LAIV IMMUNIZATION RATES AT BUH HOLZ HIGH BY WORKING WITH STUDENT LEADERS

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**Background:** School-located influenza vaccination programs (SLIVs) are increasingly recognized as a key component of community-based efforts to control annual influenza epidemics. Schools are effective virus exchange systems and children are super spreaders, shedding more of the virus for longer periods of time than adults. Computer modeling suggests that immunizing 70% of school children will protect an entire community from the flu.

In 2006, the Health Department, working in collaboration with the School System and the University of Florida, began exploration of a non-mandatory community-wide SBIIP, with the goal of achieving high levels of flu immunization of public and private pre-K through grade 8 students in the county. The program began offering the vaccines to high school students in 2010/11, achieving a 16% immunization rate, higher than the national average of 9%. Due to the low levels of immunization, the SLIV program began to work with Buhholz high school, an area school to explore ways to increase the rate.

**Methods:** During the summer of 2011, five student leaders from Buhholz high school students participated in the SLIV's Flu Ambassador's program. The students worked with the program to develop, appear, and promote flu educational videos for their school and the school system. Throughout the program, students gained flu knowledge needed to successfully develop their videos.

**Results:** The immunization of rates at Buhholz high school (n= 2,097) increased by 3%, from 16% to 19%. However, the immunization rates at control high schools receiving no intervention aside from viewing the videos produced stayed the same or declined from the previous year.

**Conclusions:** The pilot study suggests educating and involving the high school student body leaders will increase the immunization rates of their school. The goal of the Alachua County program is to expand this pilot to three schools in the county and measure the effectiveness of the intervention.

### 33. ANALYSIS OF PLASMODIUM FALCIPARUM DHFR AND DHPS GENES FOR SULFADOXINE PYRAMITHAMINE RESISTANCE IN HAITI.

**Tamar Carter**, Anthropology; Epidemiology, Liberal Arts and Sciences; Public Health and Health Professions, University of Florida; **Connie J. Mulligan**, Department of Anthropology, College of Liberal Arts and Sciences, University of Florida; **Alexander Existe**; **Yves Saint Victor**; **Gladys Memnon**; **Jacques Boncy**; **Roland Oscar**; **Mark Fukuda**; **Bernard Okech**, Environmental and Global Health, College of Public Health and Health Professions, University of Florida

**Introduction:** Each year malaria kills an estimated half a million people worldwide. In Haiti, close to 30,000 malaria cases are reported yearly. Forty years ago, sulfadoxine pyrimethamine (SP) in combination with chloroquine (CQ) was used widely in Haiti as a nationwide effort to control malaria. However, the initiative was abandoned due to low success rates. Subsequently, Plasmodium falciparum resistance to SP was reported and may have led to the malaria treatment policy that favored the use of CQ over SP. However, to date no genetic study has investigated SP resistance across Haiti. The goal of this study is to evaluate mutations in genes of P. falciparum associated with SP resistance. These data provided vital information for shaping future guidelines of malaria treatment in Haiti.

**Methods:** Dried blood spots were collected from 22 malaria infected patients in two clinics in Haiti. DNA was extracted from these samples to identify mutations in the dihydrofolate reductase (DHFR) gene at codons 51, 59, 108 and 164 and the dihydropteroate synthetase (DHPS) gene at codons 436, 437, 540 in P. falciparum. These are known markers for SP resistance.

**Results:** No mutations in the DHFR at codons 51, 59, 164 were found in any of the samples tested. However, 22.7% (5/22) of samples had a mutation at codon 108 (S108N). These five samples were collected from an urban clinic located near the city of Port-au-Prince while other samples were collected from a rural clinic. No mutations were detected in the DHPS gene.

**Conclusion:** For more than 35 years, the endemic P.falciparum strain in Haiti has been sensitive to CQ but new data (see Warner et al.'s abstract) indicates a changing trend towards CQ resistance. The results of this study suggest that the very low prevalence of SP resistance in Haiti may open additional options for malaria treatment in Haiti.

### 34. MOLECULAR ECOLOGY OF THE PLANT AND ANIMAL PATHOGEN PYTHIUM INSIDIOSUM

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There are very few fungal pathogens known to be trans-kingdom, infecting both plants and animals, but these encompass several emerging pathogens of

humans and provide unique opportunities to study the processes behind pathogen emergence. Pythiosis is a deadly disease of horses and dogs in tropical and subtropical regions, including Florida and the southeast United States. It also infects humans in Southeast Asia and is considered a potential emerging pathogen in the United States due to its apparently expanding geographic and host range. The causal agent of pythiosis is *Pythium insidiosum*, a fungal-like organism that is also a plant pathogen and is the only mammalian pathogen in a genus of plant pathogens. We expect that there are quantifiable environmental conditions that promote growth of *P. insidiosum* and infection of animals. We are beginning to investigate the ecology and population dynamics of *P. insidiosum* in the Florida environment to understand these conditions. Specifically, we are examining the geographic distribution, genetic diversity, population structure, and migration of *P. insidiosum* in Florida to elucidate the population dynamics and dispersal of the pathogen. Initial environmental sampling indicates that *P. insidiosum* is common in shoreline areas of lakes and ponds in the Gainesville area and that three of four known evolutionary lineages of the species are present in Florida. Preliminary inoculations suggest that various common aquatic plants, grasses, and sedges are hosts for *P. insidiosum*.

### 35. PHYLOGEOGRAPHY OF PHYTOPHTHORA INFESTANS, THE IRISH POTATO FAMINE PATHOGEN

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The Oomycete pathogen *Phytophthora infestans* has a long and notorious history as an emerging and re-emerging pathogen, causing devastating epidemics of late blight on potato and more recently tomato. Given the history of repeated emergence and global movement of new virulent strains of this pathogen, the prevention of future emergence events will require understanding global sources of genetic variation and migration patterns. The evolutionary origin and center of diversity of the pathogen has been thought to be central Mexico, a center of diversity of the genus *Solanum*, since a sexually reproducing and genetically diverse population was discovered there in the 1950s. However, *P. infestans* is also present in the Andean highlands of South America, which is the center of diversity of potatoes. This has led to the suggestion that the Andes are the evolutionary origin and global source of the pathogen. A recent analysis of one nuclear and two mitochondrial loci indicated that the Andean lineages are older than the Mexican population, providing support for the Andean origin hypothesis. Yet this analysis raised many questions, because it is well established that the Andean population is limited to several asexual lineages. To investigate this dichotomy, we sequenced three additional nuclear genes and genotyped microsatellite loci from Mexican and Andean *P. infestans* populations as well as

from species closely related to *P. infestans*. These data are being used to test phylogeographic models for the evolution of *P. infestans* in the Americas.

### 36. PREVALENCE AND GENETIC DIVERSITY OF MICROSPORIDA ACROSS SOLENOPSIS SPECIES IN SOUTH AMERICA

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Two species of fire ants: *Solenopsis invicta*, the red imported fire ant, and *S. richteri*, the black imported fire ant were accidentally introduced to the United States from South America. *Kneallhazia solenopsae* and *Varimorpha invictae* are microsporidia that infect fire ants, causing low weight of queens and reduced fertility. Thus, these microsporidia were identified as potential biological control agents against fire ants. In this study, the prevalence of *K. solenopsae* and *V. invictae* in *S. richteri* and *S. daguerrei*, a social parasite, from colonies in Argentina was analyzed. A total of 323 *S. richteri* colonies were surveyed for *K. solenopsae* infection encompassing 9 sites. Eight sites (89%) showed microsporidia infection with 40 (12.4%) colonies positive for *K. solenopsae* and 4 (1.2%) colonies positive for *V. invictae*. Out of 221 *S. daguerrei* ants distributed in 6 sites, 7 (3.2%) ants were positives for *K. solenopsae* from 3 sites (50%), and 1 (0.5%) ant was positive for *V. invictae*. Further studies analyzing the molecular diversity of *K. solenopsae* among different hosts will be discussed. Our study shows that *K. solenopsae* is prevalent in native populations of *S. richteri* and shows promise as biological control agent for invasive populations of this ant.

### 37. MOLECULAR ANALYSIS OF CHLOROQUINE RESISTANCE IN HAITI

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Malaria in Haiti is meso-endemic although in some areas it exists in an epidemic state that coincides with rainfall patterns. There is a general lack of detailed knowledge about Malaria in Haiti especially about anti-malarial drug resistance. The first line drug choice for the treatment of malaria in Haiti is chloroquine (CQ). This treatment policy is based on the previous drug sensitivity studies conducted more than 30 years ago that showed sensitivity of *P. falciparum* to chloroquine. The Haiti Ministry of Health does not conduct routine surveillance



of anti-malarial resistance but has recently agreed to re-invigorate surveillance activities for drug resistance especially after the one recent report of CQ resistance genotypes in malaria samples from one location in Haiti. There is need for additional data on chloroquine resistance followed up by in vivo studies. Resistance to CQ is mainly conferred by single nucleotide polymorphism (SNPS) in *pfcr* gene a chloroquine transmembrane transporter that is found in the digestive vacuole membrane of the malaria parasite. We analyzed the amino acid mutations in the *pfcr* gene at positions 72, 73, 74, 75 and 76. The most widely used and also considered a reliable marker for CQ resistance is the amino acid (AA) mutation at position 76, K->T or simply K76T. We analyzed for K76T plus AA mutations at positions 72-75, which have been used to type malaria strains; and so we used them to characterize *P. falciparum* from Haiti. In this continuing study, we have collected 120 malaria positive samples from five locations across Haiti but have analyzed 22 samples for *pfcr* gene and 14 for the *pfmdr* 1 gene. For the *pfcr* gene, we identified five K76T mutations. Analysis of the mutations at positions 72-75 revealed a new haplotype CVMMDT in Haiti. This haplotype was only recently been reported in Africa. Seventeen samples were wild type (CVMNK), 3 were CVIET, 1 was CVINT and 1 was CVMMDT. The analysis of SNPs in the *pfmdr* 1 gene, which modulates resistance to CQ, identified no mutations at amino acid positions 86 (N86 for wild type) and 1246 (D1246 for wild type). There is a great need for surveillance and monitoring of CQ resistance to provide the much needed data for the management of malaria in Haiti.

### 38. IMPROVED ISOLATION OF PATHOGENIC AMOEBIA FROM ENVIRONMENTAL SAMPLES

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Serious central nervous system infections with pathogenic amoebae including *Naegleria fowleri* (NF), *Acanthamoeba castellanii* (AC), and *Balamuthia mandrillaria* (BM) occur rarely following environmental exposure such as swimming in warm freshwater lakes. Amoebae live in the water column as trophs which are actively feeding and moving, but are sensitive to desiccation and chemiCollege of Agricultural and Life Sciences. Starvation causes the amoeba to form thick walled cysts that sink to the bottom of lakes and are highly resistant to disinfectants. Studies of the environmental distribution of these pathogenic amoebae are hampered by the labor intensive methods required for purification of amoeba from environmental samples; especially from soil or sediment. Isolation of amoeba requires many (e.g. 20 repeats) subcultures to dilute out contaminants, and the amoebae may easily be overgrown by other organisms in the sample, particularly fungi.

We examined the ability of *A. castellanii* and *B. mandrillaria* cysts to survive exposure to ethanol. A thirty minute of exposure to ethanol resulted in minimal cyst mortality. Based on these results, we have developed a rapid method for recovery of amoeba from soil or sediment samples. Briefly, the samples can be

refrigerated for several days to allow most of the amoebae to encyst. Then the sample is mixed with sterile sucrose and pelleted to allow removal of larger dirt particles. The supernatant is mixed with sterile PBS and centrifuged at a higher speed to pellet cysts. This pellet is resuspended in ethanol and incubated at room temperature for 30 minutes. The pellet is then washed and inoculated into appropriate growth media for each species of amoeba with antibiotics. This method was tested on soil samples spiked with BM and AC. Traditional methods and the ethanol enrichment methods were compared. Culture results were confirmed by PCR. The ethanol enrichment resulted in recovery of clean cultures of the amoebae after as few as a single passage. This new method was particularly useful when the sample had fungal contamination. There was some loss of sensitivity as shown by MPNs and this method is not expected to recover the troph form. However, even if both methods are run simultaneously the ethanol enrichment saved labor and supplies and improved recovery from highly contaminated samples.

### 39. EXPLORATORY PHARMACOKINETICS-PHARMACODYNAMICS (PK-PD) TARGET ATTAINMENT ANALYSIS OF PA-824

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**Background:** PA-824 is a new nitroimidazole chemotherapeutic agent in clinical trials for improving the treatment of tuberculosis, including multidrug resistance TB. In mice, it has demonstrated dose-dependent bactericidal and sterilizing activity. The efficacy of the drug was mainly associated with the PK/PD index %T>MIC. According to a recent clinical study, the maximum efficacy was unexpectedly achieved at the lowest dosage tested, indicating activity of lower doses should be explored. Therefore, the present study had the aim of performing an exploratory study that could aid dose optimization.

**Methods:** The population pharmacokinetic parameter estimates associated with the pharmacokinetic variability of PA-824 were used in Monte Carlo simulations (MCS). MIC and PK data derived from a pre-clinical study were used, where PA-824 PK was described as a one compartment body model. The magnitude of the %TÁ MIC was used as the index that best predicted microbial kill based on the inhibitory sigmoid Emax model. Different levels of free drug (5, 7.5 and 10 %) and inter-individual variability as a whole (10, 20 and 30%) were simulated at different doses. The target attainment (TA) rates were calculated for each regimen by conducting 10,000 MCS. The software Model Risk® (Vose Software, Belgium) was used for the simulations.

**Results and Conclusions:** The fraction of simulated subjects who achieved a decrease in 1.5 log CFU was calculated for the range of MICs from 0.031 to 0.125

mg/liter and range of percent free drug (5-10%). The results showed that an average T>MIC of 50% is required for at least 80% of simulated subjects to attain target. The change in free fraction from 5 to 7.5% with 10-30 percent inter individual variability did not show much change in %T>MIC required for TA. The change in free fraction from 5 to 10%, though, increased the probability of attaining target by 100%. The average %T>MIC varied largely by dose. However, the obtained percentage of animals with the decrease in CFU values (in relation to non-treated animals) higher than 1.5 log CFU count did not change extensively at doses higher than 75 mg.

#### 40. THE PHENOMENON OF SUPERSPREADING IN MYCOBACTERIUM TUBERCULOSIS

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**Introduction:** Experimental and epidemiological data have suggested considerable variability of transmission of Mycobacterium tuberculosis, the causative agent of tuberculosis (TB). However, uncertainty about the implications of these data has resulted in a lack of translation into policies and practices by TB control programs. We hypothesized that less than one-third of TB patients transmitted infection to their household contacts, and that the distribution of the proportion of household contacts that are secondarily infected with TB is log normally distributed.

**Methods:** Our study includes de-identified demographic, epidemiological, and laboratory data on index cases from household contact investigations in the state of Florida from January 1, 2008 to December 31, 2008. The t test was used to compare means, and Pearson X<sup>2</sup> to test for differences in categorical variables. Associations between the outcomes and index case-specific predictors were assessed using logistic regression analysis. Adjusted odds ratios (AORs) and corresponding 95% CIs were calculated for all variables.

**Results:** Among 290 index cases with culture-confirmed, smear positive pulmonary TB, 31.4% were documented transmitters of TB infection. In the univariate analyses, those index cases whose contacts converted their TST were more likely to have cavitation on their chest radiograph compared with those individuals who did not spread disease (OR: 1.70 [1.01-2.86]). The total number of contacts per index case was higher in those who transmitted disease compared to those who did not (OR: 1.04 [1.02-1.07]). In the adjusted model, the only variable which remained significantly associated with whether or not an index case spread to their contacts was total number of contacts (AOR: 1.05 [1.02-1.07]).

Among index cases with baseline positive contacts, it was observed that 80.3% had contacts who were TST positive at baseline. In the univariate analysis, index cases who had baseline positive contacts were more likely to have consumed alcohol in the past year (OR: 1.93 [1.04-3.59]). They were also more likely to be

foreign-born (OR: 1.96 [1.01-3.78]) and more likely to be younger (OR: 0.97[0.95-0.98]). In the multivariate analysis, both age (AOR: 0.97[0.95-0.99]) and total number of contacts (AOR: 1.08[1.01-1.14]) remained significant in the model.

**Conclusion:** Approximately 30% of index cases were confirmed disseminators of TB using TST conversion as the outcome. Using baseline TST positivity as the outcome, the data were less clear but still indicated a skewed spreading pattern. If the index cases who are the most infectious could be targeted, TB control practices could be implemented more cost-effectively.

#### 41. THE EFFECT OF RIFAPENTINE ON PLASMA CONCENTRATIONS OF RALTEGRAVIR

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**Background:** The number one infectious killer of patients with HIV is tuberculosis (TB). Treatment of both diseases results in multiple drug interactions, some of which have not been previously studied. Rifapentine (RPNT) is currently being considered for first-line treatment of tuberculosis. Raltegravir (RALT), an integrase inhibitor used in the treatment of HIV, undergoes metabolism via glucuronidation by the enzyme UGT1A1. The rifamycins are potent inducers of UGT1A1. Rifampin (RIF) reduces the C<sub>max</sub>, C<sub>min</sub> and AUC<sub>0-12</sub> of RALT (400 mg single dose) by 38%, 61% and 40% respectively, while rifabutin (RBN) 300 mg daily increases RALT's C<sub>max</sub> and AUC<sub>0-12</sub> (39% and 19% respectively) and reduces RALT's C<sub>min</sub> by 20%. In vitro studies suggest RPNT's inductive abilities are between those of rifampin and rifabutin.

**Methods:** A sequential, bioequivalence study was performed to assess RPNT's effect on RALT. This pharmacokinetic analysis was performed in 16 healthy volunteers treated with 400 mg raltegravir (RALT) every 12 hours for three days (Period 1). Following a washout period of at least ten days 900 mg rifapentine (RPT) administered once weekly was added to 400 mg raltegravir (Period 2). Following a washout period of at least ten days 600 mg rifapentine administered daily (5/7 days) was added to 400 mg raltegravir (Period 3). Plasma concentrations of rifapentine, 25-desacetyl-rifapentine and raltegravir were determined using HPLC, and pharmacokinetic data were analyzed using non-compartmental techniques (WinNonlin 5.2.1).

**Results:** The geometric mean ratio (GMR) of raltegravir's C<sub>max</sub>, C<sub>min</sub>, and AUC<sub>0-12</sub> (±90% confidence interval) for period 2/1 was 1.89 (0.97-3.68), 0.56 (0.20-1.56) and 1.73 (0.99-3.01) respectively. The geometric mean ratio (GMR) of raltegravir's C<sub>max</sub>, C<sub>min</sub>, and AUC<sub>0-12</sub>, (±90% confidence interval) for period 3/1 was 1.04 (0.53-2.02), 0.57 (0.20-1.59) and 1.01 (0.58-1.76) respectively. RALT when administered with RPNT once weekly reduced RALT's C<sub>min</sub> by 44%

while increasing RALT s Cmax and AUC0-12 (89% and 73% respectively). RALT when administered with RPNT daily reduced RALT s Cmin by 43% while having minimal effect on RALT s Cmax and AUC0-12 (3% and 1% respectively).

Conclusion: Both RPNT regimens reduce RALT trough concentrations less than that of RIF but greater than RBN. RPNT 600 mg once daily had a negligible effect on RALT Cmax and AUC0-12 while RPNT 900 mg given once weekly increased both parameters substantially. Further studies are warranted to determine the appropriate dosing regimen for TB treatment.

## 42. EPIDEMIOLOGY OF PULMONARY NONTUBERCULOUS MYCOBACTERIAL DISEASE IN THE U.S. VETERANS HEALTH SYSTEM

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**INTRODUCTION.** Nontuberculous mycobacteria (NTM) are opportunistic pathogens found in the environment. Sensitization to Mycobacterium avium complex, the most common NTM, has been highest in the Southeast than in other regions, and hospitalization rates for pulmonary NTM infections (pNTM) were recently found to be higher in Florida than in California, New York or Massachusetts. We hypothesize that rates of pNTM disease in the U.S. Veterans Health System (VHS) are higher in the Southeast than in other regions, and that the geographic distribution is similar for Legionella cases, as both NTM and Legionella pneumophila can survive in free-living amoebae. Pseudomonas aeruginosa is another opportunistic pathogen that is aquatic, but it does not survive in amoebae. We hypothesize that the distribution of hospitalizations for Pseudomonas pneumonia is random.

**METHODS.** In this pilot study, we obtained administrative data on hospitalizations, outpatient visits and number of persons with each disease for each administrative unit of the VHS. We then compared rates among regions.

**RESULTS.** There were 10,410 outpatient visits and 2,555 hospitalizations for pNTM infections. There were 802 visits and 684 hospitalizations for Legionella pneumonia, and 1,350 visits and 11,997 hospitalizations for Pseudomonas pneumonia. The rates of hospitalization for pNTM were significantly highest in the Southeast (72.8 per 100,000 veterans) and lowest in the West (5.9). The rates for Legionella pneumonia were highest in the Midwest and Northeast (14.1 and 14.8). The distribution of hospitalizations for Pseudomonas pneumonia appeared to be evenly distributed except lower in the Northeast.

**CONCLUSIONS.** These preliminary data suggest that there is a considerable burden of pulmonary NTM disease in the VHS, where pNTM is primarily an outpatient disease. The Southeast is disproportionately affected by pNTM.

pNTM accounts for 3.7 times the number of hospitalizations and 13 times the number of outpatient visits than Legionella. Contrary to our hypothesis, pNTM and Legionella pneumonia cases do not appear to share a similar geographic distribution. These preliminary data suggest the need to confirm these findings with more rigorous data analyses from the VHS.

## 43. BIOCHEMICAL CHARACTERIZATION Hip1, A MYCOBACTERIUM TUBERCULOSIS VIRULENCE FACTOR

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Hip1 is a cell-envelope predicted protease that is a virulence factor for Mycobacterium tuberculosis (Mtb). Absence of Hip1 results in compromised intracellular survival of Mtb in macrophages.<sup>1</sup> Although it was reported that Hip1 is not a protease<sup>2</sup>, here we report compelling biochemical evidence indicating that Hip1 is, in fact, a serine protease. Proteolytic activity was observed by monitoring the cleavage of the following synthetic chromogenic substrates: Ala-Pro-Ala-pNa, Gly-Pro-Leu-pNa, Ala-Pro-Ala-Arg-pNa, and Ac-Ala-Pro-Ala-Arg-pNa (pNa = p-nitroanalide). Inhibitor profiling indicates that Hip1 is a serine protease. However, Hip1 also appears to exhibit esterase activity when assayed with the chromogenic ester substrate, p-nitrophenylbutyrate. It is unclear whether the esterase activity originates from the Ser-Asp-His catalytic triad or from a non-active site serine residue. Nonetheless, the biochemical characterization and the assay development for this important drug target will facilitate our efforts to discover novel lead compounds that may serve as tuberculosis therapeutics.

## 44. TEMPERATURE AND DENGUE VIRUS INFECTION IN MOSQUITOES: INDEPENDENT EFFECTS ON THE IMMATURE AND ADULT STAGES

**Barry Alto**, Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **David Bettinardi**, Department of Microbiology, School of Molecular and Cellular Biology, University of Illinois

Holometabolous insect vectors of human pathogens such as biting flies occupy entirely different environmental niches during immature and adult stages. Environmental conditions acting on these stages may modulate vectorial capacity. The reproductive performance (fitness) and risk of disease

transmission can be impacted by temporal and spatial variation in environmental temperature. Asian tiger mosquito *Aedes albopictus* is an invasive species that has undergone rapid expansion of geographic range throughout the world. As a potential vector of numerous arthropod-borne viruses, its range expansion poses as a formable public health concern. We investigated the independent effects of environmental temperature acting on the immature and adult stages on life history traits and vector competence for dengue-1 virus. Immature and adult stages reared at 20°C, 25°C and 30°C differed in development time, adult life span and vector competence for dengue-1 virus. Cool larval rearing conditions buffered some of the life-shortening effects of warm adult rearing conditions as well as led to approximately 21% reduction in rates of viral dissemination, a prerequisite for transmission, from the midgut. Our results suggest that environmental temperature during ontogeny plays an essential role in modulating risk of disease transmission through alterations in life span of adults as well as through interactions with dengue-1 virus.

#### 45. DENGUE SEROTYPES 1-4 EXHIBIT UNIQUE HOST SPECIFICITY IN VITRO

**Kelli Barr**, Environmental and Global Health, Public Health and Health Professions; **Benjamin Anderson**, Environmental and Global Health, College of Public Health & Health Professions; **Gary Heil**, Environmental and Global Health, Public Health and Health Professions; **John Friary**, Environmental and Global Health, College of Public Health and Health Professions; **Gregory Gray**, Environmental and Global Health, College of Public Health and Health Professions; **Dana Focks**, Department of Environmental and Global Health, College of Public Health and Health Professions

**Background:** Over 3,000 thousand cell lines from over 150 species are commercially available today from American Type Culture Collection. These cell lines offer alternative approaches to investigating the interactions between arboviruses and other vertebrates at the cellular level. The various cell origins, types, and morphologies can be valuable resources for studying viral ecology and examining hypotheses regarding viral reservoirs. Dengue viruses (DENV) are major re-emerging pathogens that classically have been studied in only a few cell lines.

**Methods:** We evaluated the susceptibility of 19 distinct mammalian, avian, and reptilian cell lines to DENV infection. Cell lines were infected with DENV serotypes 1-4 and evaluated for susceptibility via focus forming unit assays and quantitative, reverse transcription polymerase chain reaction.

**Results:** Both methods demonstrated the ability of DENV to replicate in 14 cell lines derived from various vertebrates with viral titers ranging from 1X10<sup>3</sup> to 1X10<sup>7</sup> infectious units per ml. Cell line susceptibility to DENV infection was serotype specific with DENV-1 and DENV-4 infecting more cell lines than either DENV-2 or DENV-3. Cellular type also seemed to impact the infectivity of DENV. Human endothelial cells were only susceptible to DENV-4. 100% of 6 fibroblast lines were susceptible to at least one DENV serotype whereas only 62% of 13 epithelial lines were susceptible to DENV serotypes 1-4.

**Conclusions:** These data indicate that a variety of cell lines from human and animal species can be used to culture DENV. The serotype-specific susceptibility

for certain cell lines may provide a tool to help to characterize specific DENV serotypes or strains and may help substantiate the co-circulation of DENV strains and serotypes in a specific region or individual.

#### 46. MODELING THE CONTROL OF DENGUE WITH VACCINES IN THAILAND

**Dennis Chao**, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center; **Scott Halstead**, Dengue Vaccine Initiative; **M. Elizabeth Halloran**, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center; **Ira Longini**, Department of Biostatistics, College of Public Health and Health Professions, University of Florida

Dengue is a mosquito-borne infectious disease that constitutes a growing global threat with the habitat expansion of its vectors *Aedes aegypti* and *A. albopictus* and increasing urbanization. It is believed that 2.5 billion people are at risk of dengue infection worldwide. Candidate dengue vaccines, now in phase II and III trials, may constitute the best control measure for the foreseeable future. With four interacting dengue serotypes, the development of an effective vaccine has been a challenge. Before the widespread deployment of a new dengue vaccine, one needs to consider how best to use limited supplies of vaccine given the complex dengue transmission dynamics and the immunological interaction among the four dengue serotypes. Here we describe a simulation model for dengue transmission and control in a semi-rural area in Thailand where one phase III trial has started.

#### 47. MODELING FLUSH-TO-FLUSH TRANSMISSION OF HUANGLONGBING IN A CITRUS TREE AND EFFECTS OF CONTROL STRATEGIES ON DISEASE DYNAMICS

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Huanglongbing (HLB) (also known as citrus greening) is one of the most devastating diseases of citrus, and has caused serious economic damage to the citrus industries in Africa, Asia, South America and currently Florida. Since its discovery in Florida in 2005, citrus acreage in that state has declined significantly. Currently there is no cure for HLB and to understand the transmission dynamics together with the factors that make it difficult to curb the disease, a mathematical model of the transmission of HLB between its psyllid vector and citrus host has been developed. The model characterizes the dynamics of the vector and disease development, focusing on the spread of the pathogen from flush to flush (a newly developing cluster of very young leaves on the expanding terminal end of a shoot) within a tree. This approach differs from prior

models for vector transmitted plant diseases where the entire plant is the unit of analysis. The model considers two infection routes of the pathogen from flush to flush, via the vector and through the tree, which has not previously been done for a vector-borne plant pathogen. Dynamics of vector and host populations are simulated realistically as the flush population approaches complete infection. Model analysis indicates that vector activity is essential for initial infection but is not necessary for continued infection since infection can occur from flush to flush through internal movement in the tree. Flush production, within-tree spread and latent period are the most important parameters influencing HLB development. The model shows that the effect of spraying of psyllids depends on time of initial spraying, frequency and efficacy of the insecticides. Similarly, effects of removal of symptomatic flush depend on the frequency of removal and the time of initiation of this practice since the start of the epidemic. Within-tree resistance to spread, possibly affected by inherent or induced resistance, is a major factor affecting epidemic development, supporting the notion that alternate routes of transmission besides that by the vector can be important for epidemic development.

#### 48. ARE CARBONIC ANHYDRASES MAJOR PLAYERS IN BLOOD MEAL PROCESSING AND HOST SEEKING BEHAVIOR IN ADULT FEMALE MOSQUITOES?

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Mosquitoes represent a major threat to global health due to their rapid spread of diseases such as yellow fever, dengue fever, and of course the deadly malaria parasite *Plasmodium falciparum*. A major virulence factor that contributes to this massive disease spread is the adult female mosquito's ability to sense CO<sub>2</sub> from our breath. Once the female has located its human host and taken a blood meal, proper digestion of the blood meal is necessary for egg development. Carbon dioxide (CO<sub>2</sub>) is ingested with the blood meal at a high concentration and is also a form of metabolic waste produced while digesting the blood meal. We hypothesize that carbonic anhydrases (CA) optimize the processing of CO<sub>2</sub> during host seeking behavior and blood meal digestion. This poster presents studies on the localization of carbonic anhydrase using confocal microscopy and results from data mining of RNA-seq transcriptomes. Two isoforms of CA are of a main focus to this study: CA9 and CA10. Studies using the confocal microscope reveal that CA9 is found in the cytoplasm of gut and malpighian tubule epithelial cells. CA10 was found in the axonal processes of the nervous system and the cell bodies of chordotonal cells within the Johnston's organ. RNA-seq and microarray data revealed that multiple isoforms of CA are expressed in the gut, tubules, and head. These studies suggest that CAs play a major role in gut and nervous system physiological processes within adult female mosquitoes.

#### 49. CHARACTERIZATION OF VOLTAGE-SENSITIVE ION CHANNELS IN ANOPHELES GAMBIAE SUA-1B CELLS

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*Anopheles gambiae* is a complex of at least seven morphologically distinguishable species of mosquitoes in sub-Saharan Africa and the most efficient malaria vectors known. Previous studies in our laboratory found that *Anopheles gambiae* Sua-1B cells, an essentially immortal cell line, showed cytotoxicity when exposed to a mixture of the sodium channel toxin, veratridine (a plant toxin) and fenvalerate (a synthetic pyrethroid insecticide). In the current work, we present electrophysiological analysis of *Anopheles gambiae* Sua-1B cells and discuss our findings with regard to new insecticide screening. We performed electrophysiological analysis of 79 *Anopheles gambiae* Sua-1B cells with neuron-like morphologies using the patch clamp method. Of recorded cells, 63% were unipolar, 22% bipolar, and 15% multipolar neurons. No currents consistent with voltage-activated Na<sup>+</sup> channels were observed. Further studies are needed to assess whether veratridine/fenvalerate mixtures stimulate tetrodotoxin-sensitive inward currents by activating electrically silent sodium channels in Sua-1B cells, similar to previous work in NIE-115 neuroblastoma and C9 cells. However, a slowly activating outward current was observed in 76 out of 79 cells, especially at depolarized potentials. The amplitude of the current was greatly reduced (average 69%) in the presence of DIDS, a known voltage-sensitive chloride channel blocker, suggesting that this current was mainly mediated by chloride ions. Moreover, recordings made with Cs<sup>+</sup> (a K<sup>+</sup> current inhibitor) in the intracellular solution did not differ qualitatively from those made with normal physiological solution, indicating a deficiency of outwardly directed potassium currents. Additionally, recordings made with Ca<sup>2+</sup> free extracellular patch clamp solution diminished the slowly activating outward current component. Several cells had currents similar to those of volume-regulated chloride channels (VRCC). Taken together, our results suggest that outwardly rectifying voltage-sensitive currents observed in the majority of SUA-1B cells are mediated by calcium-activated chloride channels (CACCs). There is growing evidence that VGCCs are promising targets for new insecticides, and may also play a role in toxicity by pyrethroids. Given that the majority of *Anopheles gambiae* Sua-1B cells express voltage-gated chloride channels, this mosquito cell line can be a promising model for differentiated cell culture approach to novel insecticide discovery.

## 50. THE BREEDING HABITATS AND PRODUCTIVITY OF POTENTIAL DENGUE FEVER MOSQUITO VECTORS IN GRESSIER, HAITI

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**Introduction:** Dengue Fever (DF) is endemic in Haiti and continues to be a major public health concern in Haiti. While minimally important to native Haitians due to acquired immunity, to non-immune visitors with previous exposure to one serotype, it may lead to the potentially deadly hemorrhagic disease and the shock syndrome. High transmission rates of all the 4 dengue serotypes (DEN1- 4) has been reported in Haiti. Currently, there is no active surveillance of dengue transmission nor is there active dengue mosquito vector control in Haiti. With no DF vaccine available, controlling the mosquito vectors is the most effective preventive measure against transmission of DF. In Haiti there is a huge knowledge gap about the breeding habitats used by dengue mosquito and those that are responsible for the productivity of most dengue vectors. A clear understanding of container types that should be targeted in a source reduction effort can only be developed through detailed studies on container breeding types and productivities in Haiti.

**Methods:** To fill this knowledge gap, we conducted a prospective study on 41 households where we data on potential container types and presence of mosquito breeding in those containers found in those homes. In addition, ovitraps were set up inside and outside the selected homes to assess adult mosquito egg laying activity as an indicator of adult mosquito abundance in the homes.

**Results:** Overall, plastic buckets were more prevalent in the study homes, followed by plastic drums, metal buckets and metal drums. Other container types found included bottles, vases, and jars. Most of these containers were located outside homes. Of all the homes surveyed, 26 reported mosquito data. Of these 26, 18 homes were positive with larvae in the containers. Plastic buckets were commonly found with larvae, followed by plastic drums, then metal buckets and metal drums. There were no mosquito larvae found in other containers. The numbers of mosquito larvae ranged from 5-180 larvae. The SES did not influence the numbers of mosquito larvae in containers found ( $P= 0.083$ ). A comparison of the number of eggs found inside and outside study homes revealed no significant differences ( $P=0.415$ ). Additionally, the SES did not affect the number of eggs found inside or outside houses. Homes with plastic buckets had significantly higher numbers of eggs inside their homes than in homes with any other type of container (metal bucket, metal drums and plastic drums). However, the type or number of containers did not influence numbers of mosquito eggs collected outside the houses.

**Conclusion:** Many recent studies in other areas have shown that only a few, sometimes a single type of container, accounts for more than 90% of all dengue mosquito productivity. In this study, plastic buckets accounted for most dengue mosquito production in Haiti irrespective of SES. There was an equal chance for dengue mosquitoes to lay their eggs inside or outside the homes. This new knowledge on container types and productivity is important to enable mosquito source reduction and can be used in assigning homeowner responsibility for dengue vector productivity to influence community mobilization for dengue mosquito control in Haiti.

## 51. THE PATTERNS AND DRIVERS OF INTERNAL MIGRATION IN AFRICA

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Human population movements (HPM) are an important, yet poorly understood component in vector borne disease systems. A variety of data exist at different spatial and temporal resolutions that can be used to quantify HPM. In this study we sought to develop a quantitative model of movement to better understand the pattern and drivers of internal migration in individual African countries. Using freely available census microdata coupled with climate data we developed a suite of gravity-type spatial interaction (GTSI) models for 10 malaria endemic countries. The suite of models included a standard gravity model, with populations and distance parameters, that was then augmented with socio-demographic and climatic variables. Our results show that the GTSI models can in some cases explain up to 89% of the internal migration. In cases where consecutive census data was available, models fit to a previous census generated predicted flows for future census data that agreed with actual flows with an R-squared of up to 0.83. The results of this study can contribute towards optimizing malaria control, more strategic malaria elimination planning, as well as modeling the spread of drug resistance.

## 52. WEB-BASED GIS DESIGN FOR THE VECTOR-BORNE DISEASE AIRLINE IMPORTATION RISK (VBD-AIR) TOOL

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Over the past century, the size and the complexity of the air travel network has increased dramatically. Nowadays, there are 29.6 million scheduled departure flights per year and around 2.7 billion passengers are transported annually. The rapid expansion of the network and the increasing volume of international travel will inevitably cause concerns and challenges to health systems in terms of the control of infectious disease pandemics, disease vector invasion events and vector-borne pathogen importation, coupled with the challenges of climate change. The objective of this research is to develop a user-friendly Web-based GIS tool: the Vector-Borne Disease Airline Importation Risk Tool (VBD-Air), to help better define the roles of airports and airlines in the transmission and spread of vector borne human diseases. VBD-Air utilizes a three-tier server architecture in a MVC framework with distributed GIS components. This tool enables user to explore the interrelationships among the global distribution of vector-borne infectious diseases (malaria, dengue, yellow fever and chikungunya), locations of known outbreaks, and international air service routes to identify seasonal risks of vector borne infectious disease importation and spread by air travel, and to help plan mitigation strategies.

### 53. AGONISTS OF MUSCLE GLUTAMATE RECEPTOR INDUCE PARALYSIS IN Aedes Aegypti LARVAE

**Rafique Islam**, Entomology and Nematology University of Florida; **Jeffrey Bloomquist**, Entomology and Nematology, University of Florida

Designing an effective insecticide against mosquito vectors has been an enormous challenge to date. Some excitatory chemical agents activate ligand-gated ion channels followed by desensitization, a persistent, closed liganded state of the channel. Glutamic acid is a neurotransmitter capable of activating and desensitizing glutamate receptors in insect muscles. It works via the depolarizing subtype of glutamate receptor (GluRd). Prolonged activation, as well as desensitization of muscle excitatory receptors, could underlie paralysis. We have screened glutamic acid and a batch of its agonists on the yellow fever mosquito, *Aedes aegypti*. Initial efforts to test the compounds in intact larvae did not yield any effects. We explained this failure as the impermeability of the compounds for crossing the larval cuticle. Therefore, we performed our experiments on headless larvae where the thorax, the principal motor control center, was undisturbed, and the open cervical area afforded entry of compounds into the body cavity. We monitored swimming behavior of the larvae for five hours, a time period when 100% of headless control larvae displayed active swimming behavior when probed. When incubated in mosquito saline for five hours with glutamic acid, we found that some of the larvae were immobile after gentle agitation with an insect pin, and considered paralyzed. All tested compounds except domoic acid produced a flaccid type of paralysis, perhaps due to receptor desensitization. Domoic acid exposure yielded a spastic paralysis, consistent with persistently activated receptors without desensitization. While testing larvae with various GluRd agonists, we found different levels of paralytic potency, ranging from 7 ppm for L-aspartic acid to 1360 ppm for D-glutamic acid. Piracetam, a nootropic drug that modulates Na<sup>+</sup> flux at AMPA-type glutamate receptors in humans, yielded a PC50 of 8 ppm. Interestingly, we observed an enhancement of potency

when piracetam was combined with other GluRd agonists. For example, addition of 1 and 3 ppm piracetam to D-glutamic acid increased its potency 37 and 18 times, respectively. Although in lesser degree, similar enhancements were recorded for various GA agonists. Experiments are now being designed to advance these findings at the cellular level. We are particularly interested in understanding the roles of GluRd in current flow across the mosquito muscle membrane, and investigating agonist-dependent desensitization rates.

### 54. EVALUATION OF AEROSOL PESTICIDE APPLICATIONS AGAINST OLD WORLD PHLEBOTOMINE SAND FLY VECTORS OF LEISHMANIA IN KENYA

**Kenneth Linthicum**, Center for Medical, Agricultural, and Veterinary Entomology, Agricultural Research Service, USDA; **Seth Britch**, Center for Medical, Agricultural, and Veterinary Entomology, Agricultural Research Service, USDA; **Robert Aldridge**, Center for Medical, Agricultural, and Veterinary Entomology, Agricultural Research Service, USDA; **Todd Walker**, U.S. Navy Entomology Center of Excellence; **Muhammad Farooq**, U.S. Navy Entomology Center of Excellence; **Thomas Logan**, U.S. Army Medical Research Unit - Kenya; **Joshua Bast**, U.S. Army Medical Research Unit - Kenya; **Vitalice Opondo**, U.S. Army Medical Research Unit - Kenya; **Daniel N'gonga**, U.S. Army Medical Research Unit - Kenya; **Clifford Chepchieng**, U.S. Army Medical Research Unit - Kenya,

One component of the Department of Defense (DoD) pest management system is ultra-low volume (ULV) and/or thermal fog aerosol pesticide application. Despite widespread implementations of this and other components of the system, such as use of repellents and permethrin, US military operations in hot-arid regions still face substantial impacts from insect vectors of disease such as mosquitoes and sand flies. Few studies have compared ULV and thermal fog technologies, and no study has analyzed their performance or efficacy against sand flies in hot-arid environments. In this study we evaluated the Grizzly ULV (Clarke) and the Swingfog SN101E (Swingtec) calibrated on site with two pesticides, Fyfanon (malathion) and Duet (sumithrin, prallethrin, and PBO), in separate trials against caged sentinel *Phlebotomus duboscqi* sand flies and wild populations of *Phlebotomus* and *Sergentomyia* spp. sand flies in the hot-arid North Rift Valley, Kenya. Wild sand fly populations were sampled throughout the study and for all trials sentinel sand flies were arranged in 25-cage grids with five offsite control cages. Spray plots for both the sprayers and chemiCollege of Agricultural and Life Sciences were reciprocated and spray times and environmental conditions were reasonably consistent across trials. Wild sand fly population sampling showed good control in all treated plots as well as a possible repellent effect indicated by increased populations in nearby untreated areas. Wind shear effect was observed in spatial mortality patterns in thermal fog applications, but was notably absent in mortality from concurrent ULV applications. Prior trials with the Grizzly in Kenya demonstrated widespread control with Duet, but the reverse was seen in the present study. Duet applied with the Swingfog provided rapid and widespread control despite sub-optimal conditions, although uneven terrain led to longer spray time in that instance. Prior studies in hot-arid areas in California had

shown thermal fog applications superior to ULV when using Fyfanon against mosquitoes, but the present trials showed the reverse against sand flies.

## 55. CAN HORTON HEAR THE WHOS? SCALE IN VECTOR-BORNE DISEASE

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The epidemiology of vector-borne pathogens is affected by mechanisms and interactions at different scales, from individual level molecular processes to ecosystem interactions between species and their environment. This is of particular interest in the development of mathematical models to understand pathogen dynamics or develop intervention strategies. Choosing the scales and interactions included in models is critical for the conclusions drawn. We illustrate this using a key aspect of vector-borne disease, transmission of the pathogen between vectors and vertebrate hosts. A model of mosquito infection is expanded to illustrate the types of studies needed. Each mosquito has a number of virions needed for infection sampled from a gamma distribution and ingests a number of virions in its blood meal sampled from a separate gamma distribution. The two distributions are considered jointly in their effects on the resulting number of infectious mosquitoes. The parameters of the gamma distributions affected the number of infectious mosquitoes, with higher numbers occurring when the distributions were different. Assumptions about individual level characteristics (parameters of the gamma distributions) affected population level characteristics (number of infectious mosquitoes). Similar effects occur between other scales. Population interactions can affect community structure, while heterogeneity in community structure and population interactions with the environment can modify vector-borne disease transmission cycles. The interaction of communities of vectors, vertebrate hosts, and pathogens within the context of changing environmental conditions will influence individual life histories and population characteristics. Although complex, it is critical that interactions at different levels of scale are understood in order to fully integrate laboratory or small-scale field studies into an improved understanding of disease transmission at all scales, with the ultimate goal of improving risk prediction and reducing vector-borne disease.

## 56. DOCUMENTING THE POTENTIAL INTRODUCTION OF DENGUE VIRUS INTO KEY WEST, FLORIDA THROUGH AIRLINE AND CRUISE SHIP PASSENGERS FROM DE

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For the first time in decades, sporadic cases of locally-acquired dengue were reported in Key West in 2009 and again 2010. Current hypotheses regarding this continuance include vertical transmission, the establishment of an endemic state with undetected transmission between years, and multiple introductions via visitors from endemic countries during both years. Regarding the third hypothesis, country and year specific dengue incidence data (PAHO) and the numbers of airline passengers originating in dengue endemic countries in this hemisphere with a final destination of Key West were used to estimate the relative the magnitude of potentially viremic passenger days experienced per year. These estimates suggest multiple introductions per year are not uncommon and that potential introductions in 2009 and 2010 were higher than in 2007 and 2008 as a result of an increase in air travel and major dengue activity in the Caribbean and Central America. Both years were El Niño years that historically are associated with elevated temperatures and higher dengue activity in the region. A similar analysis of potential introductions via the cruise ship industry will also be presented.

## 57. MOLECULAR ANALYSIS OF PLASMODIUM FALCIPARUM CHLOROQUINE RESISTANCE IN HAITI

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Background: Malaria in Haiti is meso-endemic although in some areas it exists in an epidemic state that coincides with rainfall patterns. There is a general lack of detailed knowledge about malaria in Haiti especially about anti-malarial drug resistance. The first line drug of choice for the treatment of malaria in Haiti is chloroquine (CQ). This treatment policy is based on previous drug sensitivity studies conducted more than 30 years ago that showed sensitivity of P. falciparum to chloroquine. The Haiti Ministry of Health does not conduct routine



surveillance of antimalarial resistance but has recently agreed to re-invigorate surveillance activities for drug resistance especially after the one recent report of CQ resistance genotypes in malaria samples from one location in Haiti. There is a need for additional data on chloroquine resistance followed up by in-vivo studies. Resistance to CQ is mainly conferred by single-nucleotide polymorphisms (SNPs) in *pfcr* gene a chloroquine transmembrane transporter that is found in the digestive vacuole membrane of the malaria parasite.

**Methods:** We analyzed the amino acid mutations in the *pfcr* gene at positions 72, 73, 74, 75 and 76. The most widely used and also considered a reliable marker for CQ resistance is the amino acid (AA) mutation at position 76, K76T or simply K76T. We analyzed for K76T plus AA mutations at positions 72 - 75, which have been used to type malaria strains; and so we used them to characterize the *P. falciparum* from Haiti **Results:** In this continuing study, we have collected 120 malaria positive samples from five locations across Haiti but have analyzed 22 samples for the *pfcr* gene and 14 for the *pfmdr1* gene. For the *pfcr* gene, we identified five K76T mutations. Analysis of the mutations at positions 72 - 75 revealed a new haplotype CVMMDT in Haiti. This haplotype was only recently been reported in Africa. Seventeen samples were wild type (CVMNKK), 3 were CVIET, 1 was CVINT and 1 was CVMMDT. The analysis of SNPs in the *pfmdr1* gene, which modulates resistance to CQ identified one sample with a mutation at amino acid position 86 - N86F. No mutations were observed at amino acids position 1246 (D1246 for wild type).

**Conclusions:** There is a great need for surveillance and monitoring of CQ resistance to provide the much needed data for the management of malaria in Haiti

## 58. MALARIA ELIMINATION: THE IMPLICATIONS OF POPULATION MOVEMENTS IN NAMIBIA

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**Introduction:** Namibia has seasonal or unstable *P. falciparum* malaria primarily in the northern part of the country. The national malaria control program has set a malaria elimination target of 2020. Among the challenges that the control program faces in achieving and maintaining elimination, are population movements from neighbouring malaria-endemic Angola, that may bring imported infections. Quantifying population movements contributes to assessing the feasibility of elimination, as health facility-based surveillance systems are often not effective in detecting all imported cases, for example, infections imported by asymptomatic parasite carriers and non-health seekers.

**Methods:** Travel history data from a cross-border survey in Oshikango, Namibia, was used to assess cross-border travel between Angola and Namibia. Population movement patterns, together with malaria endemicity maps, population distribution maps and mathematical models, were analysed to assess the implications of population movements on imported infection rates into Namibia,

as relevant for strategic elimination planning. With detailed information on individual travellers' demographics, time of travel and duration of stay in higher transmission locations, estimates of the number of imported infections from Angola, seasonal adjustments for months in which largest number of imported infections are likely, and the destinations in Namibia which are most likely to receive imported infections were obtained.

**Results:** Most individuals did not travel in the survey period. Furthermore, most cross-border travellers spent only a few hours or a day across the border and as *Anopheles* mosquitoes bite at night, daily movements pose minimal threat to local transmission. However, individuals that stay longer than a day risk being bitten by a susceptible *Anopheles* mosquito and therefore may instigate onward transmission. Such short term travellers were most likely to travel to and from Santa Clara and Ondjiva in Cunene Province that borders Namibia. Most imported infections occurred between the months of January and April. Imported infections from Angola and locally acquired infections in individuals moving in and out of Namibia's northern malarious areas may result in imported infections and threaten elimination success in parts of Namibia where endemicity is low but environmental and climatic factors can sustain local transmission. **Conclusion:** Detailed spatial and temporal information on population movements can inform the strategic development of elimination tools in Namibia. Cross-border implications of population movements contribute to improved between-country control programmes to more efficiently reduce the burden of malaria at a regional level, and thereby improve the chances of elimination.

## 59. THE EFFECT OF URBANIZATION ON PLASMODIUM VIVAX MALARIA TRANSMISSION

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**Background:** Urbanization has been shown to reduce *P. falciparum* malaria transmission. Many recent studies have examined the impact of urbanization on *P. falciparum* malaria endemicity, disease burden estimation and risk mapping. However, none have examined the effect of urbanization on *Plasmodium vivax* malaria, which is the most widely distributed malaria species and can also cause severe clinical syndromes in humans. In this study, the community-based *P. vivax* parasite rate (PvPR) surveys from the Malaria Atlas Project (MAP) database are used to explore the PvPR relationships between urban and rural areas. **Methods:** A total of 10,003 geo-referenced PvPR surveys were included in the analysis. Firstly, all the PvPR surveys were overlaid onto the Global Rural Urban Mapping Project Urban Extents (GRUMP-UE) map to derive an urban/rural assignment. Then, sets of spatially and temporally associated urban-rural pairs of PvPR values were obtained to assess if significant differences between PvPR values in urban and rural areas exist. Finally, groups of PvPR surveys inside

individual city extents (urban) and surrounding areas (rural) were identified and tested to examine the local variations in PvPR values.

Results: Significantly higher PvPR values in rural areas were found globally, in Africa and Asia, while in the Americas, significantly lower values of PvPR in rural areas were found. Moreover, except for the countries in Americas, the trends found in all the other countries in Africa and Asia were consistent, with significantly lower values of PvPR in urban areas. However, the patterns among 13 specific cities were less consistent, with five cities having significantly lower PvPR values in urban areas, three cities showing lower PvPR in rural areas (though not significant), and the remainder were either not significant or zero values.

Conclusions: The tremendous environmental changes caused by urbanization have profound influences on malaria transmission. Except for the Americas, the patterns of significantly lower *P. vivax* transmission in urban areas have been found globally, regionally and nationally. However, more heterogeneity exists among the urban-rural PvPR of individual cities. To further understand these patterns, more epidemiological, entomological and parasitological analyses of the disease are needed in the future.

## 60. ALTERED BLOODFEEDING BEHAVIORS OF SINDBIS VIRUS-INFECTED Aedes Aegypti (DIPTERA: CULICIDAE) TO DEET AND NON-DEET REPELLENTS

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Changes in the bloodfeeding behavior of adult female *Aedes aegypti* (L.) mosquitoes following dissemination of Sindbis virus (SINV) were observed after exposure to repellents with the active ingredients (AI) DEET, picaridin, 2-undecanone (2-U), and oil of lemon eucalyptus. There are four stages involved in mosquito bloodfeeding: activation, orientation, probing, and engorgement. Significant ( $P < 0.0001$ ) changes in activation, probing, and engorgement times were observed in SINV infected mosquitoes after exposure to the four repellents compared to uninfected mosquitoes. Mosquitoes exposed to DEET and had a disseminated SINV infection located a bloodmeal  $2.2 \pm 0.2$  h before their uninfected counterparts. A decrease in activation time was also observed in mosquitoes with a disseminated SINV infection after exposure to picaridin with activation occurring on average  $1.7 \pm 0.1$  h sooner than in uninfected mosquitoes. Probing times in mosquitoes with a disseminated SINV infection were increased after exposure to DEET and picaridin. This increase in probing time was a result of mosquitoes with a disseminated SINV infection demonstrating the phenomenon of interrupted feeding which may result in an increase in virus transmission. Engorgement times of mosquitoes with a disseminated SINV infection were increased by  $245 \pm 18.5$  s and  $176 \pm 40.5$  s after exposure to DEET and picaridin, respectively. A decrease in the total time to complete the four

bloodfeeding stages will lessen the prey-status of the mosquito host and enhance both the chances of mosquito survival and arbovirus transmission.

## 61. A REVIEW OF WEST NILE VIRUS DISEASE IN DUVAL COUNTY, 2011

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In 2011, Duval County had a resurgence of West Nile virus (WNV) activity and had the highest number of WNV illness cases in Florida. Twenty cases of WNV illness with onset ranging from June 23 through October 4 had exposure to the virus in Duval County; three asymptomatic Duval blood donors were also identified. The report describes the public health response to a WNV illness outbreak in Duval County, Florida and associated risk factors.

West Nile virus illness is a reportable condition in Florida. Data were collected on cases reported to the Duval County Health Department and included demographics, laboratory information, medical history at diagnosis, and risk factors for infection.

The median age of cases was 55 years with a range of 38 to 85 years. The attack rate for the 55 and older age group (5.0 cases per 100,000 population) was higher than that of the 35 to 54 age group (4.0 cases per 100,000 population). Sixteen (80%) of the patients presented with neuroinvasive illness, and four (20%) presented with less severe non-neuroinvasive signs and symptoms. Eighteen (90%) of the patients were hospitalized, and two (10%) died. Risk factors included smoking (11, 55%), spending time outside (15, 75%), being homeless (4, 20%), and having pre-existing medical conditions (11, 55%). Twelve of the cases (60%) and the three asymptomatic blood donors resided in the 32210 and 32205 zip codes. The attack rate in these two zip codes (17.9 cases per 100,000 population) was much higher than for the remainder of Duval County (1.3 cases per 100,000 population).

Aggressive outreach to health care providers should be conducted to ensure reporting of arbovirus cases, and may be done via fax, participation in medical rounds, or other methods. Providing information related to factors increasing disease risk such as immune suppression, advanced age, smoking, and outdoor occupation or activities will encourage health care providers to emphasize prevention among patients at risk for severe disease. Also, engaging the local homeless coalition and attempting to secure insect repellents or mosquito netting for the homeless population may help prevent mosquito-borne illness.

Furthermore, county health departments should coordinate with the local mosquito control division to provide the public with timely information and effective mosquito control efforts. Finally, communities should be educated to minimize mosquito breeding environments, to wear protective clothing, and to regularly use insect repellent to reduce the incidence of the WNV infection and other mosquito-borne illnesses.

## 62. GENOME SEQUENCE CHARACTERIZATION OF A DENGUE-1 VIRUS ISOLATED FROM KEY WEST, FL

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Dengue virus (DENV) is a fatal disease that is transmitted to humans through the bite of mosquitoes. In November 2009, a dengue outbreak was reported from Monroe County in southern Florida, including 20 confirmed human cases. The DENV isolated from the human cases in Key West were identified as DENV-1. RNA was extracted from the DENV-1 isolate and was used in RT-PCR reactions to amplify PCR fragments to sequence. Nucleic acid primers were designed to generate overlapping PCR fragments that covered the entire genome. The DENV-1 that was epidemic in Key West has been sequenced for whole genome characterization. The sequence assembly, analysis and Genbank searches have been performed to verify the identity of the genome sequences and to determine percent identity to known DENV-1 sequences.

## 63. LOCALLY ACQUIRED DENGUE CASES IN FLORIDA

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**BACKGROUND:** In 2009-2010 an outbreak of local dengue fever with 93 cases occurred in Key West after more than 60 years without detected local dengue transmission in the state. Subsequent increased surveillance has uncovered at least eight additional dengue virus introductions. Epidemiologic, ecologic, and laboratory findings from these introductions and associated prevention efforts are discussed.

**METHODS:** Data were collected by the Florida Department of Health (FDOH) Vectorborne Disease Surveillance Program from county health departments and local mosquito control programs. Laboratory testing was performed at FDOH Bureau of Laboratories or Centers for Disease Control (CDC) per national guidelines.

**RESULTS:** Introductions occurred in 6 of Florida's 67 counties: Broward (1), Hillsborough (1), Martin (1), Miami-Dade (3 or 4), Monroe (1) and Palm Beach (2). It was not established whether two cases in Miami-Dade were linked or were individual introductions. In all other instances, virus typing and epidemiological data confirmed that cases were isolated. In two instances (Martin and Hillsborough) autochthonous infections followed international travel by another

household member. While at least six introductions occurred in counties with the highest number of imported dengue cases (Miami-Dade, Broward and Palm Beach), two were in counties with relatively low numbers of imported cases detected (Martin and Monroe). In two instances the outbreak area included popular domestic and international tourist destinations (Monroe and Miami). Likely exposure for index cases occurred while at the case home (6 or 7), lodging at a bed and breakfast (1), engaged in an out-of-doors occupation (1), and socializing outside a popular restaurant (1). Exposure sites for all cases primarily were in neighborhoods with yards and ample vegetation. Other exposure sites involved an urban apartment complex, a downtown area with abandoned buildings, tourist area, and a construction site. *Aedes albopictus* was believed to have been the primary vector in Martin County and for least one of the Palm Beach introductions. *Aedes aegypti* appeared to be most important in the others and is the only dengue vector present in Monroe County.

**CONCLUSIONS** Prevention efforts targeting travelers, international airports and cruise ship ports are needed. Emphasis should also be placed on using prevention practices when travelers become sick after returning home. Outreach is particularly important in counties with high numbers of imported dengue cases or that have robust populations of *Ae. aegypti*.

## 64. ELUCIDATING UNIQUE PHARMACOLOGICAL PROPERTIES OF RHIPICEPHALUS (BOOPHILUS) MICROPLUS ACETYLCHOLINESTERASE: PROVIDING LEADS FOR T

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The cattle tick, *Rhipicephalus (Boophilus) microplus* (Canestrini; Bm), is a potentially deadly pest of cattle as they are primary vectors for *Babesia*, a protozoan parasite that causes the deadly hemolytic disease known as babesiosis. Economic losses are furthered substantially as tick infestations lead to reduction in milk production and weight gain, as well as overall declines in cattle health. Although Bm has been eradicated within the United States, Mexican cattle still suffer infestations of Bm that harbor *Babesia* sp. and pose a threat to cattle populations within the United States through reintroduction. To compound the issue, control has become increasingly difficult due to escalating organophosphate and pyrethroid resistance through metabolic enzyme up-regulation and target site insensitivities. The purpose of this study was to characterize the structural biology of Bm acetylcholinesterase (AChE) with molecular homology modeling and to determine the potency of experimental N-methylcarbamates toward BmAChE. Pharmacological and structural analyses of AChE have revealed that AChE contains two binding sites for inhibitors: one at

the catalytic site (CS) and one near the entrance to the catalytic gorge, the peripheral site (PS). Results indicate that BmAChE has low sensitivity ( $IC_{50} = 200 \mu M$ ) toward tacrine, a monovalent CS inhibitor with mid nanomolar blocking potency in all previous species tested. Similarly, a series of bis(n)-tacrine dimer series, bivalent inhibitors (E2020 and methoxyphenyl sulfamoyl-4-methylbenzamide) and peripheral site AChE inhibitors possess poor potency toward BmAChE. Molecular homology models suggest the residue F384 has been mutated to W384 near the catalytic site. The W384 residue possesses a large side chain and obstructs the access of larger ligands to the active site and therefore, reduces potency of these larger molecules. Also, the model suggests a W286Y mutation in the Bm peripheral site decreases the enzyme sensitivity to peripheral site ligands, such as ethidium. This finding suggests a unique AChE gorge structure, a phenomenon that can further support the possibility for design of selective inhibitors.

Secondly, results indicate that several experimental carbamate inhibitors possess up to 350-fold selectivity for BmAChE over human AChE. This selectivity for AChE inhibitors could provide leads for Bm control due to its poor mammalian activity. Also, these compounds can provide leads toward designing acaricides that mitigate the target site insensitivities observed in some Bm populations.

## 65. FLUORESCENT ASSAY OF ACETYLCHOLINESTERASE LIGAND INTERACTIONS FOR DESIGN OF INSECTICIDES TARGETING MOSQUITO VECTOR OF MALARIA

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There is an urgent demand to discover and develop alternatives to conventional insecticides used in mosquito control due to their high toxicities to non-target organisms, and development of resistance. Here we present fluorescence data on the interactions between various acetylcholinesterase (AChE) inhibitors and the AChE peripheral anionic site, which is considered to be a potential target for new insecticides acting on this enzyme. The assay uses thioflavin-t (TFT) as a probe, binds to the peripheral anionic site of AChE and yields an increase in fluorescent signal. A drop in TFT fluorescence may occur either by inhibiting binding of TFT in the peripheral site, or indirectly by reducing rotational rigidity of bound TFT. In the present study, we screened effects of AChE inhibitors in different categories, including catalytic site inhibitors, peripheral site inhibitors, and bivalent inhibitors that occupy both sites. All were screened upon *Anopheles gambiae* AChE and human recombinant AChEs by using the TFT assay as well as Ellman's assay to compare and contrast changes of peripheral site conformation and catalytic ability responding to various inhibitors. Our results showed that all the inhibitors reduced TFT fluorescence with human recombinant AChE, indicating negative allosteric coupling between the catalytic site and the peripheral site of human AChE. With mosquito AChE, all the peripheral site

inhibitors, two bivalent inhibitors (PRC472 and BW284C51), and one catalytic site inhibitor, edrophonium, showed inhibitory effects on the TFT fluorescence. However, all the carbamates (which alkylate the active site Ser203) and two bivalent tacrine dimers, potentiated the TFT fluorescent signal. This finding suggests a different mechanism for catalytic site ligands modulating the conformation of the peripheral site of mosquito AChE. This finding may provide some insight into the structural differences between mosquito AChE and human AChE, which is a key point for the design of novel, selective insecticides with higher affinity to the protein target in mosquito than in human.

## 66. GENETIC MONITORING OF AMERICAN CROW AFTER THE EMERGENCE OF WEST NILE VIRUS IN UNITED STATES: PRELIMINARY DATA

**Claudio Verdugo**, Infectious Diseases and Pathology, Veterinary Medicine, University of Florida; **Maureen Long**, Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida

Pathogens play a significant role in the evolution of the host genome by influencing the maintenance of much genetic variation in natural populations (Antonovics et al. 1994). Novel or newly introduced pathogens can exert a strong selective pressure on naïve host populations (Dobson et al. 2001) modulating the genetic composition in relatively short timescales. The American crow, *Corvus brachyrhynchos*, is well recognized to be one of the most negatively affected species by the emergence of West Nile virus (WNV) in North America in 1999. Genetic monitoring of such declining population processes after the introduction of an infectious disease can provide insights into demographic and evolutionary impact of the pathogen in a natural host population over time. In this study, the levels of genetic diversity in American crows after the emergence of WNV were assessed using polymorphic microsatellite markers. We used a temporal sampling approach to examine whether the populations of crows have lower genetic diversity after few generations under the strong pressure of WNV. Variation at 38 species-specific and cross-species microsatellites were used to analyze 345 samples collected from 2000 to 2010 in New York. Levels of microsatellite polymorphism were evaluated for each locus across each year cohort. Then, the patterns of genetic structure among cohorts over all loci were analyzed to determine temporal stability among samples collected at different time of epizootic spread. Populations that have experienced a recent reduction of their effective population size exhibit a correlative reduction of the allele numbers and gene diversity (i.e. heterozygosity) at polymorphic loci. The detection of possible bottleneck in American crows due to the emergence of WNV will be further discussed.

## 67. MOLECULAR EPIDEMIOLOGY OF AVIAN MALARIA IN GRASSLAND NESTING BIRDS

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Migratory avifauna traverse multiple ecosystems across different continents which exposes them to many pathogens and has implications for the global spread of infectious diseases. Migratory birds have been implicated in the transmission and emergence of infectious diseases such as high pathogenicity avian influenza virus, West Nile virus and blood parasites (*Plasmodium* and *Haemoproteus*). Our study system in the grasslands of the Great Plains provides nesting habitat for both non-migratory grassland species such as the Greater Prairie-chicken (*Tympanuchus cupido*), and migratory species like the Upland Sandpiper (*Bartramia longicauda*), and provides the opportunity to examine how migratory behavior affects exposure to and transmission of vector-borne pathogens. We compared prevalence and examined the molecular epidemiology of the blood-borne pathogens, *Plasmodium* and *Haemoproteus* (the causative agents of avian malaria) in 1479 Prairie-chickens and 605 Upland sandpipers that nested sympatrically in the Flint Hills of Kansas. Using molecular surveillance techniques (amplification of a portion of the cytochrome b gene in the mitochondrial genome of the pathogen), we found that 8% (n=118) of Prairie-chickens and 6.6% (n=40) of Upland sandpipers were infected with haemosporidians. While the majority of *Plasmodium* infections within a species were haplotypes unique to each species, Upland sandpipers had a higher diversity of *Plasmodium* lineages with a broader pattern of phylogenetic divergence as expected for a highly vagile species. Haplotype KC82 was the most common haplotype found in the Greater Prairie-chicken (with a prevalence of 90.8% in infected birds) but was also found in several Upland sandpipers, suggesting transmission from Prairie-chickens to Upland sandpipers. In addition, a *Plasmodium* haplotype found in 40% of infected Upland Sandpipers was closely related to haplotypes reported in the endangered Galapagos Penguin (*Spheniscus mendiculus*) suggesting that migratory Upland Sandpipers may have the capacity to transport blood-borne pathogens across continents from South to North America. The global distribution of migratory birds likely increases their probability of encountering diverse pathogens and transmitting these to other avian species. Our study indicates that cross-species transmission of *Plasmodium* has occurred between the migratory Upland Sandpiper and Greater Prairie-chicken in the Flint Hills and alludes to a complex global transmission network.

## 68. PREVALENCE OF ORAL HPV INFECTIONS AND ASSOCIATED RISK BEHAVIOR IN COLLEGE WOMEN

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Center and Research Institute; **P. Daniel Obesso**, Department of Internal Medicine, College of Medicine, University of Florida; **Anna Giuliano**, H. Lee Moffitt Cancer Center and Research Institute

**Background:** HPV-related cancer prevalence of the head and neck is increasing, but little is known about when or how oral HPV is acquired. Some data suggest oral sexual behavior and smoking are associated with risk of oral HPV infections. However, data are inconsistent and no previous study reported oral HPV rates in college women, who represent a population with high rates of genital HPV infections. The objectives of this study were to determine the prevalence of oral HPV infection in a sample of college women, and to identify characteristics associated with prevalent oral HPV.

**Methods:** The sample included 1030 women, currently enrolled at a large southeastern university, who provided an oral rinse/gargle specimen and completed a computer-based questionnaire in June–November, 2011. Oral specimens were tested for 37 high- and low-risk HPV genotypes using a commercial linear array assay. A participant was noted as HPV positive if the sample amplified HPV on PCR and hybridized with a specific HPV type upon genotyping.

**Results:** Participants' mean age was 21.9 years; the racial distribution was 51.0% white, 15.2% black; 14.2% Hispanic, 9.8% Asian, and 9.7% other. Of the 1011 women whose results were  $\alpha$ -globin-positive, 10 women had oral HPV (1.0%), representing HPV types 16, 51, 59, 62, 73, and 84, of which three (16, 51, 59) were carcinogenic. 467 (45.4%) of women in the survey had received at least 1 HPV vaccine; neither of the two positives with oral HPV type 16 had been vaccinated. Prevalence of genital HPV (1.85% of those with genital HPV vs 0.94% of those without,  $p=0.424$ ) was not significantly associated with HPV. However, ever smoking was significantly associated with oral HPV (1.75% (n=8) of ever smokers vs. 0.36% (n=2) of never smokers,  $p=0.049$ ). Increasing use of alcohol per month ( $p<0.01$  for trend), increasing lifetime number of vaginal sex partners ( $p<0.01$  for trend), oral sex partners (with penile contact,  $p<0.01$  for trend), sharing of alcohol cups (1.65% (n=10) of those who shared, vs. 0.00% of those who had not,  $p<0.01$ ) were also associated with HPV.

**Conclusion:** Oral HPV infections appear to be uncommon in college women, despite the relatively high prevalence of genital HPV and risk behavior believed to be associated with oral HPV. Oral HPV infections could be latent (not shedding), acquired later in life, or truly be rare in young women. Oral HPV screening in this population may be unwarranted.

## 69. METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) COLONIZATION IN CHILDREN WITH CONGENITAL HEART DISEASE (CHD).

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**Background:** Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization has been recognized as a significant problem among hospitalized patients. Available data about MRSA prevalence among children with congenital heart disease (CHD) is minimal.

**Objective:** To determine the prevalence of and identify risk factors for MRSA colonization among children with CHD admitted to the pediatric intensive care unit (PICU).

**Design/Methods:** Children <19 years old with CHD admitted to the PICU between 4/1/2008 & 3/31/2011 had admission & weekly MRSA nasal surveillance cultures. Patients were stratified into three groups based on the complexity of their CHD (JACC, 2001. 37:1161-98). The MRSA colonized children were compared to the MRSA non-colonized children.

**Results:** During the 3-year study, there were 380 admissions to the PICU by children with CHD. Of the 380, 74 (19.5%) had no surveillance cultures or prior history of MRSA colonization and were excluded from further analysis. A total of 29 (9.5%) of the remaining 306 admissions (269 individuals) were considered colonized: 11 (3.8%) via surveillance cultures, 16 (4.2%) had prior history of MRSA colonization/infection, and 2 had non-surveillance MRSA positive cultures (an eye swab and an incision culture) .

The mean age was 3.53 yrs (range 0.01-18.76 yrs, median 0.72). Age distribution was not significantly different between the colonized and non-colonized groups. Analysis of the 269 children (30 having multiple PICU admissions during the study period) demonstrated that gender ( $P=0.621$ ), race ( $P=0.817$ ), & CHD complexity ( $P=0.087$ ) were not significantly associated with colonization status. There were 142 patients who had previous known hospitalizations and 164 who did not, with 21 (14.8%) and 8 (4.9%) MRSA colonized respectively. The odds of being colonized if previously hospitalized were 3.38 times greater than if not previously hospitalized (95% CI: 1.45, 7.90).

**Conclusions:** Among children with CHD, age, gender and complexity of heart condition are not predictors for MRSA colonization. Prior hospitalization increases the likelihood of MRSA colonization by >3 fold. Therefore, routine MRSA surveillance in patients with CHD should be performed to identify colonized patients.

## 70. SPATIO-TEMPORAL AND GENETIC PATTERNS OF ANTHRAX OUTBREAKS IN TEXAS AND MONTANA

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Since 2002, we have investigated wildlife and livestock anthrax throughout Texas and defined two ecological zones based on climatic conditions and outbreak epizootiology. We define the western counties around Val Verde as the "enzootic zone" based on evidence of at least some cases each year with frequent large epizootics (prevalence rates upward of 10%). In contrast, southern Texas is defined as the "sporadic zone", where few (if any) cases occur each year with less frequent epizootics and generally poor soil conditions for *Bacillus anthracis* spore survival. Additionally, we have been studying the apparent emergence of this pathogen in western Montana since 2008, with a large epizootic of bison, deer, and elk found dead between 23 July and 24 August 2008. Since then, we have confirmed an additional two bison cases associated with wolf kills in 2010. Since 2002, we have mapped a large number of carcasses across the Texas and Montana landscape and developed a detailed geographic information system to identify ecological patterns associated with outbreaks. We have also constructed an NDVI-based model of annual spring and summer time conditions to distinguish between epizootic and sporadic years within the enzootic zone of Texas and emerging zone of western Montana. We have also performed high resolution MLVA-25 genotyping on samples from across these zones and linked spatio-temporal epizootic data with specific genotypes. This paper will delineate these three zones and compare and contrast habitat conditions and specific genotypes associated with each zone. These data provide a significant move toward identifying pre-season conditions that may be associated with epizootic conditions and may provide a framework for predicting seasonal fluctuations in case numbers. Additionally, we provide a framework for linking multi-scale models from ecological niche-based geographic models to individual animal movement models that improve our understanding of this pathogen in North American wildlife.

## 71. ARTIFICIAL SELECTION ON SIGMA VIRUS TITER AND CORRELATED RESPONSE OF VIRULENCE

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One hypothesis to explain the evolution of parasites toward high levels of virulence (where virulence corresponds to a decrease in the host fitness) is that parasites maximize intra-host replication rate in order to increase transmission. Here, using the vertically transmitted Sigma virus (Rhabdoviruses) of *Drosophila melanogaster*, we propose to directly study the effect of within host growth rate on the virus evolution. Using QPCR, this experimental evolution work consisted of selection over eleven generations of host for both increased and decreased viral titer in seven replicates of a single effectively isogenic line of *D. melanogaster*. We observed an increase over time in the difference in virus titer between the two treatments, including significant difference in the last generation. This difference in virus titer had also effects on parasite virulence, whose three proxies showed significant differences between the two treatments. These results illustrate how parasite virulence can be

positively linked with increase in intra-host replication rate, as stipulated by the trade-off hypothesis for the evolution of virulence. The sequencing of the full genome of the viruses from the two treatments suggested that most of the differences between the two treatments are due to fixation of new mutations in the treatment consisting of decreasing the virus titer. Evolution of the host genome during the experiment is also suspected.

## 72. PORPHYROMONAS INFECTION UPREGULATES ANTIOXIDANT RESPONSE IN PRIMARY GINGIVAL EPITHELIAL CELLS FOR PERSISTENCE

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*Porphyromonas gingivalis* infection has the ability to modulate and inhibit danger signal extracellular ATP (eATP)-induced Reactive-Oxygen-Species (ROS) production and the associated oxidative stress in Gingival Epithelial Cells. The major antioxidant system utilized by eukaryotic cells to combat oxidative stress is production of Glutathione (GSH), which can directly reduce ROS. Objectives: To study GSH antioxidant response and its mechanisms in primary GECs treated with eATP during *P. gingivalis* infection. Methods: Fluorescent ThiolTracker Violet was used to quantify intracellular reduced GSH levels. Total cellular GSH and oxidized glutathione (GSSG) levels were measured via chemiluminescent detection. Glutamate-cysteine ligase (GCL), Glutathione synthase (GS), and Glutathione reductase (GR) mRNA expression levels were determined via real time Q-PCR. Results: ThiolTracker staining shows that 24h infected cells were able to overcome eATP-mediated loss of reduced GSH which was statistically significant at  $P < 0.05$  t-test compared to eATP-treated uninfected cells. eATP-treated cells exhibited a reduced GSH/GSSG ratio over the time course, while *P. gingivalis*-infected cells with and without eATP pre-treatment showed elevated GSH/GSSG ratios greater than untreated-control levels at 6h, 24h p.i. The GS mRNA levels exhibited slight elevation over the time course for eATP-treated cells, while infected cells with and without eATP had a more dramatic increase in mRNA at 3h p.i. returning to untreated-control levels at later time points. The GR mRNA levels were elevated in infected cells with and without eATP as well as in eATP-treated cells relative to untreated-control at 6h. The GCLc and GCLm mRNA were elevated in infected cells with and without eATP at 6h p.i. Conclusion: Results suggest that the GSH antioxidant pathway is likely an important mechanism for successful intracellular life of *P. gingivalis* in GECs and may contribute to observed infection-mediated inhibition of ROS and protection of host cells from eATP-mediated oxidative stress. This research was supported by NIDCR-R01DE016593 and R01DE019444.

## 73. SPATIAL AND ECOLOGICAL NICHE MODELS FOR TULAREMIA HOSTS AND VECTORS IN UKRAINE

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Tularemia is a widespread zoonosis in Ukraine, maintained in wild small mammals (typically rodents, insectivores, and hares) and sometimes in water. The primary vectors of transmission are ticks. The Central Sanitary Epidemiological Station maintains a database containing information on 3,395 culture isolates. Of these, we created a database totaling 421 geographic locations representing 893 culture isolates from 30 species of primary hosts and vectors or environmental samples. Each location represents between 1 and 37 individual culture recoveries of viable *Francisella tularensis* collected between 1941 and 2008. We used the spatial scan statistic to evaluate spatio-temporal clusters of culture collections. We used GARP ecological niche modeling of the vector species with the greatest sample size to predict the geographic potential of the pathogen in Ukraine. As vaccination is the main preventive measure in humans, understanding the distribution of hosts, vectors, and the pathogen, *F. tularensis*, can play an important role in determining where best to administer vaccination programs. This paper will describe these GIS efforts and introduce GARP based ecological niche models for the most prevalent mammal, *Microtus arvalis*, and tick species, *Dermacentor reticulatus*, identified in our historical database.

## 74. AKT MEDIATES RECURRENT URINARY TRACT INFECTION

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The prevalence and recurrence of urinary tract infections (UTIs) has made them the second most common type of infection. While anyone can contract a UTI, women are more than 50 times susceptible to infection. It is also more likely that a woman will have a recurrent infection within six months. Recurrent infections are defined as having at least two within six months or three within a year. Uropathogenic *E. coli* invade bladder epithelial cells forming intracellular bacterial colonies which lead to the recurrence and antibiotic resistance of UTIs. Recent work has shown that endothelial nitric oxide synthase (eNOS) phosphorylation-dependent activation leads to host cell activation of vesicle fission regulatory enzyme dynamin2 and consequent *E. coli* internalization. A number of kinase pathways have been shown to phosphorylate eNOS, including

the cAMP dependent protein kinase PKA as well as phosphatidylinositol 3-OH kinase (PI3K) and its substrate Akt. Here we examine these pathways to determine their role in bacterial infection. Our findings show time dependent activation of both PKA and Akt. Use of complementary pharmacologic inhibitors of PKA excluded its contribution to the bacteria invasion of host cells. However, infection assays performed in the presence of complementary pharmacologic or biologic inhibitors of PI3K/Akt inhibitors evidenced decreased bacteria invasion. These results lead to the conclusion that Uropathogenic E. coli infection proceeds via a PI3K ð Akt "ð eNOS "ð dynamin2 signal pathway that encompasses multiple drug targeting components."

## 75. ANALYZING THE SPATIAL PATTERNS OF LIVESTOCK ANTHRAX IN KAZAKHSTAN IN RELATION TO ENVIRONMENTAL FACTORS: A COMPARISON OF LOCAL (G

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Bacillus anthracis the causative agent of anthrax remains a threat to livestock and wildlife globally. A local clustering statistic and a cluster morphology statistic were utilized to compare the spatial distribution of anthrax outbreaks in large (cattle) and small (sheep & goats) domestic ruminants across Kazakhstan. In addition, we tested for associations between environmental factors and anthrax outbreak cluster locations. The Getis-Ord ( $G_i^{*}$ ) statistic and a multidirectional optimal ecotope algorithm (AMOEBa) were compared using 1st, 2nd, and 3rd order Rook contiguity matrices. A Kruskal-Wallis and a Mann-Whitney U tests were used to compare the environmental signatures between clusters and non-clusters from each test as well as differences between the AMOEBa and  $G_i^{*}$  clusters. Additionally, logistic regression was used to model the presence/absence of anthrax outbreaks and define a risk surface for large ruminants. Cluster comparisons revealed differences in the spatial distribution of clusters as well as the total number of clusters in large ruminants for AMOEBa (n= 149) and for small ruminants (n= 9). In contrast,  $G_i^{*}$  revealed fewer large ruminant clusters (n= 122) and more small ruminant clusters (n= 61). Significant differences in the environmental variables were found between groups using the Kruskal-Wallis and Mann-Whitney U tests. The logistic model predicted 32.2% of the landscape as high risk. Approximately 75% of AMOEBa clusters corresponded to predicted high risk, while ~64% of  $G_i^{*}$  clusters corresponded. In general, AMOEBa did predicted irregular shaped clusters of anthrax outbreaks in both livestock groups, while  $G_i^{*}$  tended to predicted larger circular shaped clusters. There were notable differences in both tests' abilities to detect outbreak clusters in both groups. While both cluster techniques had significantly different environmental signatures from non-cluster groups, it

is difficult to differentiate specific signatures from AMOEBa versus  $G_i^{*}$ . Here we provide an evaluation of both tests and a discussion the use of each to detect environmental conditions associated with anthrax outbreak clusters in domestic livestock. We also provide a review of core cutoff criteria for AMOEBa and critical distances of  $G_i^{*}$ . The application of local clustering statistics may provide an additional resource for analyzing spatial determinants of risk. More research is needed on the appropriate application critical thresholds when using local spatial statistics and their role in more advanced statistical modeling.

## 76. LIFE-LONG INFECTION BY THE SAME JC VIRUS STRAIN

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John Cunningham virus (JCV) is a neurotropic human polyomavirus. It is the etiologic agent of progressive multifocal leukoencephalopathy (PML), a fatal demyelinating disease of the central nervous system that occurs in a minority of immunocompromised humans. JCV is widely dispersed in humans: greater than 80% of adults worldwide exhibit JCV-specific antibodies, and adults over the age of 25 often excrete small quantities of JCV in their urine. Infection with this virus is typically benign and asymptomatic despite viral persistence in the kidneys. It is not clear how JCV enters the body, and why it is maintained in the kidneys. There are different genomic types of JCV that cluster according to geographical region/race. For example, Type 1 strains are of European origin, whereas type 2 and 3 strains are of Asian and African origin. A previous report of Japanese residents and of a few Europeans living in Japan showed that JCV strains were shed in a pattern that indicates a persistent model of infection: the same virus strain/type was shed by the same subject over a 6-year period. We asked a somewhat different question: what happens when a JCV shedder living in the United States translocates residences in non-adjointing states? Does the subject acquire new JCV strains, considering the USA, unlike Japan, has a very heterogeneous population? The subject of this study is a healthy, immunocompetent Caucasian who was born in an Asian country and sheds an Asian-type JCV strain that has unique polymorphisms useful for genetic tracking. He has lived in four different American states over a 15-year study period. We report that the subject has continued to shed the same JCV strain, with no nucleotide changes within the consensus sequence of key subgenomic markers when sequenced directly following PCR amplification of virus in urine specimens, or in cloned full-length JCV genomes prepared from the subject over a 15-year period. The JCV particles are easiest to detect in centrifuged urine sediment, not in the resulting supernatant, suggesting association of the virus with epithelial cells or debris that are shed in the urine. To-date, only an archetypal regulatory region has been detected in the shed virus, and at least a portion of the virus particles are viable as shown by slow replication over two months of the virus in



COS-7 cells, This study supports the notion of a lifelong infection by the same JCV strain in immunocompetent humans.

## 77. OPTIMUM FREQUENCY OF MRSA SURVEILLANCE CULTURES IN NICU

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**Background:** MRSA is an important pathogen in NICU. Risk factors for MRSA colonization and infection among NICU infants have been described (AJIC 2011;39: 35-41). Routine surveillance for MRSA is increasingly used as a strategy to identify colonized infants and limit spread of MRSA in NICU. Optimal frequency of surveillance testing in NICU has not been determined.

**Objective:** To determine the optimal frequency of MRSA surveillance screening in the NICU.

**Methods:** Since 2004, we performed admission & weekly MRSA screening of all infants admitted to NICU until they became MRSA colonized or infected or were discharged. Any MRSA affected infant was isolated, cohorted and no longer screened until discharge. Infections were identified when MRSA was isolated in clinical specimen as part of routine care. Infection control measures (cohorting, isolation, nasal mupirocin, hand hygiene & limited visitations) were applied uniformly during the entire study.

**Results:** From 1/1/04 to 12/31/09, 4004 infants were admitted to NICU and included in our 6-year analysis. 2241 (56%) were male, 2802 (70%) were White, and 2396 (60%) were delivered by caesarean (C/S). Mean birth weight (BW) was 2.4 Kg (median 2.4, range: 0.3-5.9), mean gestational age (GA) was 34.5 weeks (median 35, range: 22-42) & mean LOS was 2.4 days (median 13, range: 1-273). Prevalence of MRSA colonization was 3.62/1000 pd (95%CI 2.88-3.59). Among 317 (7.9%) MRSA colonized infants, non-Whites (RR 1.36; 95%CI 1.10-1.70, P=0.005) and C/S delivered infants (RR 1.81; 95%CI 1.43-2.31, P<0.0001) had a higher risk of colonization. Colonized infants had a significantly lower GA and BW and longer NICU stay than non-colonized.

Colonization was detected at a mean of 19 days (median 12, range: 1-172) following admission. Only 27% of colonized infants were detected by week 1 screening; however 90% were detected within 5 weeks of screening.

MRSA infection developed in 59 (18.6%) colonized and 27 (0.7%) non-colonized infants at a prevalence of 0.62/1000 pd (95%CI 0.46-0.77). Average time to infection after colonization was 11 days (median 6, range: 0-70) with 75% of infections occurring within 12 days and 90% within 31 days of colonization.

**Conclusions:** Weekly universal MRSA screening is important in detecting MRSA colonized infants in NICU. Over 70% of colonized infants are identified after the first week screening. The majority are detected within 5 weeks of admission. We recommend weekly MRSA surveillance cultures in NICU for the first four weeks of hospitalization and monthly surveillance cultures after the first four weeks.

## 78. ECOLOGICAL NICHE MODELLING OF THE BACILLUS ANTHRACIS A1.A SUB-LINEAGE IN KAZAKHSTAN

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**Bacillus anthracis**, the causative agent of anthrax, is a globally distributed zoonotic pathogen that continues to be a human health problem in Central Asia. We used a database of anthrax outbreak locations in Kazakhstan and a subset of genotyped isolates to model the geographic distribution and ecological associations of *B. anthracis* in Kazakhstan. The aims of the study were to test the influence of soil variables on a previous ecological niche based prediction of *B. anthracis* in Kazakhstan and to determine if a single sub-lineage of *B. anthracis* occupies a unique ecological niche.

We used a dataset of 258 outbreak locations and a genotyped dataset of 39 A1.a sub-lineage isolates. Ecological niche models were built independently for each dataset using the genetic algorithm for rule-set prediction. Adding soil variables to the previously developed outbreak based ecological niche model did not appreciably alter the predicted geographic or ecological distribution of *B. anthracis* in Kazakhstan. The A1.a sub-lineage experiment predicted presence over a larger geographic area than did the outbreak based experiment. This difference was most pronounced in the northern half of the country. When values of environmental variables were extracted from geographic areas predicted by all ten best subset models, the A1.a sub-lineage occurrences were associated with a wider range of ecological conditions than the outbreak points. Analysis of rule types showed that logit rules predominate in the outbreak-soil experiment and range rules in the A1.a sub-lineage experiment. Random sub-setting of locality points suggests that models of *B. anthracis* distribution may be sensitive to sample size.

Further investigations into the environmental affinities of individual lineages and sub-lineages of *B. anthracis* will be useful in understanding the ecology of the disease at large and small scales. With model based predictions serving as approximations of disease risk, these efforts will improve the efficacy of public health interventions for anthrax prevention and control.

## 79. DIFFERENT RATES OF DISEASE PROGRESSION HIV-1 IN HLA-B\*5701+ SUBJECTS CAN BE EXPLAINED BY THE INTERPLAY BETWEEN SPECIFIC PHYLODYN

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HLA-B\*5701 is the most consistent host factor associated with slow disease progression in HIV-1 infected individuals. The underlying mechanisms are not fully understood, but likely involve the interplay between the immune system and the evolution of the viral quasispecies. To test such a hypothesis, a multi-level analysis was developed to investigate HIV-1 evolutionary patterns, fitness and epitope-specific CD8+ T cell (CTL) responses in a unique group of six HLA-B\*5701+ untreated patients from the OPTIONS cohort. Three subjects were characterized as slow progressors (SPs) and three as progressors. Evolutionary profiles of the HIV-1 Gag p24 region and multifunctional CD8+ T cell responses were evaluated by high-resolution phylogenetic analysis and flow-cytometry (8-colors), respectively. In both SPs and progressors, substitutions were detected more frequently in flanking regions than in HLA-B\*5701-restricted epitopes. However, p24 sequences in progressors showed significantly higher diversity compared to SPs, where evolution appeared to be more constrained. Evolution in the SPs was characterized by the emergence of variants during sequential population bottlenecks in a specific order, first in the TW10 and/or ISW9 epitope, then in the CypA-binding loop. Moreover, disentangling synonymous and nonsynonymous substitution rates showed that viral replication rates were one order of magnitude higher in the progressors, as confirmed by increased virus RC over time. Polyfunctional CD8+ T cell responses, especially towards the TW10 and QW9 HLA-B\*5701-restricted epitopes, were higher in the SPs, who also showed a significant inverse correlation between perforin and IL-2 production. Overall, the data showed that specific immunological responses can constrain viral evolutionary pathways and relate to differences in disease progression in HLA-B\*5701+ subjects. They also demonstrate the power of a multi-disciplinary approach integrating high-resolution evolutionary and immunological data to understand the mechanisms underlying HIV-1 pathogenesis.

## 80. PHYLODYNAMICS OF COMMUNITY-ASSOCIATED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN NORTHEAST FLORIDA, USA

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**Background:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is the leading cause of healthcare-associated infections (HAIs), and a significant contributor to healthcare cost. In 2008, the CDC estimated that MRSA was responsible for 89,785 cases of invasive disease causing 15,249 deaths in the US. Historically, two genetically and epidemiologically distinct MRSA categories represented community-associated (CA) and hospital-associated (HA) etiologies. However, CA-MRSA strains have now invaded healthcare settings and are recognized as an important cause of HAIs, emphasizing the need to evaluate current control measures and elucidate the changing epidemiology of MRSA within the community and healthcare-community interface. Phylogenetic and population genetic inference (phylodynamics) is a powerful tool to investigate the origin and spatiotemporal spread of bacterial pathogens (Gray et al., *Mol. Biol. Evol.* 2011), as well as to elucidate transmission dynamics. In our study, we applied phylodynamic analyses of hospital-sampled MRSA isolates to investigate population dynamics and transmission between hospitals.

**Methods:** Ninety-seven clinical MRSA isolates were obtained from seven hospitals in northeast Florida, USA from during a one week period in 2010. Our analysis employed an innovative framework integrating *Staphylococcus* protein A (*spa*) molecular typing of all isolates and full-genome next-generation sequencing (Illumina GAII-X®) data of randomly selected *spa*-type t008 strains. Single nucleotide polymorphism (SNP) alignments were analyzed with several phylodynamic and phylogeographic algorithms based on coalescent theory and a Markov Chain Monte Carlo Bayesian framework.

**Results:** Twenty-six HA (t002) strains, 48 CA (t008) strains, and 23 strains of other/unknown type were identified. Phylodynamic analysis of SNP data including 30 t008 strains provided evidence of an ongoing exponential growth of the MRSA effective population size (i.e. number of effectively infectious genomes). No evidence of hospital-specific clades or directional gene-flow from/to hospitals was found by phylogeography.

**Conclusion:** The present study represents the first phylodynamic characterization of MRSA transmission at the hospitals-community interface. The high prevalence of CA isolates and lack of phylogeographic clustering within hospitals suggest that community based transmission and colonization pressure, rather than a breakdown of infection control, may significantly contribute to the emergence of CA-MRSA strains in Northeast Florida hospitals. Moreover, the findings indicate an intricate and complex dynamic of MRSA transmission, possibly driven by a growing epidemic at the community level in hidden reservoirs, which deserves further investigation. Additional phylodynamic studies of MRSA distribution and

transmission dynamics will be required to understand the factors driving the evolution and continuing emergence of new, virulent strains.

## 81. A MACHINE LEARNING APPROACH TO PREDICT DRUG RESISTANCE USING HIGH-RESOLUTION MLST DATA OF *S. AUREUS* HOUSEKEEPING GENES

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**Background:** Resistance to antimicrobial agents is a major problem for the treatment of pathogens in the hospital and community setting. Methicillin-resistant *Staphylococcus aureus* (MRSA) is currently the most commonly identified antibiotic-resistant pathogen in US hospitals. Resistance to methicillin is caused by the *mecA* gene which encodes for a penicillin-binding protein with low affinity for  $\beta$ -lactam drugs and is located on a mobile genetic element, the *Staphylococcal Cassette Chromosome mec* (SCCmec). Multilocus sequence typing (MLST) is a nucleotide sequence-based method for characterizing, subtyping, and classifying bacteria. The MLST scheme for *S. aureus* uses internal fragments of seven housekeeping genes (*arc*, *aro*, *glp*, *gmk*, *pta*, *tpl*, and *ygi*) to assign allelic profiles to isolates. In conjunction with SCCmec typing, MLST has been used to create an international nomenclature for *S. aureus*. MLST sequence types with a single nucleotide polymorphism (SNP) are considered distinct. In this study, we associated SNPs to methicillin/oxacillin resistance or susceptibility.

**Methods:** *S. aureus* MLST data, including drug resistance antibiograms, were downloaded from the international public repository (<http://saureus.mlst.net/>). The allele sequences for all seven loci of each strain were compiled in series. SNPs among the concatamers were analyzed using chi-square cross-tabulation tests, Random Forests (RF) machine learning and multivariable logistic regression (LR) to predictor antibiotic resistance. Performance of unseen data were evaluated via the cross-validation. Hierarchical clustering of SNPs was employed to analyse mutational covariation.

**Results:** The final data set included 1526 and 649 distinct instances with at least an oxacillin or methicillin antibiogram result known. Top 10 SNPs significantly ( $P < 0.05$ ) associated to susceptibility/resistance were: *aro\_153*, *aro\_101*, *tpi\_68*, *aro\_86*, *glp\_58*, *arc\_183*, *aro\_22*, *tpi\_242*, *tpi\_48*, *aro\_141* to methicillin; *aro\_101*, *aro\_153*, *aro\_86*, *arc\_183*, *tpi\_68*, *glp\_58*, *yqi\_504*, *yqi\_95*, *yqi\_512*, *aro\_419* to oxacillin. A RF model predicted correctly the resistance/susceptibility to methicillin/oxacillin in 75% and 63% of cases (cross-validated). Results were similar for LR. Hierarchical clustering of the aforementioned SNPs yielded a high level of covariation both within the same and different genes.

**Conclusions:** Machine learning approaches have successfully been used to predict phenotypic drug resistance from viral genotypes. This is the first application to the study of large bacterial pathogens that may help investigating the relation between antibiotic resistance and genomic evolution. The unexpected association between drug resistance and house-keeping genes points out that unknown determinants of resistance may be present in bacterial genomes. These techniques could provide the knowledge base for the development of novel therapeutic agents.

## 82. PHAGE COCKTAIL LYtic FOR *YERSINIA PESTIS* SIGNIFICANTLY REDUCES THE LEVELS OF THE BACTERIA ON VARIOUS HARD SURFACES EXPERIMENTALLY

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Intentional exposure of U.S. military and civilian populations to agents of biologic warfare and bioterrorism is an alarming reality and no longer an abstract theoretical consideration. Decontamination of buildings, air handling systems, equipment, and personnel presents considerable challenges, particularly when time is of the essence to restore critical assets to functional use and traditional disinfection techniques may themselves damage or imperil those assets. Thus, novel approaches to deal with infections caused by the intentional dissemination of pathogenic bacteria are urgently required. Bacteriophages may provide one such environmentally friendly approach. Here, we report the characterization of five bacteriophages lytic for *Yersinia pestis* (a class A agent of significant bioterrorism importance) and their ability to decontaminate various hard surfaces intentionally contaminated with *Y. pestis*.

Five *Y. pestis*-lytic phages (YpsP-G, YpP-Y-ATCC, YpP-R-ATCC, YpsP-PST-ATCC, and YpP-G) were obtained from various sources and were characterized for their basic biological properties, including taxonomy, host range, burst size, and lysis kinetics. Four (YpsP-G, YpP-Y, YpP-R, and YpP-G) of the five phages were also fully sequenced. The phages belonged to the Podoviridae (YpsP-G, YpP-G, YpP-Y, and YpP-R) or Myoviridae (YpsP-PST) families of DNA-containing, tailed phages. The genome size of four phages was ca. 40 kb, and it was ca. 180 kb for the fifth phage (YpsP-PST). At a concentration of ca. 109 plaque-forming units (PFU)/mL, each phage lysed 100% of the *Y. pestis* strains. The YpP-G phage was highly specific for *Y. pestis*, even more so than the PhiA1122 phage used by the CDC for diagnostic purposes. The lysis time for all was ca. 60 to 90 min at 28°C, and the burst sizes for YpsP-G, YpP-Y-ATCC, YpP-R-ATCC, YpsP-PST-ATCC, and YpP-G were ca. 121±30, 138 ± 351, 118 ± 46, 166 ± 62, and 211 ± 30 PFU/cell, respectively. The five phages were then combined in a phage cocktail (tentatively designated YPP-100), and the cocktail was used to decontaminate 3 surfaces (glass, steel and gypsum board) intentionally contaminated with a mixture of 3 genetically diverse *Y. pestis* strains CO92, KIM

and 1670G. The undiluted version of YPP-100 (ca. 109 PFU/mL) completely eliminated *Y. pestis* contamination on all surfaces in as little as 5 minutes contact time. Even when phage cocktail was diluted 1000-fold to ca. 106 PFU/mL, it significantly (Pd 0.01) reduced *Y. pestis* by 99.9%. Our studies suggest that YPP-100 can be effective in decontaminating various hard surfaces that were naturally or intentionally contaminated with *Y. pestis*.

### 83. METAGENOMIC APPROACHES TO MOLECULAR IDENTIFICATION OF PATHOGENS

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Metagenomics is a field that has rapidly expanded with regards to application to diagnostic function. The marriage of molecular techniques with bioinformatics has resulted in the development of advanced platforms for genomic identification and characterization of agents such as Lujo virus in South Africa using unbiased pyrosequencing and Reston ebolavirus in the Republic of the Philippines by pan-viral microarray (Barrette et al, 2009). These systems tend to be very information intensive due to the extensive quantities of data that are utilized in the design process, and resulting from analysis. High level programming languages such as Perl and Python are particularly well suited to developing algorithms which can be used to study these large data sets. Bioinformatics, paired with molecular methods such as random reverse-transcription PCR can produce information which can assist in the guidance of a disease investigation.

Here we describe the capture of viral nucleic acid and characterization of a novel porcine teschovirus by pan-viral microarray. Initial tests indicated the presence of a related virus, however attempts to characterize the virus by sequence analysis were originally unsuccessful. Therefore, the microarray was employed as a tool to assist in obtaining the genomic sequence of the virus.

### 84. CHARACTERIZATION OF SIV INTRA-HOST EVOLUTIONARY AND POPULATION DYNAMICS IN THE CD8-DEPLETED RHESUS MACAQUE MODEL OF NEUROAIDS

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**Williams**, Boston, Boston College; **Marco Salemi**, Pathology, Immunology, and Laboratory Medicine, College of Medicine, University of Florida

Evolutionary factors driving the emergence of neurovirulent strains during disease progression is important for the understanding the development of neuroAIDS. Experimental infection of CD8-depleted rhesus macaques with the SIVmac251 viral swarm provides a rapid disease model for studying the evolutionary and population genetic dynamics of AIDS-related neuropathology. Approximately 1700 gp120 SIV sequences derived from multiple tissue types were collected from six intravenously infected macaques. Two macaques were euthanized at early at 21 days post infection (dpi), while the remaining four macaques were followed throughout the infection. Phylogenetic and amino acid signature pattern analyses were performed to determine the frequency and persistence of viral strains. Recombination analysis was carried out to assess the percentage of recombinant sequences in the tissue types. Phylodynamic analyses were conducted to evaluate the tempo and mode of viral infection of the brain. Transmission and replication of SIV variants was not entirely stochastic. Two under-represented motifs (<4%) in the swarm were found at high frequencies (up to 15%) in all six macaques as early as 21 dpi. In addition, a macrophage tropic variant not detected in the viral swarm (<0.3%), was present at high frequency (29-100%) in sequences derived from the brain of two macaques with neuropathology. Recombinants were not linked to a specific tissue type. They were evident at all time points and macaques. Timeline plots for the number of recombinant sequences varied and may be associated with differing SIV-associated disease manifestations. The number of breakpoints within the C1-C2 domains was significantly larger than in the V3-V4 domains. Identical breakpoints were identified in sequence populations derived from different tissues in the same primate and within different primates. Phylodynamic, molecular clock analysis showed the relaxed clock was the best model for five out of six macaques. Gene flow analysis showed multiple brain seeding events occurring throughout the course of the infection, mostly involving viral strains isolated from macrophage subpopulations, with the initial brain infection occurring as early as 14-21 dpi. At about 40 dpi there was a peak in Ne in the three primates that eventually developed SIVE, while the remaining animal showed a steady rising immediately following the initial infection. This study showed the how the infecting swarm influences the progression of SIV infection. Depletion of CD8 immune response in the infected macaques results in an increase of genetic heterogeneity and the emergence of viral variants that may play a role in the development of neuropathogenesis.

### 85. CAENORHABDITIS ELEGANS NEUTRALIZES SMALL MOLECULE TOXINS PRODUCED BY PSEUDOMONAS AERUGINOSA

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The nematode *Caenorhabditis elegans*, which is found in soil and decaying fruit, is exposed throughout its life to a variety of pathogenic microbes, which makes it an ideal candidate to study bacterial resistance. Although *C. elegans* has a well-developed innate immune system, it still remains susceptible to many pathogens, including many that affect humans, such as *Pseudomonas aeruginosa*, a common pathogen of both plants and animals. *P. aeruginosa* possesses a variety of virulence factors, including toxic phenazine compounds such as pyocyanin and 1-hydroxyphenazine (1-HP) which are often found in lungs of *P. aeruginosa*-infected cystic fibrosis patients. These redox-active compounds are thought to cause damage to cells by producing reactive oxygen species and disrupting normal redox reactions.

*P. aeruginosa* grown on high-osmolarity media is able to kill L4 stage *C. elegans* in as little as 6 hours through the production of phenazines such as phenazine-1-carboxylic acid (PCA), 1-HP, and pyocyanin. We have found that upon exposure to 1-HP, the worms modify the compound into at least 5 metabolites. These metabolites, all glycosides of 1-HP, are found in differing concentrations and forms in the worm media and in worm bodies.

After exposing young adult *C. elegans* to 200  $\mu$ M of 1-HP for 24 hours, the media contains phenazine compounds made up of approximately equal concentrations of 1-( $\beta$ -glucopyranose)-phenazine (mono), 1-(6- $\beta$ -glucopyranose)- $\beta$ -glucopyranose)-phenazine (di), and two phenazine-trisaccharides (tri) which currently have not been unambiguously identified. The homogenized worm pellet contains the majority of all phenazine compounds, about 80%. Comprising the worm pellet are 1-(3-phospho-glucopyranose)-phenazine, mono, and small amounts of di and tri. We have shown that at concentrations of 200  $\mu$ M, 1-HP kills ~80% of L4s after 6 hours, while the mono, di, and tri glucosides kill less than 20%. We are currently investigating the role of the glucoside-phosphate and are working in collaboration with the Schroeder Laboratory at Cornell University to identify other compounds metabolized in a similar way.

## 86. ACQUISITION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN A PEDIATRIC INTENSIVE CARE UNIT (PICU)

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**Background:** Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization and infection is recognized as a significant problem in intensive care units. Available data on the risks for acquiring MRSA while in the pediatric intensive care unit (PICU) is minimal.

**Objective:** To determine the prevalence of and identify risk factors for new MRSA colonization and infection in a PICU population.

**Design/Methods:** Children 19 years old admitted to the PICU between 4/1/2008 & 3/31/2011 had admission & weekly MRSA nasal surveillance testing done. MRSA affected (colonized or infected) children were compared to MRSA unaffected children.

**Results:** During the 3-year study, there were 2861 admissions to the PICU. The mean age was 6.7 yrs (range: 0-19 yrs, median 4.7) and 1444 (50.5%) were male. Of the 2861, 2632 (92%) either had a known history of MRSA or had an admission surveillance test and were included in the analysis. 415 (15.8%) were MRSA affected; 264 had a known history of MRSA, 132 were found to be MRSA positive on admission and 19 became MRSA affected while in the PICU (18 colonized & 1 infected). Of the 18 colonized patients 14 (77.8%) were identified on weekly surveillance testing, 4 (22.2%) had a positive non-surveillance culture. The 19 children who became MRSA affected were further analyzed. There was no significant difference in gender or ethnicity between the MRSA affected and unaffected groups. MRSA affected children were significantly younger than the unaffected (3.68 vs 6.79 yrs,  $p=0.03$ ). Mean length of hospital stay prior to PICU admission was significantly longer in the MRSA affected group (2.3 vs 0.6 days,  $p=0.04$ ). Receiving systemic steroids ( $p=0.009$ ), mechanical ventilation ( $p=0.001$ ) and a central venous catheter ( $p=0.001$ ) were all significantly higher in the MRSA affected group, but surgery & antibiotic use were no. Mean length of stay (LOS) in the PICU was 4.3 days, 87.5% of admissions spent 7 days. Mean LOS in the PICU before becoming MRSA affected was 18 days; 6 (31.6%) identified at 7 days, 12 (63.2%) 14 days, 14 (73.7%) 21 days & 16 (84%) 28 days. **Conclusions:** Longer hospital stay prior to PICU admission, steroid use, mechanical ventilation and a central venous catheter are all significantly associated with becoming MRSA affected while admitted to the PICU. Children should be screened for MRSA upon admission to PICU and whenever their stay is 2 weeks.

## 87. DISCONTINUOUS GENETIC DETERMINANTS IN HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 SUBTYPE B R5X4 ENVELOPES REGULATE CXCR4-DEPENDENT VIRAL ENTRY

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**Discontinuous genetic determinants in Human Immunodeficiency Virus Type 1 subtype B R5X4 Envelopes regulate CXCR4-dependent viral entry and preferential CCR5 use on host cells. Tropism by HIV-1 using CXCR4 (X4) for entry into CD4 T cells and macrophages (MDMs) is associated with disease progression. Yet, genotypic determinants for X4 envelopes (Env), particularly on macrophage tropic viruses, remain unclear. To track the genetic and functional**

evolution of X4 Envs during the natural history of infection, subtype B HIV-1 envelope genes were amplified and sequenced from cell-associated or plasma viruses for as long as 10 years of infection among 8 therapy-naïve individuals. 831 Envs were analyzed by phylogenetic and bioinformatic algorithms, which showed: (1) X4 emergence from CCR5 (R5)-using Envs prior to CD4 decline, (2) R5 and X4 coexistence, and (3) exclusive R5 persistence during disease progression. Entry function was measured by titrated luciferase-gene tagged viruses (pNL4-3Luc+R+E-) pseudotyped with a subset of unique HIV-1 Env V1-V5 domains with a constant gp41 region. Some primary Envs that entered CD4 T cells failed to mediate entry into MDMs indicating that Env coreceptor use was distinct from host cell tropism. R5X4 Envs displayed novel preferential CCR5 use to enter CD4-T cells, and exclusively used CCR5 to enter MDMs. R5X4 Envs showed significant resistance to inhibition by CCR5-inhibitor (Maraviroc) and gp41-inhibitor (T20), and increased susceptibility to soluble-CD4 (sCD4) and anti-V3 monoclonal (447-52D), relative to intrahost R5 Envs. Amino acid differences between R5 and R5X4 Envs were mainly localized in V1V2 and V3. Distinct R5X4 Envs encoding glutamic acid or proline amino acids in V1 emerged overtime and were associated with decreased entry efficiency. Mutations of key residues that altered charge or complex architecture in Env V1 modified entry efficiency. Determinants in V1V2 may impact coreceptor use and efficiency, preferential coreceptor use of R5X4 Envs on CD4 T cells indicative of a novel stage of HIV-1 evolution, and cell tropism. Understanding HIV-1 Env coreceptor use evolution and cell tropism is fundamental to understanding HIV-1 pathogenesis.

## 88. MUIZENBERG FEVER: INSTRUCTIVE OUTBREAKS OF A NOVEL AGENT

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In 2010, a novel agent was associated with an outbreak in a student population in Muizenberg, South Africa. 22 out of 46 participants in the Clinic on the Meaningful Modeling of Epidemiological Data (MMED) were infected before the outbreak ceased. The same agent was associated with an outbreak in 2011, infecting 37 out of 56 MMED participants. In order to understand transmission of this agent, an unmatched case-control study was conducted among MMED participants in 2011. Risk factor analysis using multiple logistic regression indicated that attending MMED in 2010 was protective against infection in 2011 (aOR: 0.046, CI95: 0.0023-0.29). In addition, key epidemiological parameters, including the duration of infectiousness and the basic reproduction number, were estimated from infection and contact-tracing data. These estimates were used to assess deviation of the observed outbreak from expectations under the assumption of random mixing and to examine the effects of varying levels of population immunity on infection dynamics. Through investigation of these two outbreaks of Muizenberg Fever, MMED participants gained practical experience

in data collection and analysis and learned the utility of multiple approaches to the study of infectious disease epidemiology.

## 89. EFFECTS OF GALACTOOLIGOSACCHARIDES (GOS) ON THE GUT MICROBIOTA OF AGED ADULTS

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A decrease in gut microbiota diversity in aged adults has been linked to reduced immune function, and attempts have been made to increase the numbers of beneficial microbes in these individuals. The effects of specific dietary substrates on microbiota, especially in aged adults, are largely unknown. Here we report on a randomized double-blinded placebo-controlled microbiota study in 80 healthy adults aged at least 60 years. Microbiota composition was analyzed in fecal samples taken at baseline and two weeks into the intervention using various 16S rRNA based methods. Quantitative PCR (qPCR) showed an increase in the proportion of bifidobacteria in subjects consuming 5 g/day GOS ( $p < 0.05$ ). 16S rRNA sequence analysis of 719964 sequence reads resulted in 11455, 20623, and 27597 operational taxonomic units (OTUs) at the 95%, 97%, and 98% similarity levels, respectively. The prevalence of 74 OTUs was significantly affected by GOS treatment (14 OTUs at the 95% similarity level, 23 at the 97% similarity level, and 37 at the 98% similarity level). 75% of the OTUs whose prevalence was associated with GOS intake matched to the order Clostridiales. At the genus level, the numbers of sequences in OTUs matching to Ruminococcus increased with GOS intake. Our results suggest that gut microbiota, including potentially beneficial bacteria, in aged adults can be modified by intake of 5g/day GOS. Supported by Corn Products International.

## 90. DETERMINATION OF RESISTANCE MECHANISMS WITHIN FIELD POPULATIONS OF HAEMATOBIA IRRITANS THROUGH TOXICOLOGICAL AND BIOCHEMICAL TESTS

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The horn fly, *Haematobia irritans*, causes economic and physical damage to beef cattle. Horn fly adults of both sexes are obligate blood-feeding ectoparasites, the larval stadiums develop exclusively in fresh cattle dung. In the southern region of the USA, numerous generations develop each year, and can occur in extremely high numbers. Fly management is necessary in many areas of the country to maintain or increase profit and animal comfort in the cattle industry. Horn flies are thought to mechanically vector several pathogens of cattle. Horn flies are also known developmental vectors of *Stephanofilaria stilesi*, a skin dwelling nematode of cattle. Effective control methods currently utilized include applications of pour-on topical insecticide solutions or insecticide-impregnated ear tags. Resistance to insecticide-impregnated ear tags developed shortly after their initial use and has occurred in numerous insecticide groups with various modes of action. The current status of insecticide resistance in Florida has not been evaluated within the past 20 years. Horn flies were collected from beef cattle herds across Florida and evaluated resistance to the commonly used insecticides permethrin, beta-cyfluthrin, diazinon, and ivermectin using insecticide treated glass jars and impregnated filter paper discs. PCR was utilized to determine insensitive target sites that confer insecticide resistance, specifically *kdr* and insensitive acetylcholinesterase (AChE). Additionally, enzyme assays were performed to quantify AChE inhibition toward various inhibitors. Preliminary results imply field populations have higher LC50 values for some insecticides, suggesting resistance development. We found resistance ratios up to 50-fold greater than laboratory colony toxicity for permethrin, while other commonly used insecticide resistance ratios were low. Resistant genotypes of AChE and *kdr* point mutations were identified in several fly populations. However, enzyme assays did not detect differences between laboratory and field horn fly populations, suggesting the expressed AChE point mutations confer resistance to anticholinesterase inhibitors other than diazoxon. Understanding current insecticide resistance in the horn fly is critical to maintaining sustainable and economical beef production in the USA.

## 91. MORPHOLOGICAL RESPONSES TO 20-HYDROXYECDYSONE AND VERATRIDINE IN *SPODOPTERA FRUGIPERDA* (SF21) INSECT CELLS THROUGH ULTRASTRUCTURE

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A neuronal morphological phenotype can be induced in cultured insect cells by supplementing the medium in which the cells are maintained with an agent that regulates transcription of proteins specific for insect neuronal cells. The ability to induce a neural phenotype simplifies studies of insect cells, compared to either the use of primary nervous tissue or genetic engineering techniques. Ultrastructure has traditionally been a powerful tool to examine the structural, morphological, and functional development of a wide variety of tissues. In this

study the objective was to determine differences in ultrastructure between control Sf21 cells and cells treated with 20-hydroxyecdysone (20HE) and veratridine (VTD). Previous studies in our laboratory showed that 20HE induced the growth of cellular processes resembling axons in normally round cells, which was enhanced by VTD. The overall goal of this project was to determine structural and functional differences in cells between treatments, but also to determine any correlation to structural features and possible expression of synaptic contacts within these treated and untreated cells. Results showed that differentiated cells had similar somatic appearance to control cells, with slight changes in organelles and organization, such as a greater number of vacuoles and cytolysosomes. Finger-like projections were seen between control cells; however, no synaptic contacts have been shown in differentiated cells at this time. The presence of ion channels or receptors in the differentiated cells remains to be determined using biochemical and electrophysiological methods. If they are present, high throughput screening for new insecticides will be accelerated and made more economical by the utility of this method.

## 92. EXPLORING EMERGING PATHOGENS: FOUR YEARS OF EDUCATION OUTREACH

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As science literacy in the US falls to record lows, the importance of bringing engaging and relevant science education to our K-12 classrooms becomes greater. With funding from the Howard Hughes Medical Institute, the Center for Precollegiate Education and Training (CPET) and the Emerging Pathogens Institute (EPI) at the University of Florida (UF) have collaborated to offer a year-long teacher biotechnology education program focused on the theme of new and emerging diseases. The goals of the Interdisciplinary Center for Ongoing Research/Education (ICORE) Partnership Program are threefold: (1) to engage teachers in current emerging pathogen research being conducted at UF and empower them with the most up-to-date biotechnology techniques for use in their classrooms; (2) to promote science as a potential career choice to high school students; and (3) to increase public awareness of emerging diseases and their control by integrating the science of emerging pathogens into K-12 classrooms. To assess the impacts of the ICORE program on teacher professional development and teaching practices, we used a combination of pre- and post-program surveys and questionnaires, as well as focus groups for our teachers, complemented by surveys of their students. Specifically, we were interested in participant expectations of and satisfaction with the program and effects of the program on teacher confidence and interest in science teaching. We found that teacher responses to the ICORE program were overwhelmingly positive. Participating teachers stated that they attended the program in order to learn about current issues in science and develop new activities for their classrooms. The majority of participants said attending the workshop had increased their knowledge of current issues in scientific research and that they had gained a greater understanding of the practical uses of science, math, and technology.

Teachers also indicated that they became familiar with new materials and equipment that they could use in their classrooms through CPET's equipment borrowing program. We discuss the future of the ICORE program and how UF scientists can continue to be involved in this successful research outreach experience for teachers in its final year and beyond.

### 93. SUSTAINABLE FEED PRODUCTION TO SUPPORT TILAPIA AQUACULTURE IN HAITI

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Dietary protein is notably limiting for children and families in many communities throughout Haiti. In fact, amidst a myriad of remarkable challenges, lack of adequate nutrition and starvation is not uncommon throughout the country. Availability of good nutrition is key for child development and is closely linked to school attendance and graduation rate. Fish is an excellent source of healthy protein, however, a critical obstacle to successful aquaculture in Haiti is the availability of fish feed. Needed are viable and regionally-sustainable feeds to promote fish growth and health to support community-based aquaculture. This project entails controlled laboratory and field experiments to discern the feasibility of pilot feeds that may be used for sustainable, community-scale tilapia culture in Haiti. We are investigating use of ingredients that can be locally harvested in Haiti, and that are both high in digestible protein as well as resources that are not consumed by people. These ingredients include blood meal, brewery byproducts, moringa leaves, insects and cassava root. Ingredients were harvested, sun-dried and pelleted in different formulations for application in controlled laboratory studies (ongoing). Experimental feeds were formulated to have similar crude protein concentrations (29.1-29.6%), as well as lipids and fiber. Observations on sinking rate (floating feeds are preferred), palatability, and fish feed consumption and growth rates in the different laboratory feed trials will help guide the development of feed formulations for application in field trials in Haiti. School feeding programs associated with our study, that can be supplemented with animal protein from fish and/or eggs, are currently providing nourishment for over 1,500 children in two school communities. Future studies will document enhanced attendance and graduation rates associated with these feeding programs. These efforts have supported, in part, by FISH Ministries, the WINNER Program (US AID), Aquaculture Without Frontiers, the US Tilapia Association, the UF College of Public Health and Health Professions, the UF Emerging Pathogens Institute, the UF School of Natural Resources and Environment, and the UF Institute of Food and Agricultural Sciences Office of International Programs.

### 94. INVESTIGATING DRIVERS OF EUROPEAN HOT SPELLS USING EXTREME VALUE ANALYSIS

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Elevated temperatures for an extended duration have considerable impacts across society including heightened mortality and morbidity as a direct consequence of the temperature and air pollution from wild fires. The European heat wave of 2003 caused more than 70,000 additional deaths with comparison to other summers. While Munich Re estimated that the more recent event across East Europe and Russia in 2010 caused USD 15 billion damage in total and 56,000 additional deaths from the associated secondary impact of drought. It has been shown by others that in the future, extreme temperature events are likely to become more frequent, attain higher peak temperatures and last longer. Furthermore, there are likely to be more extreme weather events lasting for several days or weeks per season. One such event, a hot spell, is defined as a sequence of days/nights with maximum/minimum temperatures above a certain threshold. The duration and severity of hot spells, such as the Russian heat wave of 2010, are often attributed to persistent atmospheric circulation patterns such as blocking events. Quasiperiodic climate patterns such as the El Niño-Southern Oscillation (ENSO) and the North Atlantic Oscillation (NAO) are also known to influence global weather patterns and to cause or aggravate persistent hot spells. Extreme Value Analysis (EVA) is employed to investigate the frequency and intensity of extremely high temperature events (>95th percentile) at 76 stations over Europe, for the period of 1951-2010, and their relationship with blocking events, NAO and ENSO. The time-varying signals are introduced into non-stationary models as covariates in the location and log-transformed scale parameters. The improvements to the model obtained by introducing covariates are examined using the deviance statistic whereby the difference in negative log-likelihood values between two models is tested for significance using a Chi-squared distribution. Results indicate that each of the three covariates exhibit geographically distinct significant impacts on the frequency and magnitude of European hot spells. Atmospheric blocking appears to have the most significant impact of the three.

### 95. EVALUATION OF NOVEL POTASSIUM CHANNEL-DIRECTED COMPOUNDS AS NEW MOSQUITOCIDES FOR CONTROL OF AEDES AEGYPTI AND ANOPHELES GAMBIAE

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There are many diseases that are transmitted by arthropods, and mosquitoes vector a number of them, such as dengue fever transmitted by *Aedes aegypti* and malaria by *Anopheles gambiae*. The need to control the vectors is great and current control programs include indoor residual spraying (IRS), insecticidal spraying, and the use of insecticide-treated nets (ITNs). However, the success of these programs is threatened by burgeoning resistance, especially for ITNs, which are dependent upon a single class of insecticides, the pyrethroids. With the advent of mosquito resistance, there is an urgent need for novel control measures, including new insecticides with novel modes of action.

With this in mind, we have been screening known potassium channel blockers, as well as novel compounds in both *Anopheles gambiae* adult mosquito bioassays and *Aedes aegypti* headless larval assays. Many of the compounds showed poor penetration through the cuticle of the insect, thus removing the head allows us to see if the chemical has any intrinsic effects on the mosquito. A headless mosquito larva, in a control solution, will show aggressive lateral contractile movement when stimulated with a mechanical probe for at least 5 hours. However, exposed larva display a sluggish one-sided contractile motion or no motion at all. This criterion is used to describe mosquito paralysis/death. The adult assays were via topical application and observed over a period of 24 hours. The LC50 of the known potassium blockers, tetraethylammonium (TEA) and 4-aminopyridine was shown to be 9.69 ppm (95% fiducial limits 1.76-26.79 ppm) and 3.13 ppm (95% fiducial limits 0.521-7.35 ppm), respectively. The toxicity of these two compounds is much higher than other compounds that have been screened thus far, such as N,N-diethylnicotinamide, chlorzoxazone, quinine, and 4-tert-octylcatechol (LC50 > 100 ppm). A number of novel catechols were used in the adult assays, which showed some promising results. PRC 725 and PRC 728 had mortality rates on G3 of 7/10 and 14/20, respectively. Additionally, PRC 728 was run against the resistant Akron strain and showed a 5/10 mortality rate after 24 hours. It is our expectation that through novel compounds, we will find one that will be effective at blocking the potassium channel in mosquitoes while at the same time have little effect on humans, providing a safe and effective class of new insecticide.

## 96. ESTIMATING CYANOBACTERIA ACTIVITY USING REMOTE-SENSING TECHNOLOGIES

**Rebecca Lazensky**, Aquatic Toxins Disease Prevention Program; Florida Department of Health; **Andrew Reich**, Aquatic Toxins Disease Prevention Program; Florida Department of Health

The Florida Department of Health (FDOH) Aquatic Toxins Disease Prevention Program is participating in an externally-funded initiative with the National Oceanic and Atmospheric Administration (NOAA) and the National Aeronautics and Space Administration (NASA) to detect cyanobacteria blooms in freshwater environments using novel satellite technologies for public health response activities.

FDOH distributes experimental health bulletins to partner agencies for public health and environmental management to facilitate bloom monitoring and forecasting. Practical applications of bloom tracking include identifying unknown blooms, targeting water sampling, enhancement to public health surveillance and informing public water treatment facilities thereby preventing human exposures to cyanotoxins. Methods:

Satellite images are captured by a Medium Resolution Imaging Spectrometer (MERIS) sensor onboard the European Space Agency Environmental Satellite (ENVISAT). NOAA applies an algorithm to generate a color-metric scale which displays estimated cyanobacterial concentrations in inland freshwater in the MERIS images. Light reflectance is captured by the sensor and used to generate the estimated cyanobacteria content in the satellite's coverage area.

Results: Satellite imagery is distributed in satellite health bulletins produced by FDOH and emailed to a distribution list of 92 users throughout the state. The bulletins contain recent MERIS imagery, a conditions report outlining areas in the state with the highest estimated cyanobacterial concentrations, updates on recent HABs-related illness clusters, and an overview of ongoing bloom activity. Satellite health bulletins are designed to keep county health departments and environmental managers up-to-date on current conditions. When epidemiologists conduct public health investigations, they can ask about recent exposures to recreational water sources impacted by cyanobacterial blooms. This will result in a more robust knowledge base about the health impacts of cyanotoxins and provide more time to prepare educational interventions during a bloom. Discussion: Images produced are considered experimental and further confirmatory efforts aimed at gathering field observations to validate the satellite imagery are being performed. Water quality data including chlorophyll data, microcystin concentrations, and plankton speciation data are collected by Water Management Districts, the Florida Department of Environmental Protection, and the Florida Fish and Wildlife Conservation Commission. These data sources are used to test whether the satellite imagery correlates with actual environmental conditions. The projected outcomes of cyanobacterial bloom forecasting include identifying previously unrecognized algal blooms, targeting environmental sampling efforts, identifying the source of unknown illnesses in the community, linking symptoms with cyanotoxin exposures, and ensuring that water sources used as public drinking water are free of cyanotoxins.

## 97. FECAL MICROBIOTA DISTORTIONS APPEAR ASSOCIATED WITH LATE ONSET SEPSIS IN PRETERM INFANTS

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Late onset sepsis (LOS) is a major cause of neonatal morbidity and mortality. Although various etiologic agents contribute to LOS, abnormal gut physiology that includes increased gut leakage is frequently observed. Distortions in the establishment of initial commensal gut microbiota might contribute to LOS. Consequently, early detection of microbiota distortions might provide i) a novel diagnostic tool and ii) an opportunity for microbiota based early interventions. Using a case/control design nested in a cohort study of preterm infants, delivered before reaching a gestational age of 32 weeks with a birth weight of less than 1250g, we analyzed stool samples from nine cases collected within 72hrs, 1 week and up to two weeks prior to the culture confirmed diagnosis of sepsis and from 18 matched controls collected at similar days after birth. Nine cases were matched to 18 controls by. Initial DGGE based microbiota profiling was followed by 16S rRNA pyrosequencing to compare microbiota diversity and the prevalence of specific bacterial signatures.

DGGE profiling indicated that microbiota diversity was lower in sepsis cases ( $p < 0.02$ ). 180845 sequences with an average length of 474 nucleotides were binned into Operational Taxonomic Units (OTU's) using ESPRIT-tree. Overall microbiota diversity (chao1) was lower in cases two weeks before ( $p < 0.05$ ) but not one week before or at diagnosis. Overall microbiota structure (Unifrac) appeared distinct in cases one week before but not at diagnosis ( $p < 0.05$ ). Multiple OTU's were less frequently detected in cases ( $p < 0.01$ ). Our results support the hypothesis that distortions in microbiota are associated with LOS.

## 98. UNDERSTANDING PLASMA STERILIZATION

**Navya Mastanaiah**, Mechanical & Aerospace Engineering, College of Engineering, University of Florida; **Judith Johnson**, Pathology, Immunology and Laboratory Medicine, College of Medicine, University of Florida; **Subrata Roy**, Mechanical and Aerospace Engineering, College of Engineering, University of Florida

Conventional methods of sterilization such as autoclaving and ethylene oxide fumigation, have the disadvantages of using high temperatures or toxic gasses and long sterilizing times. Dielectric Barrier Discharge (DBD) plasma sterilization potentially offers a fast, low-temperature, versatile and non-toxic alternative. Plasma, in its simplest form, can be described as a mixture of charged particles, neutrals, and UV photons. However the fundamental mechanism of how the plasma mixture destroys bacterial cells is not yet understood.

We have developed a portable DBD plasma set-up for that provides a self-sterilizing surface. Preliminary results with our DBD plasma setup operating at 12 kV p-p, 14 kHz has achieved a 4- 8 log reduction within 3 minutes, for *Pseudomonas aeruginosa* 6003-7, *Yersinia enterocolitica* SSUD 4037, *S. enterica* EPI 603, MRSA WCH132, and *Acinetobacter baumannii*. Effect of DBD plasma on *Geobacterium thermophilus* spores has also been tested and have proven to be more resistant. Preliminary tests have shown a 6 log<sub>10</sub> drop in ~6 minutes. To begin to understand the mechanisms of plasma lethality we have examine kill curves. Survival curves obtained for the above pathogens depict a bi-phasic and sometimes tri-phasic behavior. This phasic behavior can be hypothesized, as the result of different stages in which different plasma components act lethally upon

the cells. However efforts to prove this are still ongoing: one approach is to isolate the different plasma components via UV filters (for the UV photons), oxidizing agents (for the chemical species) etc. Once a particular component of plasma is isolated, the effect of doing so will be evident via plasma sterilization time. The problem can also be better understood by constructing a numerical model to simulate the interaction of plasma species with the cell structure. Such a numerical model would couple the governing equations involved in plasma air chemistry and the governing equations involved in cell breakdown, due to the oxidation of complex hydrocarbons present in the cell. The coupling factor would be the plasma species common to both sets of governing equations. Thus, as is evident from this short abstract, plasma sterilization is characterized by myriad factors. Understanding them through experimental studies of control parameters integrated with numerical modeling of the fundamental plasma processes is the focus of our research.

## 99. RACE AND ECONOMIC DISPARITY IN RUPTURED AND UNRUPTURED CEREBRAL ANEURYSM TREATMENT AND OUTCOME

**William Newman**, Department of Neurosurgery, College of Medicine, University of Florida; **Dan Neal**, Departments of Epidemiology and Health Policy Research, University of Florida; **Fred Barker**, Neurosurgical Service, Massachusetts General Hospital, Harvard University; **Brian Hoh**, Department of Neurosurgery, College of Medicine, University of Florida

**Background:** Disparities exist in access to high volume hospitals (HVHs) for a variety of conditions and may affect outcomes for patients with ruptured or unruptured cerebral aneurysms.

**Objective:** Determine the impact of race and income on access to HVHs and treatment modality (coiling or clipping) for patients with ruptured or unruptured cerebral aneurysms.

**Methods:** The Nationwide Inpatient Sample database from 2002-2008 was queried for all patients with unruptured cerebral aneurysm (n=12,089) or ruptured cerebral aneurysm (n=12,918) treated with either clipping or coiling. Primary endpoints were annual case volume during the year of admission and whether the patient received clipping or coiling.

**Results:** Low median income Blacks are 0.76 times less likely to be treated at a HVH, 3.04 times more likely to die in the hospital, and incur \$4,702 more in hospital charges than their wealthiest White counterparts ( $p < 0.001$  for all). Low median income Hispanics are 0.72 times less likely to be treated at a HVH, 3.2 times more likely to die in the hospital, and incur \$8,681 in additional cost ( $p < 0.05$  for all). For ruptured aneurysms, Blacks and Hispanics are less likely to receive coiling ( $p < 0.05$ ) and Hispanics, Native Americans, and Others are less likely to be treated at HVHs ( $p < 0.05$ ).

**Conclusion:** Low-income Blacks and Hispanics with unruptured aneurysms are less likely to be treated at a HVH, have longer hospitalizations, higher total charges, and increased mortality. Blacks and Hispanics with ruptured aneurysms are less likely to receive coiling, and Hispanics, Native Americans, and Others with

ruptured aneurysms are less likely to be treated at HVHs. Further investigation into race and income-based disparities in neurosurgical care is needed for improvement in patient outcomes and health care systems.

## 100. UNDERSTANDING THE RESEARCH DATA LIFE CYCLE AT THE UNIVERSITY OF FLORIDA

**Hannah Norton**, Health Science Center Library, George A. Smathers Libraries, University of Florida; **Erik Deumens**, Chemistry & Physics, College of Liberal Arts and Sciences, University of Florida; **Rolando Garcia-Milian**, Health Science Center Library, George A. Smathers Libraries, University of Florida; **Laurie Taylor**, Digital Collections, George A. Smathers Libraries, University of Florida; **Michele Tennant**, Health Science Center Library/UF Genetics Institute, George A. Smathers Libraries, University of Florida

Research data represent a valuable resource to academic and research communities. While they often require large investments of time and money to be created, many datasets have significant value beyond their original research purposes. Access to data can facilitate scrutiny of research outcomes, lead to new collaborations, and increase impact and visibility of research. Adequate management of data is necessary in order to make the best use of these valuable resources. Among the benefits of proper data management practices are: efficiency savings, risk management, access, and reuse. Proper data management provides an idea of data storage and processing requirements as well as potential improvement of workflows throughout the data lifecycle. Given the breadth of research occurring here, the University of Florida faces many of the challenges inherent to preserving, storing, managing, and making accessible large quantities of research data. The Health Science Center Libraries, George A. Smathers Libraries, and UF High Performance Computing Center (HPCC) are committed to developing local solutions to these challenges based on a comprehensive understanding of the UF data environment. To that end, we are in the process of performing a data services needs assessment, using surveys and interviews of UF researchers. We will present the preliminary results of this assessment, with an indication of future directions for library and HPCC services supporting campus data needs.

## 101. ESTIMATING INEQUITIES IN SANITATION-RELATED DISEASE BURDEN AND POTENTIAL IMPACTS OF PRO-POOR TARGETING

**Richard Rheingans**, Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida; **Oliver Cumming**, London School of Hygiene and Tropical Medicine, SHARE; **Julia Showalter**, Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida; **John Anderson**, Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida; **Peng Jia**, Department of Geography, College of Liberal Arts and Sciences, University of Florida

Recent work has shown significant variation in sanitation access improvements across wealth quintiles in many low-income settings, suggesting certain groups are being marginalized by current strategies and investments. The objectives of this study are to create a child-level model that integrates distributions of 1) sanitation-related health burden by wealth quintile and 2) health benefits for targeting different wealth quintile groups with 3) the spatial distribution of sanitation-related health burden and benefits in low-income countries Kenya and Malawi. Household and child survey data from the Demographic and Health Surveys was used to estimate disparities in sanitation-related services, exposures, susceptibility, burden and impact of infrastructure improvements. We used Principal Components Analysis to develop a wealth index based on household assets, excluding water and sanitation. A sanitation risk index was developed from combined estimates of community-level exposure and individual susceptibility for each child under five years of age. The exposure index integrated 1) presence of household improved sanitation, 2) the population density without sanitation in the surrounding community and 3) environmental vulnerability based on the source of water and construction materials of the home. The susceptibility index was developed from estimates of 1) nutritional vulnerability measured by weight for age Z-scores, 2) vitamin A doses and 3) likelihood of treating diarrhea with oral rehydration solution. Model outcomes were compared among wealth quintiles for both urban and rural settings and entered into ArcGIS for geo-spatial analyses. The results of this modeling exercise suggest the health burden of poor sanitation in urban settings is 35 (Kenya) and 7 (Malawi) times greater in the poorest as compared to the wealthiest households and 6.5 (Kenya) and 3 (Malawi) times greater for the poorest as compared to the wealthiest households in rural settings. We conclude that health burden disparity is the result of both greater exposure to enteric pathogens and increased susceptibility among children in the poorest households. Improvements in sanitation for the poorest households may bring 9 times (Kenya) and 7 times (Malawi) greater health benefit than improvements in the richest households. While rural households generally have lower levels of access, sanitation associated risk may be greater for the urban poor due to the increased likelihood of being in areas with a high density of people without sanitation. More effective targeting strategies that reach children in the poorest households are required to maximize the potential impact of sanitation investments.

## 102. TEMPORAL PATTERNS OF VACCINATION DISPARITIES OVER IN 12 LOW-INCOME COUNTRIES

**Richard Rheingans**, Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida; **John Anderson**, Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida; **Erica Trejo**, The Center for African Studies, College of Liberal Arts and Sciences, University of Florida; **Camila Pazos**, The Center for African Studies, College of Liberal Arts and Sciences, University of Florida

The introduction of routine vaccinations through the Expanded Program on Immunizations has contributed to reducing global child mortality over the past

decades. Campaigns for immunization against measles and polio and have also significantly reduced these threats to child health. New vaccines offer additional opportunity to further reduce child mortality due to diarrhea and respiratory disease. While most countries have moved toward universal coverage, progress in reaching the children most likely to benefit has been uneven and disparities in access threaten potential health gains from immunization. We used household data from the Demographic and Health Surveys from 12 countries to examine whether improvements in vaccine coverage in low-income countries has resulted in reductions in disparities. We selected countries with data after 2005 and a previous survey within 5 to 6 years. We examined BCG, DPT1-3, Polio 0-3, and measles vaccine coverage among children 12-23 months of age. We compared coverage by economic status, based on a household asset index calculated using Principal Components Analysis and calculated equity ratios and concentration indices as overall measures of disparities in coverage. The equity ratio is the ratio of coverage in the poorest quintile compared to that of the richest. The concentration index is an overall measure analogous to the Gini Index. For each vaccine, we compared national level equity ratios and concentration index values to examine whether improvements in coverage occurred between the two time periods and if these were associated with reductions in disparities. Data from Nigeria and Mali were examined in additional detail. A visual dashboard was constructed to provide decision-makers with an interactive way of exploring the patterns. Between the early and late 2000s, the 12 countries experienced different trajectories in coverage and disparities. For example, Bangladesh, Mali, and Kenya experienced increases in coverage, particularly among the poorest children, while Nigeria and India experienced little change with increases often occurring among only richer children. Patterns differed within countries by vaccine, with campaign based vaccines often being more equitable than routine ones. The global push for universal child vaccination has resulted in increases in coverage and reductions in disparities in a number of countries, but has been less successful in others. In particular, some of the countries with the highest levels of child mortality lagged behind others in terms of disparities. In order to achieve the full potential of childhood vaccines, added efforts are needed to target reaching children in greatest need.

### 103. ESTABLISHMENT OF A MODEL FOR AN ON-SITE FERTILITY PRESERVATION CLINIC FOR CANCER PATIENTS

**Alice Rhoton-Vlasak**, Obstetrics and Gynecology, College of Medicine, University of Florida; **Gwendolyn Quinn**, College of Medicine, University of South Florida; **Devlin Murphy**, Moffitt Cancer Center; **Caprice Knapp**, Health Outcomes and Policy, Medicine, University of Florida

**Objective:** The goal is to report on a project aimed to replicate a fertility preservation clinic (FPC) at the Shands Cancer Center at the University of Florida (SCC-UF). The original model was developed and implemented at Moffitt Cancer Center (MCC). An on-site FPC, such as the one at MCC is hypothesized to increase the likelihood that newly diagnosed cancer patients of childbearing age will receive timely information about fertility preservation options. MCC is the first National Cancer Institute designated Cancer Center to have an on-site REI.

**Materials and Methods:** In developing this model of care, the following steps were undertaken. First, we followed MCC's lead and developed a FP referral process by conducting a pilot study of impact of distributing a patient education brochure about FP. This increased calls to the REI clinic from patients seeking information, but left an unmet need for young cancer patients who required actual FP services. SCC-UF increased access to an REI, by following the MCC recommendations to facilitate having an on-site REI specialist available weekly to see all patients desiring an FP consult. A clinic log documented all incoming calls for REI appointments. We established a baseline rate in June 2010 and found that April–June 2010 the REI clinic received a total of 32 phone calls (~ 10 per month) with 3 patients scheduling an appointment. Baseline data will be compared to data collected in Sept 2011 to assess the impact of on-site REI appointments.

**Results:** Based on the success of this model at the MCC, implementation of an oncofertility specialist into the SCC is being undertaken. Shands will compare phone calls and appointments scheduled in the 3-month pilot period to that of a 3-month baseline period. Anecdotal reports suggest on-site access to a REI allows for consulting.

**Conclusions:** We present a novel clinical model and referral system that allows oncologists to meet the obligation by referring and providing patient information about FP. Even patients who choose not to use FP services will have had the opportunity for outreach at this critical time in their lives.

### 104. INVESTIGATING INITIATION OF THE IMMUNE RESPONSE IN THE LUNG BY NANOPARTICLES AND VIRUSES

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Despite extraordinary advancement in manipulating materials at the nanoscale and their current use in industrial, consumer and biomedical products, we lack sound understanding of the biotoxicity associated with environmental exposures. As the use of engineered nanoparticles (ENPs) is rapidly growing there is eminent concern regarding adverse health effects. The unusual physico-chemical properties that make ENPs promising for novel product enhancement applications may also influence their ability to modulate immune defense and inflammatory responses in humans. Inhalation being a primary exposure route underscores the critical need to comprehend how ENP impact the lungs. We have a particular interest in single-walled carbon nanotubes (SWNT) as they possess a superficially resemble asbestos which may be relevant to their long-term health consequences. Additional concerns surround the ability of ENP to influence the behavior of infectious agents thereby increasing susceptibility to infections. This can have critical consequences particularly for viruses, such as influenza A (IAV) that are notorious for causing pandemics. The objective of the present work is to

characterize the mechanisms controlling the immune response of lung cells exposed to SWNT and IAV. We concentrate on toll-like receptors (TLRs) as they are an early line of defense against foreign invaders in human body. Our hypothesis is that SWNT stimulate TLRs resulting in the production of pro-inflammatory cytokines through activation of transcription factors NF- $\kappa$ B. Furthermore, combined exposures of SWNT and IAV will synergistically activate TLR-driven pathways leading to enhanced inflammation and injury. To begin to address this hypothesis, we exposed lung cells to SWNT and assessed cytotoxicity, activation and expression of TLRs and downstream immune genes using suite of molecular assays. Results show that although SWNT are not acutely cytotoxic, they activate TLR2 and NF- $\kappa$ B, alter the expression of TLR7, and stimulate immune target genes. Furthermore, if we modify the SWNT surface (oxidize), activation of TLR2 does not occur. These data indicate the ability of SWNT to alter first line defense receptors and subsequent immune responses in an ENP-specific manner, highlighting the importance of surface chemistry in biologic effects. As an effort to determine how these results impact the normal immune response of pathogens through TLRs, we have begun to characterize H1N1 pandemic 2009 virus. This research will not only lay a foundation for studying the health impacts of emerging contaminants and infectious disease, it will also generate a comprehensive understanding of how multiple aspects of ENP affect cell function and will provide reliable in vitro model systems to evaluate and engineer safe nanomaterials.

## 105. COMPUTATIONAL MODELING OF TELOMERASE IN ACTION

Mahmoud Shobair, Physics, College of Liberal Arts and Sciences, University of Florida

Telomerase is a special reverse transcriptase that adds repetitive DNA sequences, GGGTTG for *Tetrahymena thermophila*, at the 3' end of DNA strand in the telomere region to ensure DNA replication completion. This enzyme is a ribonucleoprotein complex with RNA subunit as a template for synthesis of the repetitive telomeres. Telomerase is a key element to understand cellular aging and tumorigenesis due to its direct impact on chromosome length maintenance. The mechanism with which telomerase adds the six nucleotide repeat is not well-understood with current experimental biochemical and biophysical methods. The lack of three-dimensional structure of telomerase further hinders the current to fully understand its crucial biological function. Here, we attempt to propose a 3D structural of the six catalytic steps using computational modeling with experimental constraints. We perform discrete molecular dynamics simulations with experimental constraints derived from SHAPE chemistry, FRET and crystal structure homology modeling. Our preliminary results reveal interesting structural features and dynamic properties of telomerase in action. Further simulations and detailed computational analysis will allow us to generate experimentally testable hypothesis. The synergetic approaches of computational modeling and experimental validation will help us understand the molecular mechanisms of telomerase.

## 106. INTERNATIONAL AIR PASSENGER MOVEMENTS INTO FLORIDA AS A RISK FACTOR TO THE IMPORTATION POTENTIAL OF MEDITERRANEAN FRUIT FLY

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Globalization, trade liberalization and increasing passenger traffic are playing a facilitating role in the movement of pathogens and pests between distant geographic regions. Transport network expansion provides fast connections and gateways into new regions for the spread of exotic species, and therefore results in inevitable increases in pest and pathogen arrival rates for many countries. In this study we examined the seasonal properties of international passenger movements into Florida, as a surrogate for assessing the risk pathways for importation of one of the most economically significant pest species: Mediterranean Fruit Fly *Ceratitis Capitata* (medfly). Medfly is a highly polyphagous pest with over 250 hosts, established on 5 continents and is currently not present in the continental United States. It has been found in Florida multiple times, each time resulting in very costly eradication efforts. Should medfly become established, eradication efforts, losses in crop yields and control measures will cost millions of dollars. Here, international passenger movements are examined, since medfly interception records at the ports of entry are highly associated with agricultural products found mainly in passenger luggage. The species survival is highly dependent on the encountered environment and Florida could sustain medfly populations due to their favorable climate and host availability throughout the year. In this study we look at the properties of passenger traffic movement to major airports in Florida from countries known to have medfly populations established. We hypothesize that the risk of medfly importation via this pathway is changing on an intraannual basis due to seasonal fluctuations in passenger traffic frequency, volume and environmental suitability for medfly at origin locations. This study is a component of a larger multidisciplinary framework aimed at providing risk assessments of the seasonality in human-mediated pest and pathogen movements. The results will serve as a recommendation for guiding strategic decisions in the optimal deployment of surveillance resources, including staffing levels and sampling methods - ultimately attempting to reduce the number of pest and pathogen species introductions in Florida and therefore posing a great economic benefit to agriculture industry and the state.

## 107. US NATIONAL SURVEILLANCE FOR ZONOTIC MARINE MAMMAL VIRUSES

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**Introduction:** Marine mammals evoke strong public affection as well as considerable scientific interest. However, the resultant close contact with marine wildlife poses human health risks, including zoonotic disease transmission. Marine mammal populations suffer significant morbidity and mortality from a variety of viruses including potentially zoonotic agents like influenza viruses, coronaviruses, and paramyxoviruses. The detection, management, and treatment of these emerging pathogens poses special challenges requiring the expertise of physicians, veterinarians, and wildlife biologists. Here we outline the first concerted virus surveillance effort to detect potentially zoonotic viruses among wild and managed marine mammal populations.

**Materials and Methods:** We gained the collaboration of stranding network staff, marine mammal veterinarians, and wildlife biologists by providing a diagnostic service to them in exchange for their submission of marine mammal respiratory and fecal samples. We employed previously validated PCR assays to design a 7-pathogen surveillance panel to include: orthomyxoviruses (Influenza A and B viruses), paramyxoviruses, coronaviruses, reoviruses, anelloviruses, herpesviruses, and adenoviruses.

**Results:** To date, we have tested approximately 200 marine mammal samples and have discovered novel adenoviruses in bottlenose dolphin (*Tursiops truncatus*), harbor porpoise (*Phocoena phocoena*), and California sea lion (*Zalophus californianus*). Other discoveries include an anellovirus infecting bottlenose dolphin, an orthoreovirus infecting Steller sea lions (*Eumetopias jubatus*) as well as the first poxvirus infecting managed Northern and Southern sea otters (*Enhydra lutris*).

**Conclusions:** Preliminary efforts to detect and characterize potentially zoonotic marine mammal pathogens from zoos, oceanaria, and stranded marine mammal populations revealed novel DNA (adenovirus, anellovirus, poxvirus) and RNA (orthoreovirus) viruses. Given the limited samples tested to date, continued surveillance is planned to determine the prevalence of significant human pathogens, such as influenza viruses, circulating in marine mammal populations.

## 108. A MODULARITY-BASED METHOD FOR OTU PICKING OF 16S rRNA SEQUENCES

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**Binning 16S rRNA sequences into Operational Taxonomic Units (OTUs) is an initial crucial step in analyzing large sequence datasets generated to determine the microbial community composition in various environments including that of the human gut. Various methods are currently used to achieve binning, but most suffer from either inaccuracy or from being unable to handle the millions of sequences generated in current studies. Furthermore, most existing binning methods require a priori decisions regarding binning parameters such as the distance level to define an OTU. We and others have previously shown that using a single distance level across various bacterial phyla, families etc. is inappropriate. Appropriate distance levels differ not only by the location in sequence space as reflected on the phylogenetic tree, but also by the hypervariable region that is analyzed. Here we describe the development of a modularity-based approach to address this problem. The proposed method utilizes ideas from community/pattern detection in graphs. Based on pairwise distances, sequences are viewed as vertices on a weighted graph and each pair of sequences is connected by an imaginary edge. An OTU is defined as a region containing related sequences with high homogeneity where similarity between sequences within the region is greater than that outside. The modularity-based method can thus be used to find the partitioning that best reveals the community structure in particular regions of the graph. OTUs are defined by homogeneity of edge densities and not by distance between neighboring clusters; thus, the issue of defining the appropriate distance level is solved. Distance levels within OTUs vary by OTU, depending on the density of sequences in a particular region of the graph.**

The proposed modularity-based approach includes three steps: (1) compute pairwise sequence distances, (2) form an epsilon-neighborhood graph by only considering sequence distances less than epsilon, and (3) perform modularity-based clustering on this graph. Sequence data from different hypervariable regions of 16S rRNA were chosen to verify its performance, and the results were compared with those from CROP and ESPRIT-Tee. Our results suggest that the proposed method can generate more accurate and consistent results for OTU picking that better reflect the natural variation of cluster size and densities in the 16S rRNA sequence space.

## 109. PHARMACOKINETIC/PHARMACODYNAMIC MODELING OF IN VITRO ACTIVITY OF VERTILMICIN AGAINST THREE BACTERIAL STRAINS

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**Objective:** Vertilmicin is a novel semisynthetic aminoglycoside derived from verdamicin. The goal of the present study is to develop a semi-mechanistic pharmacokinetic/pharmacodynamic (PK/PD) model to describe the relationship between bacterial responses and drug concentrations and to predict the optimized dose required to achieve maximum efficacy in the clinic based on in vitro time-kill kinetic data.

**Method:** The in vitro antibacterial activity of vertilmicin was evaluated by static and dynamic time-kill kinetic experiments against three different strains of bacteria, namely methicillin-susceptible *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. The classic aminoglycoside, gentamicin, was used as the reference drug in order to evaluate the efficacy of vertilmicin. In the static time-kill kinetic studies, the bacterial burden was determined under a wide range of drug exposures ranging from 0.25- to 16-fold of minimum inhibitory concentration (MIC) over a 24-h period. The dynamic time-kill experiments were performed at 20 µg/mL for both drugs and carried out using a manual dilution system with half-life of 2 h. The recommended clinical concentration for gentamicin was used as the comparison basis since vertilmicin has yet to enter clinical trial. The data was subsequently fitted to a population dynamic model that incorporates a saturation function for bacterial growth, a function for bactericidal effect of the antimicrobial agents, an adaptation factor for time-dependent resistance development and a natural death rate. EC50 (the concentration required to achieve 50% of the maximum effect) of vertilmicin was evaluated and compared to that of gentamicin.

**Result:** The static time-kill kinetics showed that all the three bacterial strains could be completely eliminated when the concentrations of vertilmicin and gentamicin were greater than 4 µg/mL. The resistance development in all three bacterial strains was observed in the dynamic time-kill kinetics even when the starting drug concentration was 20 µg/mL. The stark contrast in the drug concentrations in the static kill curve versus the dynamic kill kinetics, required to achieve bactericidal effect, suggested that the elimination half-life of the antibiotic agents in the human body had a dramatic impact on their antimicrobial activities. A semi-mechanistic pharmacokinetic-pharmacodynamic model was developed to elucidate the bacterial response to vertilmicin and gentamicin based on the time-kill kinetic experiment. The EC50 of vertilmicin were estimated at 0.748, 0.610, and 3.784 µg/mL against MSSA, MRSA and *P. aeruginosa*, respectively. All these values were greater than those for gentamicin.

**Conclusion:** The result of PK/PD modeling demonstrated that vertilmicin has limited prospect for future clinical application if only potency is taken into consideration. A PK/PD model can be a powerful tool to evaluate the in vivo antibacterial activity of novel antibiotics, as well as to make key decisions in drug development.

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