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Letter from the Director

Welcome to the sixth annual EPI Research Day! As you look through the abstracts in this book, and see the posters, you should get a feel for the wide range of emerging pathogens-related research conducted by EPI members and collaborators. We are particularly pleased to welcome investigators from outside of Gainesville, including collaborators with the Florida Department of Health, who are among those presenting today.

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

Dr. Patrick Concannon is a nationally recognized geneticist, and the newly appointed Director of the UF Genetics Institute; he will officially assume his duties here at UF this coming week. He has a long history of collaboration on studies of the impact of infectious diseases in the developing world, and we look forward to partnering with him on issues related to emerging infections. Dr. Bryan Grenfell is Kathryn Briger and Sarah Fenton Professor of Ecology and Public Affairs in the Department of Ecology and Evolutionary Biology at the Woodrow Wilson School, Princeton University. He has been instrumental in developing our understanding of the spatio-temporal dynamics of infectious diseases – the topic of his talk today. Dr. Grenfell is a new member of the EPI External Advisory Committee, and we welcome him to UF.

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

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

EPI RESEARCH DAY 2013

Schedule of Events

9:00 AM - 10:00 AM	Check-in (Cancer and Genetics Research Complex, 1st floor lobby) Breakfast and Poster setup (Cancer and Genetics Research Complex, 1st, 3rd, and 4th floors)
10:00 AM - 1:00 PM	Poster Session (Presenters, please stand by your posters)
12:00 PM - 12:45 PM	Lunch (Cancer and Genetics Research Complex, 1st floor lobby)
12:45 PM - 1:00 PM	Keynote Assembly (Cancer and Genetics Research Complex Auditorium 101)
1:00 PM - 1:15 PM	Welcome- Dr. David Norton, VP for Research Introductions- Dr. J. Glenn Morris Jr., Director, EPI
1:15 PM - 3:15 PM	Keynote Speeches
3:30 PM - 4:00 PM	Remove Posters

(1:15-2:15)
Dr. Patrick Concannon Ph.D.

Professor and Associate Director
Center for Public Health, Genomics, University of Virginia
Charlottesville, VA

"The Genetics of Linear Growth Faltering in Undernourished Populations"

(2:15-3:15)
Dr. Bryan Grenfell Ph.D.

Kathryn Briger and Sarah Fenton Professor of Ecology and Public Affairs
Department of Ecology and Evolutionary Biology
Woodrow Wilson School, Princeton University
Princeton, NJ

"Spatio-temporal Dynamics and Control of Acute Immunizing Infections"

01. An Expert-Based Multi-Criteria Ranking of Global Foodborne Parasites

Michael Batz - Emerging Pathogens Institute, University of Florida; **Lucy Robertson** - Norwegian School of Veterinary Science (Oslo, Norway); **Joke van der Giessen** - National Institute of Public Health and the Environment (RIVM) (Bilthoven, Netherlands); **Brent Dixon** - Health Canada (Ottawa, Canada); **Marisa Caipo** - Food & Agriculture Organization of the United Nations; **Mina Kojima** - World Health Organization; **Sarah Cahill** - Food & Agriculture Organization of the United Nations

Introduction: Foodborne parasites cause a high burden of infectious disease globally, yet generally do not receive the same amount of attention as other microbiological and chemical hazards in food. Data on disease incidence and transmission routes are lacking, a problem exasperated by symptoms that may be latent or chronic. **Purpose**: In December 2010, the Codex Committee on Food Hygiene (CCFH) requested that FAO and WHO provide the Committee with "guidance on the parasite-commodity combinations of particular concern." FAO and WHO initiated a series of activities to provide this guidance, culminating in an expert workshop in September 2012. **Methods**: During a weeklong expert workshop, a multi-criteria decision analytic approach was developed and applied. Experts screened an initial list of 95 parasites down to 24 and identified food pathways for each. A tool was designed interactively with the experts to score the importance of each parasite-commodity combination along seven criteria, including disease prevalence, global spread, trends, severity, case-fatality ratio, trade relevance, and socio-economic impact. Each parasite was scored by groups of five along these criteria, with revisions following full-group discussions. Groups provided weights for combining criteria into scores, which were then computed, averaged across groups, and ranked. **Results**: Experts ranked Taenia solium, Echinococcus granulosus, Echinococcus multilocaris, Toxoplasma gondii, and Cryptosporidium as the top five parasites from a global foodborne perspective, followed by Entamoeba histolytica, Trichinella

spiralis, Opisthorchiidae, Ascaris and Trypanosoma cruzi. Rankings were largely driven by public health impact over other criteria. **Significance**: This multi-criteria ranking is the first of its kind for global foodborne parasites, and served as a useful, systematic, and open approach to providing policy guidance. The approach itself has broader applications, as it could be adapted for regional or national use, or expanded to other infectious diseases.

02. Comparing Cost of Illness and QALY Loss as Measures of Foodborne Illness Burden

Michael Batz - Emerging Pathogens Institute, University of Florida; **Sandra Hoffmann** - Economic Research Service, U.S. Department of Agriculture; **J. Glenn Morris, Jr.** - Emerging Pathogens Institute, University of Florida

Introduction: Cost of Illness (CoI) and Quality Adjusted Life Year (QALY) measures are needed to assess and rank the public health significance of foodborne illness across pathogens or pathogen-food pairs. The National Academy of Sciences has recommended against combining these two measures, but little research has been conducted to examine the implications of this recommendation for food safety policy analysis. **Purpose**: The purpose of this study is to assess empirical differences between CoI and QALY loss estimates and rankings across pathogens and pathogen-food pairs. **Methods**: The study compares new QALY and CoI estimates and rankings for 14 foodborne pathogens and 168 pathogen-food pairs based on 2011 incidence estimates. It evaluates the influence of annual number of illnesses, hospitalizations and deaths on QALY and CoI rankings. It uses non-parametric statistical analysis to examine the relationships between rankings based on both integrated measures and on CDC incidence estimates. The study also examines the implications of data limitations on the completeness of QALY and CoI estimates and rankings. **Results**: We find that existing research and data are sufficient to estimate both QALY loss and CoI due to acute illness with most foodborne pathogens, but that data on chronic sequelae are lacking for CoI. Despite this, we find QALY and CoI

rankings to be highly correlated for pathogens, foods, and pathogen-food pairs. Both QALY and CoI rankings are driven by deaths and hospitalizations. Correlation between QALY and CoI measures falls considerably when focused on only the top 10-20 ranked pathogen-food pairs. **Significance**: For overall rankings of all pathogen-food pairs, it makes little difference if rankings are based on CoI, QALY or deaths. As burden measures, CoI and QALYs each have meaningful strengths as well as empirical limitations. This study shows how these limitations affect estimates of the burden of foodborne disease in the U.S.

03. DISCOVERING NEW VIRULENCE FACTORS IN PATHOGENIC ESCHERICHIA COLI

Miguel De la Cruz - Department of Molecular Genetics and Microbiology, Emerging Pathogens Institute, College of Medicine, University of Florida; **Jorge Giron** - Department of Molecular Genetics and Microbiology, Emerging Pathogens Institute, College of Medicine, University of Florida

Escherichia coli (E. coli), one of the most important model organisms in the laboratory, is the best studied microorganism. The primary niche occupied by E. coli is the lower intestinal tract of mammals, where it resides as a beneficial component of the commensal microbiota. Although it is well-known that E. coli resides in the human intestine as a harmless commensal, specific strains or pathotypes have the potential to cause a wide spectrum of intestinal and diarrheal diseases. Two of these pathotypes are enterohaemorragic and enterotoxigenic Escherichia coli (EHEC and ETEC, respectively). In EHEC, we found that CpxRA, a two-component system, is involved in the biofilm formation and expression/secretion of type III-effector proteins. Additionally, a Toxin-Antitoxin module was found in EHEC, being involved in the bacterial viability and biofilm formation at room temperature. ETEC produces a fimbriae named Longus, involved in the interaction to intestinal cells. We identified three transcriptional regulators involved in the Longus production: LngR and HilD encoded inside lng operon, and CpxR encoded outside this

fimbrial operon. Details of the transcriptional regulation of this operon are currently studied in the laboratory.

04. ECOLOGY OF VIBRIO CHOLERAE IN FLORIDA BAYS

Lei Fang - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Kwang Cheol Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, University of Florida; **Anita Wright** - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Introduction: Vibrio cholerae(Vc), the etiological agent of cholera, is responsible for seven pandemics since 1817. U.S. outbreaks are rare, and only eight sporadic cases were reported to the CDC between 2003 and 2007. Recent epidemics in Haiti and Cuba confirmed cases due to the 7th pandemic strain of Asiatic Vc. A Florida outbreak in 2011 was associated with raw oyster consumption and indicated emergence of a serotype (O75) that differed from pandemic Vc. **Purpose**: This study investigated the genetic structure of Vc populations in Florida relative to the toxigenic 7th pandemic Vc. **Methods**: Water, sediment, oyster, fish and various plant samples were collected seasonally from 2011 to 2012 at different sites of Tampa and Apalachicola Bays. Environmental parameters included temperature, salinity, pH, dissolved oxygen were also evaluated. Presumptive Vc was isolated from CHROMagar™ and TCBS, and confirmed by rRNA intergenic spacer region-based PCR. Confirmed isolates were compared to clinical Vc by multilocus sequence typing (MLST) of five house keeping genes (recA,gyrB,pyrH, gapA, and topA) and screened for cholera toxin gene, ctxA, by PCR. **Results**: Vc was isolated only from water samples in Apalachicola and from both water and oysters in Tampa Bay. Unlike other Vibrio species Vc was not widely distributed throughout the bay, but was mostly associated with near shore sites with lower salinity. Most strains from Apalachicola (92%) and Tampa (63%) closely associated with a genetic clade that included Vc O75 and classical Vc 395, but were distinct from the 7th pandemic Vc clade that only included

only one isolate from Apalachicola. Other strains formed unique clades. No environmental strain was postitive for ctxA. **Significance**: Vc is endmic to Florida waters but is divergnet from the current 7th pandemic, and virulence potential appears limited due to the absence of genes for cholera toxin.

05. APPLICATION OF CHITOSAN MICROPARTICLES FOR THE REDUCTION OF VIBRIO SPECIES

Lei Fang - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Kwang Cheol Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, University of Florida; **Anita Wright** - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Introduction: Vibrios are gram-negative bacteria that commonly occur in costal estuarine environments. V. vulnificus (Vv), V. parahaemolyticus (Vp), and V. cholerae (Vc) are the principle pathogens known to cause at least 75% bacterial seafood-borne disease, and 95% of fatalities in the U.S. Consumption of undercooked seafood, especially raw oysters, can result in a severe systemic Vibriosis. The primary response by the seafood industry to reduce Vibrios relies on the implementation of postharvest processing (PHP), including thermal treatments and high hydrostatic pressure. Although effective, currently approved PHP will also kill the oysters and is not suitable for the live "half shell" market. **Purpose**: This study investigated the efficacy of chitosan microparticles (CM) for reduction of Vv, Vp and Vc both in vitro and in live oysters. **Methods**: The three Vibrio species were inoculated (ca. 104 CFU/ml) in to a nutrient medium with different concentrations of CM (0, 0.1, 0.3, and 0.5%) to determine effects on growth over 12 hours. Artificial seawater was inoculated (ca 107 CFU/ml) to examine survival over 48 hours. All samples were processed, serially diluted and spread plated to culture media to enumerate viable bacteria. **Results**: Growth of three species was completely inhibited within three hours of treatment with 0.3 % and 0.5 % CM. Furthermore,

treatment with 0.5 % CM successfully reduced three species by ca.7.5 log CFU/ml in artificial seawater by 48 hours. Vv was observed to be the most sensitive to CM treatment, while Vc was the most resistant of the three species. Numbers of Vibrios and total number of bacteria also significantly declined (p=0.012) in treated oyster samples (0.1, 0.3 and 0.5% CM) compared with untreated cntrols. **Significance**: These results show that CM treatment might be an effective postharvest process to reduce spoilage or pathogenic microbial threat in seafood industry.

06. CONTROLLING OF ESCHERICHIA COLI O157:H7 INFECTION IN CATTLE BY CHITOSAN MICROPARTICLES

Soo Jin Jeon - Department of Animal Sciences, Emerging Pathogens Institute, University of Florida; **Manhwan Oh** - Emerging Pathogens Institute, University of Florida; **Klibs Galvão** - Department of Veterinary Medical Sciences, College of Veterinary Medicine, University of Florida; **Kwang Cheol Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, University of Florida

Despite efforts to improve food safety, multi-state outbreaks caused by foodborne pathogens are continuous public health challenges. The reason can be found in biased intervention strategies that focus on postharvest food safety. The objective of this study is to develop antibacterial materials at pre-harvest level. Implementation of combined pre- and postharvest intervention strategies will be practical to decrease the number of outbreaks. We developed chitosan microparticles (CM) for reducing Escherichia coli O157 in the gastrointestinal tract in cattle. Here, the mechanisms by which CM reduce the levels of this pathogen in cattle was studied. CM showed remarkable and effective antibacterial activity against gram negative and positive pathogens, including E. coli O157:H7, Salmonella, Vibrio, and Streptococcus. OmpA was the primary binding target of CM, resulting in disruption of the bacterial membranes. The ΔompA mutant presented significantly reduced the CM susceptibility but was restored by complementation with the ompA gene. The vivo experiment, using the dairy cows with the pelvic inflammatory

disease, was conducted to test if CM can be used to treat disease as an alternative antibiotic. CM treatment showed reduced level of intrauterine pathogenic E. coli (IUPEC) and high treatment rate of the uterine disease. Therefore, we propose that CM could be a potential candidate for non-antibiotic alternatives, reducing the risk of pathogens in the preharvest level.

07. A SUPER-SHEDDER PLAYS A ROLE IN THE HIGH PREVALENCE AND TRANSMISSION OF ESCHERICHIA COLI O157:H7

Soo Jin Jeon - Department of Animal Sciences, Emerging Pathogens Institute, University of Florida; **Mauricio Elzo** - Department of Animal Sciences, Institute of Food and Agricultural Sciences, University of Florida; **Nicolas DiLorenzo** - Department of Animal Sciences, Institute of Food and Agricultural Sciences, University of Florida, North Florida Research and Education Center; **Cliff Lamb** - Department of Animal Sciences, Institute of Food and Agricultural Sciences, University of Florida, North Florida Research and Education Center; **Kwang Cheol Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, University of Florida

The prevalence of Escherichia coli O157:H7 in cattle herds is positively correlated to outbreaks of this pathogen, causing severe diseases in humans. Controlling the prevalence of Escherichia coli O157 in cattle is critical in reducing outbreaks of this pathogen. The prevalence of E. coli O157:H7 could be determined by bacterial (colonization), environmental (level of exposure to E. coli O157:H7) and animal factors (breed). Here, we hypothesized that animal genotype or phenotype contributes to the prevalence of E. coli O157 in cattle, and susceptible animals (named as a super-shedder) serve as a source of contamination. The purpose of this study was to evaluate animal genetic and physiological factors modulating the prevalence of E. coli O157 among an Angus-Brahman multibreed herd and to predict the effect of a super-shedder on transmission. Rectal anal junction swab samples were collected from 91 cattle. The swab samples were re-suspended in 2 ml of tryptic soy broth and serially diluted in 0.1% (w/v) peptone. The diluted samples were plated

on CT-SMAC to isolate and determine the number of E. coli O157. Multiplex PCR targeting stx1, stx2, hly, and rbfE was conducted to confirm E. coli O157. The lowest number of E. coli O157 was observed in the Brahman breed among an Angus-Brahman multibreed herd, and bulls excreted more E. coli O157 than steers in the pens. The high shedding of E. coli O157 was related to the presence of a super-shedder, defined as cattle excreting >105 CFU/rectal anal swab. To investigate the role of super-shedders in transmission, 44 animals at a pasture were transported to a feedlot and housed together with feedlot cattle. After six months, animals were randomly examined for identification of E. coli O157, and Pulsed-Field Gel Electrophoresis (PFGE) was performed to subtype farm isolates. Molecular subtyping analysis showed 96.6% similarity between two strains isolated from the pasture and feedlot. Taken together, super-shedders play a pivotal role in the high prevalence of E. coli O157 as well as farm-to-farm transmission of E. coli O157. These results provide insights for the development of mitigation strategies to reduce prevalence of E. coli O157 in cattle or at farm.

08. SURVIVAL OF VIBRIO CHOLERAE IN NUTRIENT-POOR ENVIRONMENTS IS ASSOCIATED WITH A NOVEL "PERSISTER" PHENOTYPE

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

In response to antibiotic and/or environmental stress, some species of bacteria shift to a "persister" phenotype. Although toxigenic Vibrio cholerae, responsible for the disease cholera, can be found in nutrient-poor aquatic environments in endemic areas, the underlying mechanism(s) by which culturable cells persist in these environmental reservoirs is largely unknown. Here we report that the introduction of V. cholerae into a nutrient-poor

filtered, sterilized, lake water (FSLW) microcosm promoted a shift to what we have defined as a "persister" phenotype (PP), which was culturable for over 700 days. Direct transfer of PP of V. cholerae from original microcosms to freshly prepared FSLW resulted in the same pattern of persistence seen in the original microcosms. Scanning electron microscopy of cells persisting for over 700 days demonstrated cell morphologies that were very small in size with a high degree of aggregation associated with flagella emanating from all aspects of the cell. V. cholerae PP cells reverted to a typical V. cholerae morphology when transferred to nutrient-rich L- broth. Cell-free supernatants, obtained from microcosms at 24 hours, 180 days, and 700 days, all showed >2-fold increase in CAI-1 signaling molecules, consistent with quorum sensing activity, as has been described for Pseudomonas aeruginosa persister cells. Chitin and phosphate promoted cell growth. Our data suggests that nutrient stress can select a V. cholerae persister phenotype in environmental reservoirs with these strains, then seed subsequent cholera epidemics in response to chitin and phosphate availability.

09. ISOLATION OF VIBRIO VULNIFICUS USING DIFFERENTIAL MEDIUM AND FLUOROGENIC SELECTIVE

Myoung-Su Kim - Department of Food Science and Human Nutrition, University of Florida

Enzymatic profiles of Vibrio species related with specific reference to the β-galactosidase reaction. 4-Methylumbelliferyl β-D-galactopyranoside is known as a substrate for β-galactosidase and becomes fluorogenic by cleavage of the free 4-methylumbelliferyl moiety when exposed to long-wave UV radiation. This research will compare thiosulfate-citrate-bile salts-sucrose agar (TCBS), cellobiose colistin (CC) agar, and modified cellobiose-polymyxin B-colistin (mCPC) agar for maximum recovery of fluorescent and non-fluorescent Vibrio vulnificus colonies from Vibrio vulnificus environmental isolates strains in oysters at 37°C and 40°C for 24 and 48 h. A newly developed medium will be compared for the efficacy of isolation for the species and for optimal β-galactosidase activity with the

fluorogenic selective marker, 4-Methylumbelliferyl β-D-galactopyranoside, and other selective markers. The fluorogenic selective marker, secondary fluorogenic selective markers, and using will prove to be a specific powerful tool for isolation in V. vulnificus environmental isolates strains.

10. Detection of Cronobacter Sakazakii in Powdered Infant Milk Formula Using Real-Time PCR

Myoung-Su Kim - Department of Food Science and Human Nutrition, University of Florida

Cronobacter sakazakii is a neonatal pathogen that has been found commonly in contaminated dried infant milk formula and milk powder. The fluorogenic selective marker, 4-Methylumbelliferyl-α-D-glucoside and secondary selective markers, sodium thiosulfate & ferric citrate have been used in differential media to indicate the presence of C. sakazakii based on α-D-glucosidase enzymes unique to this pathogen. This research will compare four enrichment broths for maximum recovery from powdered infant milk formula: C. sakazakii – Enterobacter sakazakii enrichment (ESE) broth, Tryptic Soy Broth (TSB), Enterobacteriaceae enrichment (EE) broth, and M-Coliform broth. Differential selective and nonselective agars including Trypticase soy agar (TSA), Violet red bile agar (VRBA), Violet red bile D-glucose agar (VRBDGA), and a newly developed KJ medium will be compared for the efficacy of isolation for the species and for optimal α-D-glucosidase activity with the fluorogenic selective marker, 4-Methylumbelliferyl-α-D-glucoside and secondary selective markers. C. sakazakii strains ATCC 29544, ATCC 29004, ATCC 12868, and ATCC 51329 will be utilized as positive controls to run in artificially contaminated powder infant milk formula (PIMF) with each enrichment broth. DNA will be extracted from enrichments, which will be examined using real-time PCR in order to compare to culture-based detection to determine relative sensitivities between the two approaches. The fluorogenic selective marker, secondary selective markers, and using a PCR protocol will prove to be a rapid and specific powerful tool for the

detection of Cronobacter sakazakii in powdered infant milk formula.

11. Is Cefotaxime Resistance Endemic in Farm Animals?

Raies Mir - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **WonSik Yeo** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Todd Bliss** - Department of Public Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Judy Johnson** - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **J. Glenn Morris Jr.** - Department of Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Kwang Cheol Jeong**

- Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Antimicrobial resistance is a growing concern in animal and public health. The number of antibiotic resistant microorganisms (ARMs) is increasing and will continue to increase due to the slow development of new antibiotics and lack of alternative therapies for bacterial diseases. Extended spectrum β-lactamase (ESBL) producing microorganisms are resistant to third-generation cephalosporins including cefotaxime and present a new challenge to the food animal industry. Here, we report that cefotaxime resistant microorganisms are prevalent in the cattle farms. Cefotaxime resistant microorganisms were isolated from nine different locations and farm settings in Florida. 16S rRNA gene sequencing was conducted to identify the resistant microorganisms. Seventeen different species of microbes including animal, human, and plant pathogens, as well as soil bacteria, were identified. The prevalence of cefotaxime resistant microorganisms in cattle was varying among farms, ranging from 5.2% to 100%. Animals reared in loose housing systems show lower prevalence of ARMs compared to animals in intensive

housing systems, indicating animal-to-animal transmission plays a role in the ARM transmission. Interestingly, cefotaxime resistant microorganisms were isolated from animals, which have never been exposed to this antibiotic through their entire life span, suggesting the acquisition of resistance might have originated from nature. Taken together, our findings provide the first occurrence of cefotaxime resistance in animals and shows that the development of antibiotic resistance is a continuous natural process.

12. THE ABILITY OF A REPORTER YERSINIA PHAGE TO CONFER A BIOLUMINESCENT SIGNAL TO Y. PSEUDO STRAINS UNDER DIFFERENT CONDITIONS

Nino Mitaishvili - Branch of Battelle Memorial Institute in Georgia; **Chythanya Rajanna** - Department of Molecular Genetics and Microbiology, Emerging Pathogens Institute, College of Medicine, University of Florida; **Tamara Revazishvili** - Emerging Pathogens Institute, College of Medicine, University of Florida; **Alexander Sulakvelidze** - Department of Molecular Genetics and Microbiology, Emerging Pathogens Institute, College of Medicine, University of Florida

Recombinant reporter phage may provide a rapid and specific approach for the detection of Y. pseudotuberculosis, as phage bacteria interaction is highly specific. The method describes the phage-mediated transduction of a bioluminescent phenotype to cultivated Y. pseudotuberculosis cells which are subsequently measured using a microplate luminometer. The major advantage of this method is the ease of use, the rapid results, and the ability to test multiple samples simultaneously in a 96-well microtiter plate format. Experiments were performed to determine the ability of a reporter Yersinia phage to detect Y. pseudotuberculosis strains and the best conditions (media, temperature, enrichment) for detection. Further, to determine if the Yersinia phage can detect Y. pseudotuberculosis in presence of other bacterial species. Experiment was conducted on three Y. pseudotuberculosis strains at room temperature, 28°C and 37°C. The results showed that room temperature was not an effective

way to detect. Y. pseudotuberculosis could be rapidly detected within 30 minutes at 28°C. The bioluminescent signal to different concentrations of Y. pseudotuberculosis strains was examined. The reporter phage assay could detect luminescence within 45 minutes when the bacterial cells were at 105 cells/ml. Yersinia reporter phage was highly specific for Y. pseudotuberculosis strains. The reporter phage assay could detect Y. pseudotuberculosis within 30 minutes in presence of other enteric bacteria without selective enrichment.

13. ANIMAL FACTORS: POTENTIAL TARGET DEVELOPING PRE-HARVEST INTERVENTION TO REDUCE SHIGA TOXIN-PRODUCING ESCHERICHIA COLI

Manhwan Oh - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Maria Cevallos** - Department of Animal Sciences, College of Agricultural and Life Sciences, University of Florida; **Min Young Kang** - Department of Animal Sciences, Emerging Pathogens Institute, University of Florida; **Seung Cheon Hong** - Department of Plant Pathology, North Carolina University; **Mara Brueck** - Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Matthew Taylor** - Emerging Pathogens Institute, University of Florida; **Jennifer Fore** - Department of Microbiology and Cell Science, Emerging Pathogens Institute, University of Florida; **Laura Henry** - Department of Animal Sciences, Emerging Pathogens Institute University of Floria; **Kwang Cheol Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, University of Florida

Controlling the prevalence of Shiga toxin-producing Escherichia coli (STEC) in cattle at the pre-harvest level is critical to reducing outbreaks of this pathogen in humans. A multilayer of factors, including environmental and bacterial factors, modulates the colonization and persistence of E. coli O157 in cattle, which serve as a reservoir of this pathogen. However, understanding the animal factors, which affect the prevalence of this pathogen remains unknown. The purpose of this study was to identify animal factors that affect the prevalence of STEC in cattle to

provide insights for the development of mitigation strategies at the pre-harvest level. Cattle fecal swab samples were collected at the rectal anal junction from 90 animals, which were used twice in this project over the course of two years. Swab samples were plated on MacConkey agar, and incubated at 37°C for 24 hours to isolate lactose fermenting colonies. Twenty pink colonies per plate were analyzed for STEC using multiplex PCR amplifying stx1 and stx2 genes. Pearson's Chi-squared test was used to compare the prevalence of stx1, stx2, or stx1/2 positive microorganisms in cows and heifers. The total number of STEC from the RAJ varied between animals, ranging from 0 to more than 106. The prevalence of STEC (positive with either stx1 or stx2) was 47% and 50% in the 1st and 2nd year, respectively, and the majority of positive samples contained this pathogen at 102-105 CFU/swab. The prevalence of STEC was significantly lower ($p<0.01$) in heifers compared to cows, indicating animal age plays a key role in the prevalence of STEC. Our data reveal that animal age affects the prevalence of STEC in cattle, providing a potential mitigation strategy to reduce STEC at the pre-harvest level.

14. BACTERIOPHAGE-BASED PROBIOTIC PREPARATION FOR MANAGING SHIGELLA COLONIZATION OF THE GASTROINTESTINAL TRACT IN MICE

Yura Park - Emerging Pathogens Institute, University of Florida; **Maria Ukhanova** - Emerging Pathogens Institute, University of Florida; **Alexander Sulakvelidze** - Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida, Intralytix, Inc., Baltimore, Maryland; **Volker Mai** - Department of Epidemiology, College of Public Health and Health Professions, University of Florida

Bacteriophages against enteric pathogens offer an alternative to antibiotic drugs. Lytic phages frequently exhibit high host specificity, which is in contrast to broad spectrum antibiotics that also affect commensal microbes. We hypothesized that bacteriophages can be developed into a phage-based probiotic aimed at establishing a beneficial microbial gut environment that is resistant against enteric pathogens infections.

In this study, we observed the efficacy and toxicity of ShigActive phage in reducing Shigella in the gut environment. Shigella is a common enteric pathogen that, especially in developing countries, can cause severe dysentery and fever with high morbidity and potential mortality. To test for the efficacy of the phage, mice were challenged with an antibiotic-resistant strain of Shigella S43 NalR. Phage mix was administered by oral gavage based on different schedules to test for the most effective mode of delivery (1 hr after, 3 hr after, 1hr before and after, and 1 hr before Shigella challenge) while control animals received PBS. Stool samples were collected postchallenge (day 1 and day 2), cecum and small intestinal contents were collected upon sacrifice. Shigella CFU counts were determined on McConkey agar plates containing selective antibiotic. To test for toxicity, mice were administered ShigActive phage twice a day for 7 days and then once a day, every other day for 28 days. The animals were sacrificed on day 7 or day 28 and stool and cecum contents were collected for microbiota analysis. Urine tests, blood tests, and histopathology examinations were also conducted to evaluate potential toxicity. Fecal counts were reduced in mice receiving the ShigActive phage. The greatest reduction in Shigella colonies was detected after phage was administered 1 hr before and 1 hr after challenge. DGGE results showed no significant effect of phage on the diversity of the gut microbiota composition. After 28 days of treatment, the serum albumin level (ALB) was significantly higher ($p < 0.05$) and blood urea nitrogen (BUN) was lower ($p < 0.05$) in phage-treated mice than in the control group. None of the phage-treated mice however, showed any other biological alterations or histopathological changes in their tissues. In conclusion, ShigActive consumption appears to be efficient in minimizing growth of Shigella with few associated side effects.

15. CATCH LOCATIONS AND FISH TYPES ASSOCIATED WITH CIGUATERA ILLNESSES IN FLORIDA

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Introduction: Ciguatera fish poisoning is a food-borne illness that results from consumption of reef fish containing toxins produced by Gambierdiscus dinoflagellates. Florida is the U.S. state with the most reported cases of ciguatera, with case numbers increasing over the past decade. Case reports likely represent only a small percentage of true ciguatera illnesses and it is important to improve ciguatera surveillance to better understand the disease burden and to identify areas with fish at risk of being toxic for prevention messaging. We compared the catch locations and types of fish that caused ciguatera illness in outbreaks reported to the Florida Department of Health (FDOH) and among Florida fishermen with a history of ciguatera illness. **Methods:** We performed an email-based survey of recreational saltwater fishing license holders in Florida to identify unreported cases of ciguatera illness. Participants were asked whether they had ever been diagnosed with ciguatera or experienced characteristic symptoms after eating saltwater fish caught by themself or someone they know. Those with a past ciguatera illness were asked where the fish was caught and the type of fish. We compared the survey results to de-identified cases of ciguatera reported to FDOH from 2000-2011 with available information about the fish meal. Reported cases were combined into outbreaks, defined as one or multiple cases linked by a common fish meal. **Results:** There were 65 presumptive ciguatera cases identified through the email survey and 41 outbreaks reported to FDOH with catch location reported. The Florida Keys and the Bahamas accounted for 73% of reported outbreaks and 52% of fishermen cases. Fishermen cases originated from a wider variety of countries in the Caribbean. One (2%) reported outbreak and 8 (12%) fishermen cases were associated with a fish caught north of South Florida (above St. Lucie County). Fish type was reported in 72 presumptive cases and 96 reported outbreaks. Barracuda were identified in 36 (38%) reported outbreaks and 10 (13%) fishermen cases. Grouper, amber jack, and snapper were commonly identified in

both data sources. Other or unknown fish accounted for 15 (16%) reported outbreaks and 29 (39%) fishermen cases. **Conclusions:** Cases of ciguatera reported to FDOH exhibit less variation in catch location and type of fish than cases identified in recreational fishermen. This suggests a possible bias in reporting practices towards those who consumed barracuda obtained in the Florida Keys or the Bahamas. An expanded version of the email survey for an additional 200,000 license holders is currently in progress to better estimate underreporting and reporting biases.

16. ELUCIDATING THE DETERMINANTS OF PROTECTIVE NOROVIRUS IMMUNITY

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Volunteer studies performed in 1970-1990 in which volunteers were challenged multiple times with a human norovirus (genogroup I, genotype 1; GI.1) demonstrated that this virus failed to elicit protective immunity. In contradiction to this result, recent pandemic norovirus strains (all GII.4 variants) appear to elicit herd immunity that drives the evolution of the virus and consequent emergence of new viral variants. One possible explanation for these apparently contradictory results is that specific human norovirus strains (of which there are over 100) interact with the host immune system differentially. Our hypothesis is that there are virus strain-specific differences in the induction of protective immunity. To test this hypothesis, we utilized two murine norovirus strains – MNV-1 which is relatively virulent and MNV-3 which is relatively attenuated. Here we demonstrate that MNV-3 induced robust protective immunity against both homologous and heterologous re-challenges, whereas MNV-1 failed to elicit a significant protective immune response. The two strains reached similar peak titers during primary infection, allowing us to exclude differential viral

replication efficiency as the explanation. We have determined that CD4+ T cells and B cells are essential to MNV-3 protective immunity whereas type I interferon signaling, type II interferon, and CD8+ T cells are not. Furthermore, our findings demonstrate that MNV-3 induced higher serum IgG and mucosal IgA titers than MNV-1 following infection. Additionally, MNV-3 virus-specific serum antibody conferred partial protection from homologous and heterologous challenge when used in passive transfer experiments; however MNV-1 specific serum antibody did not. We are now testing whether MNV-3-specific mucosal IgA also confers protection upon passive transfer. Altogether, our findings have revealed that norovirus strain-specific disparities in protective immunity induction do exist and have shed light on the determinants involved in norovirus protective immunity. These findings will allow us to better understand the role of specific factors involved in protective immunity and will advance our understanding of norovirus immunity and rational vaccine design.

17. NEW OBSERVATIONS ON FECAL MICROBIOTA DEVELOPMENT IN PRETERM INFANTS WITH NECROTIZING ENTEROCOLITIS (NEC)

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Necrotizing Enterocolitis (NEC) is a major cause of neonatal morbidity and mortality especially in premature infants. A critical role of gut microbiota development in NEC pathogenesis has been suggested, and we have previously shown a late bloom in

Proteobacteria in NEC cases. Distortions in the initial establishment of normal gut microbiota might increase disease risk by i) compromising the mucosal barrier causing leakage of luminal contents, and ii) failure to develop immune tolerance. To investigate such correlations we established a prospective cohort in which we enrolled to date more than 900 preterm infants delivered at three Florida hospitals. Starting with the first stool weekly specimens were collected from infants born at gestational ages ≤32 weeks or birth weights≤1250 g. We used a nested case/control design to study microbiota associations in 27 NEC cases matched to 44 controls. We generated PCR products from 121 samples using a bar-coded primer set based on universal primers 27F and 338R. After removing low quality sequences or sequences shorter than less than 150 nucleotides we retained 335626 sequences (2774 /sample) with average length of 288 nucleotides and clustered them using ESPRIT- tree algorithm for analysis at varying similarity levels. We obtained 2976 and 705 OTUs at the 98% and 95% similarity levels. We detected OTUs unique to each group and a microbiota composition distinct by case status as well as by hospital. In the control babies, the proportions of the major bacterial phyla did not change significantly over time. In contrast, in NEC babies we observed an increase of Firimicutes and decrease of Proteobacteria over time. In NEC babies Proteobacteria was dominant two weeks before diagnosis, which is in contrast to our previous observation. The levels of Bifidobacteria, detected by qPCR, were significantly lower in cases two weeks and one week before of NEC diagnosis. The lower levels of Bifidobacteria may prevent a timely development of colonization resistance and predispose infants to infection by pathogenic bacteria.

18. DISPARATE FUNCTION OF NOVEL PARALOGOUS EFFECTOR PROTEINS FROM ENTEROHEMORRHAGIC ESCHERICHIA COLI O157:H7 CAUSES CELL DAMAGE

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Many pathogenic bacteria inject multiple effector proteins into host cells via the type III secretion system (TTSS) to trigger signal transduction pathways leading to a multitude of host cell responses including actin cytoskeletal rearrangement and rewiring transcription. Enterohemorrhagic Esherichia coli (EHEC) possesses at least 41 effector genes encoded in the outside of the locus of enterocyte effacement (LEE) (non-LEE) where many of those are paralogous proteins that have arisen by gene duplication and may have different biological activity due to mutations. However, only few cases have been elucidated their precise biochemical roles and functional specificity of those are poorly understood during infection. Here we show a set of paralogous effectors in the non-LEE from the previous studies of in silico analysis in EHEC genome. Most of non-LEE effectors exhibit different extent of the cytotoxicity when they expressed in mammalian cells in trans and/or deleted from EHEC genome. Based on the sublocalization prediction of those paralogous effectors, they target to different compartments, suggesting that subtle differences in amino acid sequences determine their host targets. Thus, our findings provide a potential role of uncharacterized non-LEE effectors whereby paralogous effectors in pathogenic bacteria may have evolved in distinct way to manipulate host signaling pathways via unknown mechanism yet and reflect functional specificity during infection.

19. CD4+ T CELLS ARE OF VITAL IMPORTANCE TO NOROVIRUS PROTECTIVE IMMUNITY

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Observations from human norovirus volunteer studies and natural norovirus outbreaks have led to conflicting conclusions regarding the question of whether noroviruses induce protective

immunity. One possible explanation is that norovirus strains - of which there are over 100 genetic variants - differ in their interaction with the host immune system. We utilized two murine norovirus strains, relatively virulent MNV-1 and relatively attenuated MNV-3, to test whether genetically related but pathologically distinct norovirus strains induce differential protective immunity. Our findings demonstrate that MNV-3 induced robust protective immunity against both homologous and heterologous re-challenges, whereas MNV-1 failed to elicit a significant protective immune response. This difference was not due to variable viral replication efficiency since MNV-1 and MNV-3 reached similar titers during primary infections. Our findings further indicate that CD4+ T cells and B cells are essential to MNV-3 protective immunity while type I interferon signaling, type II interferon, and CD8+ T cells are not required. Based on the observations that CD4+ T cells are even more critical to MNV-3 protective immunity than B cells and that antibody confers only partial protection upon passive transfers, we tested whether CD4+ T cells play a helper-independent role in MNV-3 protection. Interestingly, adoptive transfer of CD4+ T cells purified from immunized mice mediated partial protection from a MNV-3 challenge. We are now dissecting what subset of CD4+ T cells mediate this effect and why MNV-1 fails to induce these cells. Our recent results demonstrate that Th17 cells are not involved in MNV-3 protection so ongoing studies are focused on cytolytic CD4+ T cells and pathogenic T regulatory cells. Altogether, our findings have revealed remarkable norovirus strain-specific disparities in protective immunity induction and the determinants involved in the protective response. These findings advance our understanding of norovirus immunity and may inform the development of effective norovirus vaccines.

20. EVALUATION OF QUIDEL® INFLUENZA ASSAYS IN SWINE INFLUENZA VIRUSES

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Background: Detections of swine influenza viruses (SIVs) in humans and human influenza viruses in pigs are increasing. This has been particularly evident since the 2009 H1N1 pandemic virus rapidly spread around the world to man and pigs. Reassortant variants of SIVs are particularly troublesome, especially a H3N2v which has infected more than 300 humans in the United States and is thought to be enzootic in US swine herds. As recent reports have indicated that SIVs may be missed with human diagnostics, we sought to compare World Health Organization (WHO) qRT-PCR, and Quidel Corporation's (San Diego, CA) commercial influenza diagnostics (Molecular Influenza A, QuickVue Influenza, and Sofia Influenza A assays) in detecting SIV from pig swabs. **Methods**: Iowa State University Veterinary Diagnostic Laboratory (ISUVDL) shipped, on icepacks, a blinded panel of 200 pig clinical nasal swab specimens (0.2mL undiluted aliquot each) to the Global Pathogens Laboratory. Collected from 2010 to 2012, 147 of these specimens had been previously identified to be influenza A-positive by molecular assays. In 2010, specimens were tested using a homebrew qRT-PCR assay for influenza A. Beginning in 2011, samples were tested with a USDA-approved veterinary qRT-PCR VetMAX-Gold SIV detection kit (Life Technologies Corporation, Carlsbad, CA). **Results**: Compared to the ISUVDL influenza A assays, the WHO and Quidel assays lacked sensitivity but had excellent specificity in detecting influenza A in pig swab specimens. **Conclusions**: While we likely had some degradation of influenza A virus from the freeze-thaw of the pig swab specimens, it seems clear that molecular and

immunological assays, that are designed for detection of human influenza A virus, may not be equally effective in detecting SIV.

21. MAXIMIZING INFLUENZA IMMUNIZATION RATES IN ALACHUA COUNTY SCHOOLS K-12

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Objective: Computer modeling studies suggest that immunizing 70% of school aged children against influenza will protect the entire community. Time and resources need to be utilized and allocated effectively to achieve this community immunity. In order to optimize the utilization of resources, this study aims to identify demographic factors that are associated with low influenza vaccination rates in Alachua County schools to adapt future interventions to reach an overall 70% immunization rate. **Methods**: This study aims to identify demographic factors associated with low vaccination rates by comparing the immunization percentages to: (1) school grades / elementary, middle, high, (2) school locations / urban vs. rural population density, (3) free and reduced meal rates, and (4) school assessment grades / A, B, C, D, F, as an indicator of student academic achievement. The immunization percentages were calculated by taking the number of LAIVs (Live Attenuated Influenza Vaccines) administered at each school grade by the Alachua County's influenza vaccination program over the total

population of the school during the 2012-2013 year. Data on free and reduced meals, which is an indicator of the socioeconomic status, and school assessment/grades, were provided by the Alachua County School Board. **Results**: For 2012-2013, the overall immunization percentage for school aged children in Alachua County was between 58-63%. Data from Alachua County's school located influenza vaccination program indicates that 51% of children got vaccinated in elementary schools, 39% in middle schools, 23% in high schools, and 10-15% in private physician offices. Vaccination proportions were statistically different among elementary, middle, and high schools. Elementary schools had a significantly higher vaccination proportion than middle and high schools (p=.0066). Urban elementary and high schools and rural schools did not differ significantly in Alachua County (p=.5902), but rural middle schools had higher (though not significantly) vaccination proportion than urban middle schools (p=.2351). Schools with higher percentages of students with free and reduced meal rates did not have a significantly higher proportion of students that received FluMist, compared with schools with lower percentages of students with free and reduced meals (r= -.203). Elementary schools with a school assessment grade of A or B had a higher immunization proportion than schools with a grade of C or lower. **Conclusion**: Influenza vaccine coverage among children in Alachua county needs to be a priority. Due to limited resources, interventions to improve coverage should target all high schools, rural middle schools, and elementary schools with a low school assessment score.

22. TARGETING THE OX40 (CD134) CO-STIMULATORY RECEPTOR ENHANCES PULMONARY CD8 MEMORY T CELL RESPONSES.

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For many highly pathogenic respiratory viruses CD8 T cells can play a critical role in protecting against repeated infection. However, despite our significant understanding of vaccine design the development of effective CD8 T cell vaccines against respiratory viruses remains elusive. An explanation for this continued failure centers around the concept that a single immunization with replication defective or attenuated vaccine vectors can not elicit adequate numbers of CD8 T cells to warrant protection. This led to the development of prime boost vaccination strategies that, although capable of enhancing recall numbers, do not maintain sufficient CD8 memory T cell numbers over time. We have recently demonstrated that targeting of the OX40 (CD134) co-stimulatory receptor can enhance primary CD8 T cell responses following infection with both virulent and attenuated pox-virus vaccine strains. Suggesting that manipulating co-stimulatory signals during a vaccination boost might influence the re-activation and subsequent persistence of CD8 memory T cells. To test this hypothesis in the context of a prime boost vaccination strategy we transferred virus specific CD8 memory T cells into naïve mice that were later immunized via dermal scarification. We show that OX40 agonism 24 hours following a vaccination boost enhances both the number of virus specific secondary effector CD8 T cells, defined by their expression of KLRG, IL7-R, CD62L and CD27, located within the lung and their capacity to produce both TNF-α and IFN-γ. Remarkably, promoting OX40 signaling during memory cell reactivation (vaccination boost) augments IL-7R expression, which is critical for memory cell persistence and recall capacity, and enriches the numbers of CD8 memory T cells that persist in mucosal tissues such as the lung. These data provide evidence that targeting OX40 at the same time as an antigen boost can expand the numbers of effector CD8 memory T cells that are capable of residing within lung tissue and thus protect against mucosal viral infection.

23. CD8 T CELL DERIVED IFN-Γ: A CLUTCH PLAYER IN PROTECTING AGAINST RESPIRATORY VIRUS INFECTION.

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Respiratory viral infections remain a leading cause of global morbidity and mortality and are set to continue as global populations age, social attitudes toward vaccination alter and antigenic recombination produce novel respiratory viruses. Despite the acceptance that CD8 T cells are essential to protect against highly pathogenic respiratory viruses, in part due to their continual genetic re-assortment and replicative capacity, the required CD8 T cell effector functions and mechanisms of protection remain to be fully determined. Type II interferon, IFN-γ, plays a critical role in host anti-viral immunity as demonstrated by the existence of numerous viral encoded IFN-γ decoy receptors and virulence factors that inhibit downstream IFN-γ signaling. However, despite extensive understanding of IFN-γ signaling biology, there remain many questions surrounding the precise role and function of IFN-γ in the context of respiratory viral immunity. Using a highly pathogenic respiratory model of vaccinia virus (VACV) infection, we now demonstrate that IFN-γ produced between day 3 and 6 post infection is critical for survival through limiting VACV replication and dissemination. Furthermore we establish, through the use of T cell transfers, that CD8 T cell derived IFN-γ is both necessary and sufficient in protecting RAG-/- and IFN-γ-/- mice against systemic inflammation and rapid death. Interestingly, by using VACV-GFP+ we also highlight that respiratory epithelial cells, both in vivo and in vitro, are major sites of VACV replication and that direct IFN-γ signaling on respiratory epithelial cells reduces VACV replication and increases survival in mice that only express IFN-γR on

respiratory epithelium. Collectively we provide the first piece of evidence that establishes the critical requirement for CD8 T cell derived IFN-γ signaling on respiratory epithelial cells during a respiratory viral infection. These data identify both a possible therapeutic strategy and important implications for mucosal vaccine design against highly virulent respiratory viruses.

24. CD27 CONTROLS CD8 MEMORY T CELL REACTIVATION AND PERSISTENCE IN THE LUNG.

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Vaccines that elicit robust and long-lived CD8 memory T cell responses are thought necessary to protect against highly virulent mucosal pathogens such as highly pathogenic avian influenza, human immunodeficiency virus and severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV). However, the recent discovery that CD8 memory T cells are heterogeneous in terms of their specificity, anatomical locations, and phenotype has raised several important questions. In particular, do we understanding which memory T cell subsets are critical for protective immunity or whether specific subsets contribute to viral mediated immunopathology upon antigen re-exposure or natural infection. Consequently, it is important to examine whether existing phenotypic markers of differentiation can be used to predict the efficacy and quality of a specific CD8 memory T cell recall response. We have recently discovered that CD27, a co-stimulatory member of the tumor necrosis superfamily, defines distinct subpopulations of CD8 memory T cells generated following different routes of immunization with vaccinia virus (VACV). Therefore, to test the functional consequences of CD27 expression, we isolated memory cells, based on their anatomical location and levels of CD27 expression, and transferred them into naïve mice that were then intranasally infected with VACV. We now show that CD27 expressing CD8

memory T cells demonstrate enhanced proliferative capacity and ability to produce TNF-α and IFN-γ following re-activation. Thus suggesting that CD27 expression can be used as a functional marker to predict not only the capacity of CD8 memory T cells to mediate recall responses, but also the efficacy of the recall response. This will help and encourage further studies to characterize and improve the mucosal immunogenicity of poxvirus vaccine delivery vectors.

25. VISUALIZING T FOLLICULAR HELPER CELL, DENDRITIC CELL, AND B CELL COMMUNICATION WITHIN THE WHITE PULP OF SECONDARY LYMPHOID ORGAN

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OX40 (CD134) is a member of the TNFR family of costimulatory molecules that is highly expressed on T follicular helper cells (Tfh cells), which provide cognate help to germinal center (GC) B cells. Its ligand OX40L (CD252) is induced on activated dendritic cells (DCs) and B cells. We found that OX40/OX40L interactions during an acute virus infection play an indispensable role in T follicular helper cell-dependent anti-viral Ab production and germinal center formation. Here we investigated whether OX40/OX40L interaction simply support activation of naive CD4 T cells during initiation of responses by dendritic cells or if they directly influence Tfh and B cell communication. We found that OX40+ T cells initially formed clusters with OX40L+ DCs along marginal zone bridging channels (MZBC) from the red pulp towards the white pulp side of the spleen. After this time, they appeared in the periarterial lymphatic sheaths (PALS) near MZBC and then in the deeper PALS along the arteries by 5–10 days. OX40L+ B cells also

encounter OX40+ T cells in the PALS, move to the boundary between B cell follicles and T cell zones, and engage in cognate interactions with Tfh cells. Finally, we show that the responding OX40+ Tfh cells and OX40L+ B cells migrate into B cell follicles and form highly specialized structures within germinal centers. Our results reveal insight into the dynamic regulation of anti-viral humoral immunity and identify OX40 and OX40L signaling pathway as critical for both initiation and maintenance of Tfh cell phenotype.

26. PROSPECTIVE STUDY OF AVIAN INFLUENZA VIRUS INFECTIONS AMONG RURAL THAI VILLAGERS

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Background: In 2008, 800 rural Thai adults living in 8 sites within Kamphaeng Phet Province were enrolled in a prospective study of zoonotic influenza transmission. Serological analyses of sera collected at the time of enrollment suggested that this cohort had experienced subclinical avian influenza infections with H9N2

and H5N1 viruses. Unidentified environmental exposures may have played a role in disease transmission. **Methods**: After enrollment, participants were contacted each week for 24 months to monitor for acute influenza-like illnesses (ILI). Household contacts of cohort members confirmed with influenza A infections were enrolled in a family transmission study. Respiratory swabs and paired sera were collected from all ill participants. Cohort members also provided annual sera samples at 12 and 24 months after enrollment to monitor for changes in antibody titers. Serologic assays were performed against 10 avian, 3 swine, and 3 human influenza viruses. Respiratory swabs were examined for molecular evidence of influenza virus. **Results**: Over the 2 years of follow-up, 49 subjects withdrew their participation and 45 replacement enrollments were added; 768 participants (96%) completed the 12-month follow-up and 784 participants (98%) completed the 24-month follow-up visit. Eighty-one ILI investigations were conducted and 80 household contacts were enrolled in the family transmission study with 12 case contacts (14%) developing ILI. Thirty-two (34%) of the 93 reported ILI cases were positive for influenza A virus, although no avian influenza virus (AIV) infections were detected. Serological activity was most prevalent for the avian-like A/Hong Kong/1073/1999(H9N2) virus; 21 subjects at 12-months and 40 subjects at 24-months. Prior receipt of a human influenza vaccine was significantly associated with elevated antibodies against H9N2 for both 12- and 24-month follow-ups and likely explained much of the observed seroreactivity ([OR=11.4, 95% CI, 2.5-41.2] and [OR=5.0; 95% CI, 1.9-12.3], respectively). Only one subject had an elevated antibody titer (1:20) against H5N1 HPAI over the course of follow-up. **Conclusions**: AIV infections were rare among this rural Thai population. Clinical AIV infections were not detected, and subclinical AIV infections were indeterminate due to influenza antibody cross-reactivity. There is a critical need for improved serological diagnostics to more accurately detect subclinical AIV infections in humans.

27. AN INFLUENZA OUTBREAK SIMULATION LEADS TO AN INCREASE IN INTENT AND BEHAVIOR REGARDING INFLUENZA IMMUNIZATIONS OF COLLEGE STUDENTS

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Background: College aged students have lower influenza vaccination rates than most populations. This experiment determined the impact of a flu outbreak simulation on students' intent to be immunized and subsequent actual immunization. **Methods**: A pre-test and post-test measured the intent of 109 students in a college level virology class to receive flu vaccination. The flu outbreak simulation started by providing 16 students with a playing card. The teacher kept an identical deck. The teacher selected one card from their deck and asked the student with the identical card to stand. The student was told he/she had flu and was going to infect 2 classmates (R0=2). This first student then selected 2 cards from the teacher's deck. Students 2 and 3 stood, each selected 2 cards, etc. If a card was pulled for an already infected student (standing), that student was immune and did not pull any cards. The process was continued until all the selected cards were for students already standing. This process was repeated with half of the students immune, to demonstrate the power of immunization (i.e. those with clubs and spades). The study also determined the number of students receiving flu vaccinations at the student health care center before and after the simulation. The number of students immunized was determined by cross referencing the class roster from the virology class and the student health care immunization data. All students were asked to graph the number of students infected/'day' (incubation period = 1 day) and total number/day. The students were then

given a posttest, indicating their intentions to be vaccinated and, if appropriate, reasons why they changed their intentions. **Results**: Of the 109 students, 30 reported having already been immunized. On the pretest, of the 79 unimmunized students, 32 planned to be immunized and 47 did not. On the posttest, 16 of the 47 (34.0%, p=.017) changed their minds and planned to be immunized. The Student Health Care Center reported immunizing 22 of the 144 enrolled students before the intervention and 16 afterwards. **Conclusion**: Demonstrating a flu outbreak simulation to college students resulted in a significant increase in intention to be immunized against influenza. This was associated with students actually being immunized.

28. ANNUAL ECONOMIC IMPACTS OF SEASONAL INFLUENZA ON US COUNTIES: SPATIAL HETEROGENEITY AND PATTERNS

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Economic impacts of seasonal influenza vary across US counties, but little estimation has been conducted at the county level. This research computed annual economic costs of seasonal influenza for 3143 US counties based on Census 2010, identified inherent spatial patterns, and investigated cost-benefits of vaccination strategies. The computing model modified existing methods for national level estimation, and further emphasized spatial variations between counties, in terms of population size, age structure, influenza activity, and income level. Upon such a model, four vaccination strategies that prioritize different types of counties were simulated and their net returns were examined. The results indicate that the annual economic costs of influenza varied from $13.9 thousand to $957.5 million across US counties, with a median of $2.47 million. Prioritizing vaccines to counties with high influenza attack rates produces the lowest influenza

cases and highest net returns. This research fills the current knowledge gap by downscaling the estimation to a county level, and adds spatial variability into studies of influenza economics and interventions. Compared to the national estimates, the presented statistics and maps will offer detailed guidance for local health agencies to fight against influenza.

29. AN ORAL VACCINE AGAINST INFLUENZA GENERATES VIRUS-SPECIFIC T CELL RESPONSE

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Seasonal influenza remains a formidable threat by contributing to the deaths of approximately 50,000 individuals each year in the United States. Influenza infection affects individuals of all ages; however, the elderly and children are particularly vulnerable. Although influenza vaccines and therapeutics are available, they have limited efficacy. Thus, to explore new avenues for an effective vaccine strategy, we combined two fundamental requirements for effective vaccination i) the targeted delivery of antigens to professional antigen presenting dendritic cells (DCs) and ii) utilization of a potent oral adjuvant and delivery vehicle. Here, we have used a 12-mer-peptide sequence to deliver viral antigens to intestinal DCs by the commensal bacterium, Lactobacillus gasseri, which not only acts as a vehicle for antigen delivery, but also acts as an adjuvant. To utilize this methodology for the development of an effective influenza vaccine, we developed a mini-gene cassette to deliver the highly immunogenic epitopes of the most common influenza A viruses (i.e., H1N1 and H3N2) to intestinal DCs in order to elicit robust T cell immune responses against influenza infection. We hypothesized that delivering immunogenic epitopes derived from the viral proteins to intestinal DCs via L. gasseri would result in a virus-specific immune response. The mini gene-DC peptide fusion cassette was cloned into a heterologous vector suitable for

expression in L. gasseri. A group of mice (n=5) were vaccinated for four consecutive weeks, rested for two weeks, and then boosted for another two weeks with L. gasseri harboring an empty vector, L. gasseri expressing mini gene-Flu vaccine fused to a control peptide (FluVac-Ctrl), or L. gasseri expressing mini gene-Flu vaccine fused to the DC-binding peptide (FluVac-DC). One week post-immunization, viral protein [nucleoprotein (NP) and the polymerase subunit, PA] specific CD8+ T lymphocytes were evaluated in the lungs, mesenteric lymph nodes (MLNs), and spleens excised from the mice. Mice that received FluVac-DC vaccine, demonstrated an increased percentage of virus-specific CD3+CD8+ T cells in the MLNs and spleen, when compared to either FluVac-Ctrl or empty vector-treated mice. Additionally, restimulation of CD8+ T cells with DCs loaded with NP and PA peptides produced pro-inflammatory cytokines (i.e., IFNγ, TNFα) and Granzyme B. In conclusion, this vaccine strategy generates a virus-specific T cell response; however, challenge studies are needed to establish its protective role as a vaccine.

30. SPATIO-TEMPORAL MODELING OF WEEKLY ILI PEAK EVENTS WITH CROSSING THEORY

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Annual influenza incidence usually peaks during one or several weeks, and reflects a remarkably high percentage of infections. Current definitions of influenza peak events, however, are incapable of comprehensively representing the patterns of influenza activity. This article proposes a new method of defining and statistically characterizing influenza peak events. These variables include: annual event density (number of such events per flu year), their timing, duration, and magnitude (peak values) over some prescribed threshold levels. On the basis of the Crossing Theory, the peak values of maximum weekly Influenza-Like Illness (ILI) events above various critical thresholds of

interest are analyzed from 5 selected counties in Florida using recent 6-year records of the outpatients Influenza-Like Illness Surveillance Network (ILINet). Data are initially extracted at a low threshold to maximize the available sample size, and the probability distributions fit to variables provide statistically satisfactory results. The characteristics of ILI events exceeding higher, more rarely experienced levels (90th and 95th percentiles) predicted on the basis of observations at the 80th percentile level, are not significantly different from observed in all cases, illustrating the proposed methodology's flexibility and ability to extrapolate to rarely observed levels of interest from more frequently observed levels.

31. MODULATION OF INFLUENZA VIRUS INFECTIVITY AND ACTIVATION OF TOLL-LIKE RECEPTORS BY CARBON NANOMATERIALS

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Recent advancement in manipulating materials at the nanoscale and their current use in industrial, consumer and biomedical products has raised public concern about the biotoxicity associated with environmental exposures to engineered nanoparticles (ENPs). The extraordinary physico-chemical properties that make ENPs promising for novel product enhancement applications, may lead to adverse immune defense

and inflammatory responses in humans. We have a particular interest in inhalation of single-walled carbon nanotubes (SWNT) as they possess a superficially resemblance with asbestos which may be relevant to their long-term health consequences. Moreover, there is a possibility that SWNTs can influence the behavior of other infectious agents thereby increasing susceptibility to pathogenic 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 determine if SWNT modulate IAV infectivity of lung epithelial cells (LECs) and characterize the immune-mediated mechanisms controlling this response. In addition, we are also testing whether the chirality of SWNTs differentially alters the immune response to IAV. We are concentrating on toll-like receptors (TLRs) as they are an early line of defense against foreign invaders in human body. Our hypothesis is that SWNT enhance IAV infectivity through modulation of TLRs resulting in the production of pro-inflammatory cytokines through activation of transcription factors NF-κβ. To begin to address this hypothesis, we exposed LECs to non-cytotoxic doses (50ug/ml) SG65 SWNT (6,5 chirality) for 24 hours followed by co-incubation with H1N1 IAV. Cells were fixed and stained with an H1N1-specific antibody to detect infected cells. Using microscopy we observed that LEC pre-exposed to SWNTs had significantly higher levels (~10%) of IAV infection which was confirmed with a viral titer assay. In efforts to define a role for TLRs, we exposed TLR2 overexpressing cells to SG65 SWNT and show a dose dependent activation of the downstream transcription factor NF-κβ. Future studies are underway to test activation of alternate TLRs and IAV infectivity by SWNT with distinct chiralities (SG76, and CG200). Overall these studies are the first to report modulation of IAV behavior by SWNT and activation of TLRs. The present research lays a foundation for comprehending the impact of ENP on pathogen susceptibility and provides data imperative for evaluating the safety of ENP.

32. PROTECTING AMERICAN PINE FORESTS: AN ASSESSMENT OF POTENTIAL BEETLE-FUNGUS PATHOGENS.

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Exotic (non-native) beetle-fungus symbioses drive tree mortality in the United States. Exotic wood borers cause $2.5 billion in damages in the U.S. annually, often as a result of the pathogenic fungi the beetles carry. For example, the redbay ambrosia beetle carries a pathogenic fungus that is eradicating American Lauraceae and threatening the Florida avocado industry. The increasing number of exotic wood borers becoming established in the US makes it difficult for regulatory agencies to make decisions about which species are dangerous. This has led to a "wait-and-see" approach, which makes it nearly impossible to eradicate invasives once they have become established. To determine if harmful invasions can be effectively predicted and prevented, symbiotic fungi of beetles in Asia will be evaluated for pathogenicity to American trees. Beetles will be collected in China and Thailand, where their symbiotic fungi will be isolated from the beetle. The fungal isolates, detached from their beetle vector, will be sent to quarantined facilities at the Emerging Pathogens Institute, at the University of Florida. Literature suggests these fungi could be pathogenic to pines (Pinus) from the southeast US. To determine pathogenicity, the most economically and ecologically important pines in the southeast (loblolly, slash, longleaf) will be inoculated with the Asian fungi. This project seeks to demonstrate the feasibility of assessing invasive pathogen potential and offers a route for regulatory agencies to effectively protect one of Florida's most valuable commodities: pines.

33. CHARACTERIZATION OF THE CITRUS BLACK SPOT PATHOGEN AND ITS POTENTIAL SPREAD IN THE US

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Citrus black spot (CBS) was recently introduced into southern Florida. The causal agent Guignardia citricarpa Kiely was isolated from infected fruits. This pathogen is a quarantine organism in Europe and the rest of the USA, and shipment of citrus fruits from infested areas is prohibited. It is therefore important to estimate the potential spread of CBS to other citrus production areas in the US. The DNA sequences of the ITS region of ten isolates of G. citricarpa from south Florida were compared with those of Guignardia species worldwide and growth rates of several isolates were determined at different temperatures. Effects of temperature and relative humidity on postharvest lesion formation on orange fruits were also determined. The climatic requirements of G. citricarpa were then used to estimate parameter values in the CLIMEX model to predict potential establishment of CBS in North America. Comparison of the ITS region of the isolates to those in international databases confirmed the identity and uniformity of G. citricarpa. Colony growth and conidia production in vitro were optimal at 27°C, while there was no growth below 4°C and above 37°C. On fruits, lesion growth and conidia production were observed at 4°C, though at a low rate, indicating a greater versatility of the fungus on fruits. Variations in humidity had little effect. Input parameters for CBS risk in CLIMEX reflecting conditions for infection in

spring/summer in Florida, predicted potential establishment in Florida but not in California. Altering the parameter values to account for survival of the pathogen in leaf litter in winter predicted potential establishment in California as well as Florida. Thus, G. citricarpa could possibly establish beyond Florida if this organism is transported outside of the current quarantine zone to other citrus production areas.

34. ASSESSMENT OF BIOLOGICAL ACTIVITY OF CAESALPINIA FERREA MARTIUS EXTRACTS (FABALES CAESALPINIACEAE) AGAINST LEISHMANIA (VIANNIA) GUYANENSIS (KINETOPLASTIDA: TRYPANOSOMATIDAE)

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The search for leishmanicids agents with fewer side effects is one of the greatest challenges in modern research. The species Caesalpinia ferrea Martius it is popularly used for many therapeutic purposes, including the treatment of various infections like those caused by leishmaniasis, making it a promising tests of biological activity against leishmania. The objective of this study was to evaluate by bioassays the activity of hexane and methanol extracts against protozoa. The fruits of C. ferrea were collected and processed in the INPA institute. The studies were performed with the whole fruit and its separate parts, coconut shell and seed. Hexane e methanol crude extracts were prepared from the fruits, coconut shell and seeds, which were tested to evaluate the biological activity in vitro against promastigotes of the stationary phase of the protozoan Leishmania (Viannia) guyanensis. The extracts were analyzed by thin layer chromatography Comparison (CCDC) and Nuclear Magnetic Resonance (NMR) to determine the chromatographic profile. All biological assays were carried out experimentally in vitro in microplates containing 106/mL promastigotes to axenic

cultures of L. (V.) guyanensis evaluating the inhibitory activity and stimulating parasite growth. The most active crude extract was submitted to CCDC and fractionation by partition líquid-líquid resulting in semi-purified fractions that were evaluated for their chemical composition. The results showed that the epicarp methanolic extract of C. ferrea, showed anti-leishmanial activity for L. (V.) guyanensis in the concentration of 20 mg/mL/24h. However, contrary to expectations, there was a stimulatory effect on L. (V.) guyanensis by the hexanic extract obtained from the fruit seed of C. ferrea. Chemically analyzing the coconut shell and fruits methanolics extracts of, which showed anti-leishmanial activity, we verified the presence of substances such as chlorophyll, flavonoids, xanthones, tannins, triterpenes and saponins as major constituents, and shows antioxidant activity. In conclusion, the methanolics extracts of coconut shell and fruitsof C. ferrea can be considered as a promising alternative in the treatment of cutaneous leishmaniasis and should be undertaken pharmacological and toxicological tests in vivo of extract, making other fractioning in order to isolate the substances responsible for the anti-leishmanial activity.

35. TEMPERATE AND TROPICAL PHYTOPHTHORA IN NORTH-CENTRAL FLORIDA FORESTS

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Phytophthora species include notorious and costly diseases of agricultural crops and forest trees, including potato late blight and sudden oak death. Most of the research on Phytophthora has focused on agricultural and exotic pathogens. Very little is understood about the native biology or ecology of this group. Recent environmental sampling in temperate forest streams has uncovered the true diversity of Phytophthora in natural ecosystems. We have begun sampling Florida natural areas to reveal the diversity of Phytophthora in Florida's varied

subtropical ecosystems, establish a baseline community to enable future detection of new exotic pathogens, and lay the groundwork for research on the ecological roles of these pathogens in their native environments. In preliminary sampling of North-Central Florida forests, we have found both temperate and tropical Phytophthora species, including known pathogens and at least 2 new species.

36. New Anthracnose Disease of Lucky Bamboo (Dracaena sanderiana) Caused by Two New Colletotrichum Species

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International trade in ornamental plants has increased worldwide over the last decade. Dracaena is the genus most frequently imported into the U.S. There are several diseases that are common on Dracaena species in the U.S. and Florida, however there are many pests and pathogens currently not in the U.S. that could potentially be imported with Dracaena plant materials. A recent example of an imported organism associated with lucky bamboo (Dracaena sanderiana) involves the plant pathogen causing anthracnose. In 2009, a diseased lucky bamboo was collected from a grocery store in Florida. The causal agent was identified as a Colletotrichum sp. that differed from Colletotrichum spp. that had previously been reported in Florida.

The disease was found to be anthracnose caused by Colletotrichum dracaenophilum, a fungus that originated from Asia. C. dracaenophilum is now considered by USDA-APHIS to be a reportable pathogen of lucky bamboo. In February and March 2012, imported lucky bamboo plants were observed in two retail stores in Gainesville, Florida, with typical anthracnose symptoms. A Colletotrichum fungus was isolated from symptomatic lucky bamboo from each store and a pathogenicity study involving Koch's postulates were performed. The two Colletotrichum strains differed in aggressiveness on lucky bamboo. PCR was performed on the ITS and 28S rDNA regions of the original isolates and the re-isolated strains and the sequences were compared with sequences of Colletotrichum spp. in GenBank. Sequence analysis indicated that the Colletotrichum isolates were a different species than the Colletotrichum dracaenophilum isolated in 2009. We are currently in the process of conducting phylogenetic studies on the new Colletotrichum isolates.

37. ENVIRONMENTAL SAMPLING REVEALS A NEW GENETIC CLADE OF THE PYTHIOSIS PATHOGEN IN FLORIDA

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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, dogs, cattle and other animals 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. There are

currently 3 major genetic clades within Pythium insidiosum. Cluster I is composed of isolates from North, Central, and South America. Cluster II contains isolates from Australia, the Americas and Southeast Asia. Cluster III contains isolates from Asia and the Americas. While clusters I and II are genetically close, it has been proposed that cluster III may represent a new species or subspecies. We sampled 13 lakes and ponds in North Central Florida for P. insidiosum and found apparently large endemic populations in multiple lakes. Three clades of P. insidiosum are present in Florida: Cluster I, Cluster III, and a 4th clade previously represented by a single isolate from a captive bear in South Carolina. Some lakes have only one cluster type present while other lakes have two. Future directions of this research are to: (1) define the environmental factors favor P. insidiosum growth, (2) describe the geographic distribution of the different P. insidiosum clades, and (3) determine which clades are responsible for pythiosis in domestic animals in Florida.

38. GENOME SIZE ESTIMATION OF AN EMERGING PLANT PATHOGEN PHYTOPHTHORA ANDINA BY FLOW CYTOMETRY

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Phytophthora andina Adler & Flier, sp. nov. , a newly heterothallic plant pathogen of Andeans solanaceous hosts including Solanum betaceum, S. muricatum, S. quitoense, and several wild Solanum spp. has been identified recently. P. andina emerged via the hybridization of the Irish Potato Famine pathogen, Phytophthora infestans and an unknown Phytophthora species also belonging to Phytophthora clade 1c by cloning four nuclear loci to obtain haplotypes. However, as the P. andina has only recently been identified, there was no report about the nuclear genome size of P. andina, which may impede the understanding of the basic genetic mechanisms in this pathogen. Flow cytometry has been

applied widely in the genome size estimations of many eukaryotes with the advantages of fast preparation, convenient observation and reliable analysis. In this study, a total of thirteen isolates of P. andina, together with one external standard reference P.infestans isolates T30-4 with a known genome size (~240Mb) determined by genome sequencing were characterized by the flow cytometry. The results indicated that the genome sizes of P. andina were, in general, various and smaller but very close to the genome size of the reference isolate of P. infestans T30-4. This preliminary estimation of genome size in P. andina suggests the feasibility of genome sequencing of P. andina and has provided a solid foundation for the further study pertaining to the genetic mechanism behind hybridization in this emerging plant pathogen.

39. PROTEIN BINDING OF RIFAPENTINE AND ITS METABOLITE, 25-DESACETYL RIFAPENTINE, IN PATIENTS WITH PULMONARY TUBERCULOSIS

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Background: For highly bound drugs, changes in protein binding may result in significant changes to the free concentration of the drug, and potentially, changes to the pharmacological activity of the drug. To date, the free concentrations of rifapentine and 25-desacetyl rifapentine have been determined in vitro, in healthy volunteers, and in patients with hepatic disease. In healthy

volunteers, rifapentine is reported to be approximately 97% protein bound while its partially active metabolite, 25-desacetyl rifapentine, is reported to be approximately 93% protein bound, both primarily to albumin. We investigated rifapentine's protein binding in the plasma of patients with tuberculosis. **Methods**: Ninety-one samples were tested from 43 subjects (21 African, 22 Non-African) enrolled in the CDC Tuberculosis Trials Consortium (TBTC) Study 29. Free drug concentrations were determined by ultrafiltration of plasma samples followed by high performance liquid chromatography with tandem mass spectroscopy (LC/MS/MS). **Results**: The median protein binding for rifapentine in this study was 99.2% for all samples (range, 96.26 to 99.82%) and 97.2% for 25-desacetyl rifapentine for all samples (range, 87.03 to 99.71%). Rifapentine and 25-desacetyl rifapentine samples from African subjects showed a lower median protein binding percentage than samples from non-African subjects (rifapentine 98.8% vs. 99.4%, p-value≤0.05, and metabolite 93.9% vs. 97.7%, p-value≤0.05) respectively. The median Cmax rifapentine free concentrations of 0.14 mcg/ml for Africans and 0.10 mcg/ml for non-Africans are less than three times greater than the typical MICs associated with Mycobacterium tuberculosis, and support the further investigation into the optimal dosing of rifapentine. In analyses of multiple variables by ANOVA, between assay variation significantly affected free rifapentine concentrations. **Conclusion**: The protein binding results in this study are towards the higher end of the range previously reported. African subjects showed lower protein binding (and thus higher free concentrations) than non-African subjects. The reason for this difference currently is not known, and requires further investigation.

40. COUGH AEROSOLS: A NEW PARADIGM TO ASSESS INFECTIOUSNESS IN TUBERCULOSIS

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Introduction: Tuberculosis (TB) is transmitted by the airborne route, i.e., by fine aerosols containing Mycobacterium tuberculosis. Although TB is not transmitted by sputum, the current laboratory marker of infectiousness is microscopy of the sputum smear, which is associated with a large variability of transmission. We recently demonstrated that a novel method of collecting infectious aerosols from TB patients was feasible in Uganda, a resource-limited country with a high burden of TB. **Hypothesis**: Cough aerosol cultures of M. tuberculosis from newly diagnosed TB patients are highly associated with markers of recent transmission among household contacts. **Methods**: Cough aerosols from TB patients (TB) were cultivated. We assessed household contacts for TB infection using conversion of the tuberculin skin test as the primary outcome measure. We used multivariable logistic regression analysis with cluster adjustment to analyze predictors of new infection. **Results**: We enrolled 96 sputum culture-confirmed index TB cases and their 442 household contacts in Kampala, Uganda. Only 43 (45%) yielded M. tuberculosis in aerosols. Contacts of TB patients who produced high aerosols (≥10 colony forming units - CFU) were more likely to have a new infection compared to contacts from low aerosol (1-9 CFU) and aerosol negative cases (69%,25% and 30%, respectively) (P=0.009). A high aerosol TB patient was the only predictor of new M. tuberculosis infection in both unadjusted (odds ratio 5.18, 95% confidence interval 1.52-17.61) and adjusted analyses (OR 4.81, 1.20-19.23). **Conclusions**:

Cough aerosol cultures of M. tuberculosis are highly associated with recent transmission, even though they are produced by a minority of TB patients. Cough aerosols may help identify the most infectious TB patients and thus improve the cost effectiveness of TB control interventions, including active case finding and treatment of latent TB infection. They may also help reduce exposure assessment errors in studies of diagnostics, vaccines and drugs.

41. HIP1, A NOVEL SERINE PROTEASE DRUG TARGET FOR TUBERCULOSIS

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Hip1, (Rv2224c), hydrolase important for pathogenesis 1, is a cell-envelope hydrolase that is a virulence factor for Mycobacterium tuberculosis (Mtb). Disruption of Hip1 (Rv2224c::tn) results in compromised intracellular survival of Mtb in macrophages and modulation of pro-inflammatory responses. Mice infected with the Rv2224c::tn mutant Mtb survive longer and have reduced immunopathology compared to mice infected with wild-type Mtb. Although it was reported that Hip1 is not a protease, here we report compelling biochemical evidence indicating that Hip1 is a serine protease and also exhibits esterase activity. We have discovered 4 tripeptide substrates and 1 octapeptide substrate for Hip1. The chromogenic tripeptide substrates APA-pNa, GPL-pNa, APAR-pNa, and Ac-APAR-pNa (pNa = p-nitroanilide) were tested because they are substrates for the tripeptidyl-peptidases A, B, and C, which are members of the serine protease clan SC of which Hip1 appears to also be a member. These substrates were utilized for inhibitor profiling of Hip1. Only the serine protease specific

inhibitors PMSF and AEBSF (93% and 99% inhibition, respectively) inhibited Hip1 substrate cleavage indicating Hip1 is a serine protease. Previous data indicated that Hip1 activity is required for cleavage of the GroEL2 protein in Mtb. To test this, we had a 19 amino acid peptide synthesized and tested for Hip1 hydrolysis. LC/MS data indicates that Hip1 cleaves the synthetic peptide at two predominate cleavage sites, and this cleavage is inhibited completely by PMSF. From this information, we designed a FRET peptide substrate for screening small molecule libraries for Hip1 inhibitors. This octapeptide substrate is hydrolyzed by Hip1 (Km = 18 μM). In summary, we have determined that Hip1 is a novel serine protease, and we have discovered chromogenic and fluorescent substrates useful for screening libraries for Hip1 inhibitors. Additionally, we have gained a glimpse of the subsite specificity of Hip1 from the substrate and inhibitor studies. This information will enable our tuberculosis drug discovery efforts with the Hip1 target to continue through Hit Discovery and Lead Optimization with the ultimate goal of structure-aided drug design.

42. SUSCEPTIBILITY OF FLORIDA AEDES AEGYPTI AND AEDES ALBOPICTUS TO DENGUE VIRUSES FROM PUERTO RICO

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Dengue viruses are responsible for an estimated 50-100 million cases of dengue fever every year. Historically, locally acquired

dengue cases in the continental United States have been rare. However, outbreaks of dengue during 2009-2010 in Florida suggest vulnerability to transmission in areas where vectors Aedes aegypti and Aedes albopictus occur. Puerto Rico is an unincorporated territory of the U.S. where dengue is endemic, multiple serotypes of dengue co-circulate, and thousands of dengue cases occur every year. Also, travel between Puerto Rico and the U.S. mainland is common which may pose as a risk for traveler-imported dengue cases. Mosquitoes were collected in Florida and used to evaluate their susceptibly to dengue viruses from Puerto Rico (serotypes 1-3). Aedes aegypti and A. albopictus were fed blood infected with dengue 1, 2, or 3 virus and assayed for virus infection and dissemination following 7 and 14 days of incubation. We show that both Florida Aedes spp. were susceptible to virus infection and dissemination with dengue-1 and 2, but not 3. Aedes aegypti was significantly more susceptible to infection for dengue-2 (more than 67%) and dissemination for dengue-1 than A. albopictus (more than 82%), respectively. This study indicates that Florida would be vulnerable to transmission of dengue virus serotypes from Puerto Rico and that A. aegypti is potentially more competent than A. albopictus.

43. VERTICAL TRANSMISSION OF KEY WEST DENGUE-1 VIRUS BY AEDES AEGYPTI AND AEDES ALBOPICTUS MOSQUITOES FROM FLORIDA

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Following dengue outbreaks in Key West in 2009 and 2010, we used Florida Aedes aegypti and Aedes albopictus mosquitoes and dengue-1 virus (DENV-1) isolated from Key West to examine if

vertical transmission of DENV-1 from infected female mosquitoes to their eggs could have served as an inter-epidemic source of dengue virus between 2009 and 2010 outbreaks. Additionally, we measured infection and dissemination rates and determined the effect of DENV-1 infection on mosquito fecundity. Vertical transmission of DENV-1 was documented, with rates of 11.11% (2/18) for A. albopictus and 8.33% (3/36) for A. aegypti. Approximately 94% (113/121) of A. aegypti that fed on DENV-1 in blood meals became infected, and of those, 80% (90/113) had disseminated infections. Similarly, 93% of A. albopictus became infected (53/57), and of those, 85% (45/53) had disseminated infections. Dengue infection did not significantly affect numbers of eggs laid by either species, suggesting little cost of infection on fecundity. Our results demonstrate that Florida A. aegypti and A. albopictus mosquitoes are competent vectors for DENV-1, whose maintenance may be facilitated by vertical transmission to eggs.

44. TICK-BORNE DISEASE RISK AND PREVENTION: A TOURISM SUPPLY-SIDE PERSPECTIVE

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Tick-borne diseases are on the rise and their spatial reach is growing. From a tourism supply perspective, tick-borne disease has the capacity to immobilize the outdoor workers upon which the industry depends to guide nature tours, landscape and maintain parks and public spaces, and manage wildlife for example. Interventions such as tick control and public health campaigns can reduce tick populations and increase tick-borne disease knowledge respectively. Despite an evolving natural science perspective on the relationships between pathogens, ticks, hosts, and the environment, the human ecology of tick-borne disease is not well understood. A review of the scientific evidence confirms that exposure is the greatest risk factor for

infection while knowledge and prevention play an important role in risk mitigation. A conceptual model developed on the basis of this review is proposed to guide future research related to tick-borne disease and human health risk assessment. Occupational risk training programs are recommended to prevent tick bites and tick-borne disease infection for tourism managers and specifically those individuals who are at the highest risk: outdoor workers.

45. EVALUATION OF PLASMODIUM FALCIPARUM MULTI-DRUG RESISTANCE -1 GENOTYPES IN HAITI

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Background: Resistance to antimalarial drugs is a major threat to the management and elimination prospects of malaria in endemic countries [1, 2]. In Haiti where Chloroquine is widely used for malaria treatment, reports of clinical resistance to CQ are scarceabsent despite a long history of its use. However, presences of resistant haplotypes have been reported [3]. In this study, we examined Pfmdr1 gene mutations that have been found to closely correlate with CQ resistance [4](ref). **Methods**: We amplified the pfmdr-1 genes at five codons namely 86, 184, 1034, 1042 and 1246 using a nested PCR protocol. Products of amplification were then subjected to site-specific restriction enzyme digest and the enzymatic digestion products visualized by electrophoresis on 1-3% agarose gels correlated to a polymorphic site known to confer resistance to CQ. **Results**: Of the 356 samples obtained and confirmed positive for P. falciparum by microscopy, only 160

samples were able to amplify for the presence of P. falciparum small ribosomal subunit RNA gene. The codons at 86, 1034, 1042 and 1246 were all wild type with no mutations. However, at codon 184, all the samples that amplified at this position had a mutation (Y184F) confirmed by both restriction enzyme digestion and nested sequencing. Thirty eight samples amplified well at all the four codons (86, 184, 1034 and 1042) indicating the widespread presence of the NFSN haplotype in Haiti. **Conclusion**: The study has found the widespread presence of Y184F mutation in P. falciparum parasites in Haiti. This mutation is thought to confer resistance to other antimalarial medications. Therefore, surveillance of changes in prevalence of SNPs in the Pfmdr-1 gene is important and may serve as an early warning for the emergence of P. falciparum resistance to CQ and other antimalarial in Haiti.

46. EARLY DETECTION SAMPLING IN MULTIHOST DISEASES

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The effectiveness of disease control measures depend on when an outbreak is detected. Early detection can significantly reduce the costs associated with disease eradication and reduce financial losses when a disease affects livestock or a cash crop. Here we examine the optimal sampling design in the early stages of a multihost disease. We use how sampling effort is constrained by the cost of sampling vectors and hosts to determine the optimal allocation of sampling effort.

47. INTERNAL MIGRATION AND THE MOVEMENTS OF MALARIA IN AFRICA

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Malaria elimination is currently a key focus in the global public health agenda, with over 30 countries having stated elimination as their goal. The feasibility of elimination within any given country depends on a number of factors, including human population movements within the country and how those movements contribute the circulation of malaria parasites. Until recently, data appropriate for measuring these movements, such as migration, have been lacking or poor; recent studies have shown the utility of gravity type spatial interaction models to fill this gap. By combining both movement data and models with malaria prevalence data, the internal communities that arise from malarious movements can be mapped. Here we present results for these community maps of malaria movements for 11 sub-Saharan countries in Africa along with initial assessments of their accuracy.

48. DELAYED MORTALITY AMONG CASES OF EASTERN EQUINE ENCEPHALITIS AND WEST NILE VIRUS ILLNESS IN FLORIDA, 1999-2011

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West Nile virus (WNV) illness and Eastern equine encephalitis (EEE) associated mortality is believed to occur mostly during

acute or early convalescent phases of illness. Florida epidemiologic and vital statistics data collected from January 1, 1999 to December 31, 2011 were analyzed to determine if delayed mortality (more than 30 days after onset of illness) occurred. All nine EEE deaths were attributed to the arbovirus infection resulting in an overall mortality rate of 45%. Median interval from onset to death for EEE related deaths was 10 days (range 3-373 days), with two of nine deaths occurring one year or more after onset. The median age was 40 years of age (range 1-78 years). Eleven of 41 WNV illness deaths were attributed to WNV infection resulting in a 5% overall mortality rate. Median interval from onset to death for WNV infection attributed deaths was 24 days (range 4 - 455 days) with delayed mortality occurring in 3 cases. The median age was 68 years of age (range 40-89 years). Results of this analysis suggest delayed mortality due to EEE and WNV illness does occur and that the associated mortality rates in Florida are underestimated.

49. AGONISTS OF MUSCLE GLUTAMATE RECEPTOR INDUCE PARALYSIS IN AEDES AEGYPTI LARVAE BY BLOCKING SYNAPTIC TRANSMISSION AND DEPOLARIZING THE MEMBRANE POTENTIAL

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Our continued efforts toward designing an effective insecticide against mosquito vectors has led us to study how excitatory chemical agents cause paralysis by affecting muscle physiology. Glutamic acid is the primary neurotransmitter capable of activating and desensitizing glutamate receptors in insect muscle. It works via the depolarizing subtype of glutamate receptor (GluRd), coupled to an intrinsic cation channel. Activation of the receptor underlies generation of the excitatory junction potential and triggers contraction, while the latter a non-conducting, persistent ligand-bound state of the channel. Prolonged

activation, as well as desensitization of muscle excitatory receptors, could underlie paralysis. We have developed a method to quantify the effects of glutamic acid and a batch of known receptor agonists on 4th instar larvae of the yellow fever mosquito Aedes aegypti and found that when incubated in mosquito saline for five hours with glutamic acid agonists, the larvae were 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. Paralytic potency of the compounds varied from 7 ppm for L-aspartic acid to 1360 ppm for D-glutamic acid. Piracetam, a nootropic drug that modulates Na+ flux at AMPA-type glutamate receptors in humans, yielded a PC50 (concentration paralyzing 50% of the larvae) of 8 ppm. Interestingly, we sometimes 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. To understand the cellular mechanisms of paralysis, we performed intramuscular recordings of responses to glutamate agonist treatment. To achieve this, we dissected 4th instar Aedes egypti larvae in mosquito saline and treated them with various concentrations of GluRd agonists. Effects of the compounds on the muscle were recorded while an electrical stimulus was applied to the ganglia. We measured the changes in membrane potential (depolarization or hyperpolarization) and excitatory postsynaptic potential (EPSP). At 1 mM concentration, L-Glutamic acid, L-Aspartic acid, Cyclothiazide, Kainic acid and NMDA depolarized membrane potential by at least 50%. At the same concentration, L-Glutamic acid, L-Aspartic acid, Cyclothiazide, Kainic acid, NMDA, L-BMAA, Piracetam, and AMPA inhibited the EPSP by at least 50%. Among the most potent inhibitor is L-Glutamic acid, which blocked the EPSP and depolarized membrane potential by 100%. We aim to further these findings by testing other compounds that may enhance

GluRd agonists for controlling mosquito vectors Aedes egypti, and Anapheles gambiae.

50. UNEXPECTED ROLE FOR RAD51 IN BABESIA BOVIS ANTIGENIC VARIATION

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Babesia bovis is an intraerythrocytic protozoan parasite of cattle that causes pathology sharing many parallels with falciparum malaria. B. bovis undergoes antigenic variation of at least one virulence factor, VESA1, using a segmental gene conversion-type mechanism. Rad51 is a canonical DNA repair protein that plays a major role in gene conversion-mediated repair in most organisms. To determine whether Rad51 similarly plays a key role in the segmental gene conversion process associated with antigenic variation in B. bovis, we created a Rad51 knockout clonal line, rad51ko1, using a double crossover gene replacement strategy. The success of gene replacement was confirmed by PCR and Southern blotting, and the lack of rad51 transcripts by RT-PCR. Preliminary experiments with B. bovis rad51ko1 revealed several interesting results. First, no apparent defects in parasite growth rate or morphology were observed following recovery from transformation with the plasmid used for targeted gene disruption, despite the significance of Rad51 to DNA replication and repair. Perhaps more significantly, there was essentially no difference in the survival rate of B. bovis rad51ko1 compared with the CE11 parental line after exposure to DNA-damaging γ-irradiation. Unexpectedly, the Rad51 mutants appear to have compromised control over monoallelic transcription from the ves multigene family, which encodes for the VESA1 protein. Finally, as anticipated, transcripts encoding the VESA1 antigens revealed a lack of segmental gene conversion in the B. bovis rad51ko1 cell

line. This work was supported by NIH grant RO1 AI055864 and funds from the University of Florida.

51. MOVEMENT NETWORKS OF POPULATIONS AND MALARIA IN EAST AFRICA: IDENTIFYING THE KEY POPULATION GROUPS

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Population movement plays an important role in the transmission and importation of malaria. Parasite-carrying individuals moving between high and low transmission zones risk infection movement that may instigate local transmission and burden health systems. Different demographic and socioeconomic sub-population groups have different movement patterns and infection rates, and therefore different importation likelihoods. Identifying high risk sub-population groups, by quantifying and comparing their movement patterns, would enable intervention limited resources to be efficiently targeted. National household surveys and population censuses provide individual-level migration data, shown to be a useful proxy for inferring movement at finer temporal scales. Together with spatially referenced malaria data, mathematical models and network analysis tools, Kenyan, Ugandan and Tanzanian migration data was analyzed to construct sub-population movement networks. Origin and destination administrative units represented nodes in each country-specific sub-population movement network. Directional population and malaria movement flows between regions represented weighted network edges. Malaria movement flows were estimated using population movement flows weighted with age-specific malaria endemicity data, using a weighting index. Movement networks were analyzed and compared using local and global network centrality measures and community

detection methods for weighted directed networks. Network analysis results showed that population and malaria movements were different between regions and sub-population groups. For example, network density showed that movement networks for young adults were more dense than networks for children and elderly populations in all three countries. Node degree showed that urban centers had more connections than rural districts. Community detection analysis showed that districts closer together had more flows between them than districts far apart. Some movement networks also showed similar characteristics. For example, children under 10 years and adults between 15-24 years in Kenya had overlapping cumulative degree distributions, implying that children were likely to move with their parents. Malaria-weighted flows showed that groups, such as young adults who had low individual importation risks, had similar overall likelihoods of transporting infections as groups, such as young children who had higher individual importation risks, due to the significantly larger magnitude of adult movement flows. Census and survey data that include migration and demographic data, together with spatially referenced malaria data, GIS and network analysis tools, can be useful for nationwide population and malaria movement assessments, particularly for making comparisons between sub-population groups. Sub-population stratified malaria importation estimates provide a unique evidence base to inform control policy targeted to population groups likely to import infections.

52. JAPANESE ENCEPHALITIS VIRUS IN JAPAN: INSIGHTS FROM A DYNAMIC TRANSMISSION MODEL

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Japanese Encephalitis Virus (JEV) is a flavivirus endemic to South and Southeast Asia. The most commonly identified cause of encephalitis within vulnerable populations in these regions, JEV is an important public health concern because most clinical cases lead to death or long-term disability. JEV is transmitted through a complex enzootic cycle between Culex mosquitoes, Ardeid birds, and swine, with humans as dead-end hosts. We developed a dynamic model that reflects the basic transmission ecology of the virus in rural Japan, where the virus was intensively studied in the 1950's and 1960's. We demonstrate that in areas of intensive pig farming, a simplified version of the model including the course of infection in domestic pigs and mosquitoes as well as host and vector demography, is sufficient to reproduce the 'cyclic' patterns of swine seroprevalence and human risk described in a classic study of a JEV outbreak in Myagi Prefecture, Japan in 1964.We also explore several alternative models of mosquito population dynamics that are capable of reproducing empirical patterns of mosquito abundance and the effects of the resulting alternative demographic compositions of mosquito populations under these models. We then explore the extent to which swine and avian hosts contribute to invasion of novel areas and viral persistence in a variety of transmission contexts, which has implications for understanding the contrasting ecology of the virus in epidemic versus endemic transmission settings and for the viability of potential interventions in different transmission landscapes.

53. ANTHRAX & PLAGUE DIAGNOSTIC IDENTIFICATION, & ANTIBIOTIC SUSCEPTIBILITY TESTING USING BIOLUMINESCENT REPORTER PHAGE

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Yersinia pestis and Bacillus anthracis are Category A bacterial pathogens that are the etiological agents of the plague and anthrax, respectively. Both diseases' have a rapid clinical course, high mortality rate, and produce clinical symptoms shared by many common diseases. Therefore, prompt culture identification and administration of appropriate antibiotics are vital for a positive prognosis. Bioluminescent reporter phages hold promise for the rapid detection and antibiotic susceptibility testing of B. anthracis and Y. pestis. Recombinant "light-tagged" reporter phages were generated by integrating the bacterial luxAB reporter genes into non-essential regions of the phage genomes. The resulting reporter phages were able to confer a bioluminescent phenotype to Y. pestis or B. anthracis within 15-20 min of infection using cultured cells. The sensitivity limits of detection for Y. pestis and B. anthracis were ~102 CFU/mL from spiked human blood. Non-anthracis Bacillus species and non-pestis Yersinia species did not generate a signal, or produced significantly reduced responses upon incubation with the reporter phage. The reporter phages were unable to produce a bioluminescent signal in the presence of inhibitory antibiotic concentrations. In addition, the light response in the presence of varying antibiotic concentrations produced profiles within minutes that were comparable to the standard CLSI method for determining antibiotic susceptibility. The bioluminescent reporter phages display promise for the specific detection and antimicrobial susceptibility testing of Y. pestis or B. anthracis from clinical specimens, cultivated isolates, or environmental samples.

54. EFFECT OF PAST OVIPOSITION EXPERIENCE ON FUTURE OVIPOSITION SITE SELECTIVITY IN THE MOSQUITO *AEDES AEGYPTI*

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Mosquitoes exhibit specific preferences when searching for a suitable aquatic habitat for oviposition (egglaying). Taking advantage of these preferences, oviposition repellents and attractants have been developed and employed to reduce populations of medically-significant mosquito species by drawing mosquitoes into traps or keeping mosquitoes away from humans without neccesarily affecting fitness. These controls can help reduce local burden of mosquito-borne disease, as most mosquito-borne disease transmission occurs near aquatic habitats where eggs are laid. Previous studies have suggested that preferences for egglaying sites and egglaying behavior may be highly plastic, however, changing based on an individual's previous exposure to repellents and attractants, which may influence how these chemicals affect oviposition patterns. In this study, we examine a previously unexplored avenue of adaptation in oviposition behavior to environmental experience, by testing whether past experience with aquatic habitats influences future choosiness. Mosquitoes do not necessarily have any knowledge of the aquatic habitats that are available in an environment. As a result, if only repellent aquatic habitats are encountered, it becomes increasingly likely that most aquatic habitats in the area are repellent. In these environments, it becomes advantageous for mosquitoes to become less choosy, and lay eggs in typically repellent waters. To test whether this mode of behavioral adaptation occurs, we compared the choosiness of mosquitoes that have a previous experience with an attractive aquatic habitat, a repellent aquatic habitat, and no aquatic habitats whatsoever. We then presented these mosquitoes with a repellent aquatic habitat in a wind tunnel, and recorded the proportion of eggs that

were laid in the repellent waters. Mosquitoes that had a previous experience with repellent waters laid a significantly higher proportion of eggs in the repellent aquatic habitat (23.7%) than mosquitoes with an experience with attractive aquatic habitats (6.8%) or mosquitoes with no previous experience (13.7%). These results suggest that mosquitoes may be informing future oviposition decisions with past experiences with aquatic habitats, suggesting a potential novel mechanism of learning employed by mosquitoes. If mosquitoes are learning via this mechanism, then large-scale control measures that significantly alter the environment (such as large-scale chemical treatment campaigns) may result in unexpected changes in mosquito oviposition behavior, such as a willingness to lay eggs in waters treated with repellents.

55. PREVALENCE OF PANOLA MOUNTAIN EHRLICHIA (PME) IN FLORIDA AND EVIDENCE OF CROSS-REACTIVE ANTIBODIES AMONG RELATED EHRLICHIAE

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Panola Mountain Ehrlichia (PME) is an emerging Ehrlichia sp. reported in 10 US states. Based on sequence homology of all known PME genes, the organism is most closely related to Ehrlichia ruminantium, the causative agent of heartwater disease. Because of the proximity of E. ruminantium to the US (the Caribbean Islands), PME could play a significant role in disease monitoring and control in the event of an outbreak of Heartwater. Over 1,100 host-seeking, Amblyomma americanum were collected from 7 state parks in N. C. Florida. DNA was extracted individually from each tick and evaluated for the presence of PME using a nested PCR reaction targeting the gltA citrate synthase

gene. PME-positive ticks were confirmed by sequence analysis and occasionally by using deep sequencing techniques. Positive cyropreserved stabilates, prepared from each tick, were inoculated into cell culture flasks containing either RF6A, DH82 or ISE6 cell lines. PME was detected by PCR analysis in approximately 1% of ticks, with highest prevalence if positively identified ticks in the month of June. Culture attempts are still on-going, however all attempts thus far have been unsuccessful. To characterize the cross-reactivity between Ehrlichia spp., synthetic peptides based on the MAP1 genes of E. ruminantium, E. chaffeensis and PME were used in an ELISA to demonstrate cross-reactive epitopes on this major, immunodominant surface antigen. Although serum antibody levels were predominantly higher in samples when used in homologous reactions with the respective peptides (PME being the exception), cross-reactive antibodies were found in all samples used in heterologous reactions with samples from infected animals and peptides from the MAP1 of the related Ehrlichia. This data suggests that the MAP-1B ELISA, the serological assay of choice to diagnose heartwater, cannot distinguish PME from E. ruminantium. Additionally, the role of PME in providing protective immunity to infection with E. ruminantium needs to be further investigated and would be greatly facilitated by successful attempts to culture the organism.

56. IN-GROVE SPATIAL SPREAD OF CITRUS HLB AND NUTRIENT AND INSECTICIDE EFFECTS ON PLANT HORMONE AND PATHOGEN CONTENTS IN LEAVES

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Citrus huanglongbing (HLB) has affected the citrus industry severely since it was first observed in South Florida in 2005. No published information is available on occurrence and in-grove distribution of HLB in north Florida and on the long-term effects of complex nutrients and systemic resistance inducers on the contents of the pathogen, Candidatus Liberibacter asiaticus (Las), in citrus leaves. Citrus leaf samples were collected from one organic and two conventional groves in Citra, FL, and from an ongoing factorial field experiment in Immokalee with insecticide or nutrient treatments (including systemic resistance inducing agents) or both compared to an untreated control. Leaves were tested for Las content using qPCR. Las was first detected in leaf samples from asymptomatic organic orange trees in Citra in Spring 2012. HLB symptoms were found in the organic grove and one conventional grove in Fall 2012. Semivariograms for within-row and across-row HLB spread were constructed using Las Ct values and tree distances. Monomolecular models were fit to the semivariance data. The range (=zone of influence) of HLB infected trees was greater within rows (20m) than across rows (13m), suggesting that HLB is transmitted more readily within than across rows. There was no relation between leaf nutrient contents and HLB development. However, in the factorial nutrient and insecticide experiment in Immokalee, Ct values of Las and leaf area and weight significantly increased after long-term (>3 years) nutrient applications, implying that the Las concentrations decreased in the long-term, although they increased in the beginning of the experiment, while the insecticide treatment had the opposite effects. Leaf N, Mn, Zn and B significantly increased after nutrient applications whilst Cu significantly decreased by this treatment. Salicylic acid significantly increased in old leaves

treated with insecticides, nutrients or both whilst the difference with the control in young leaves was significant for the nutrition treatment only. The jasmonic acid concentration was highest after the nutrition treatment in both old and young leaves. Redundancy analysis of the endophytic α-proteobacteria community structure indicated that the Ct value of Las was positively correlated with nutrient applications, and contents of Ca, Mn, B, Zn, Mg, and Fe in leaf samples collected in 2012. Results from the factorial experiment suggest that effects of insecticides on HLB were significant in the early 2-year period whilst nutrients plus resistance inducing agents had significant effects on Las concentrations and leaf size and weight after at least 3 years of applications.

57. SPATIAL ANALYSIS OF CITRUS HUANGLONGBING (HLB) SPREAD IN SOUTH FLORIDA

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The citrus disease Huanglongbing (HLB), associated with an uncultured phloem limited bacterial pathogen (Candidatus Liberibacter asiaticus) was reported around Miami, South Florida, in 2005. The disease is transmitted by a pysilid insect vector. The disease has spread quickly throughout Florida, and poses a great threat to the USA citrus industry. By 2008 the disease was already detected in some commercial citrus producing counties in Florida and in 2012 it was reported in California. The purpose of this study is to examine and describe the spatial and temporal spread of citrus trees that tested positive for Ca. L. asiaticus by qPCR in South Florida from 2008 to 2010. Data on HLB symptomatic citrus trees that had been tested for Ca. L. asiaticus between 2008 and 2010 were obtained from the Southwest Florida Research and Education center in Immokalee. One composite leaf sample

per tree was sent to the HLB detection lab together with geographic coordinates and pertinent citrus grove data. A Geographic Information System (GIS) was used to identify hot spot areas and the spatial distribution of HLB positive trees. A collection events tool was used to combine coincident points before hotspot analysis. Spatial and temporal cluster analyses were performed using Moran's I spatial autocorrelation. A total of 2391 samples collected in 2008 were included in this preliminary study. HLB hotspot areas were predominantly in St. Lucie and Hendry counties (Southeast and South west coasts of Southern Florida, respectively) whereas HLB cold areas were predominantly in Polk County (Central Florida). Similarly, Morans I autocorrelation analysis showed clusters of high HLB disease on the east and west coast of Southern Florida. Thus, HLB infected trees were numerous on the east and west coast of South Florida, and the disease was spreading towards the North. Further analysis for the years 2009 and 2010 and to determine the influence of geo-physical factors such as temperature and wind (direction and speed) on disease spread is going on.

58. PLANT ESSENTIAL OILS AS SYNERGISTS AND CONTROL AGENTS FOR VECTOR MOSQUITO CONTROL

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Phytochemicals have been considered as alternatives for conventional pesticides due to their low mammalian toxicity and environmental safety. They usually display less potent insecticidal effects than synthetic compounds, but may express as yet unknown modes of action. In the present study, first we evaluated 14 plant essential oils for their 24-hour toxicities against two types of vector mosquitoes, 4th–instar larvae of Aedes aegypti as well as 5-7 day old adults and two strains of 4th–instar larvae of Anopheles gambiae (G3 and Akron strains). And then we tested their synergistic effects with carbaryl and

permethrin against 4th–instar larvae of Aedes aegypti and 5-7 day old adults. Plant essential oils showed low toxicities against Aedes aegypti both larvae and adults. Only four plant essential oils killed Aedes aegypti larvae at concentration of 50 ppm with up to 22% mortality, and no plant essential oils showed toxicities against adults at doses up to 2000 ng/mosquito. All of them showed higher toxicities against Anopheles gambiae larvae than Aedes aegypti larvae. The LC50 values ranged from 7 ppm to 204 ppm for both susceptible and resistant strains, and they did not suggest high cross resistance. Six essential oils showed significant synergistic effects with carbaryl at 10-50 ppm, but paradoxically all of them decreased the toxicity of permethrin against Aedes aegypti larvae. None showed synergistic effects on Aedes aegypti adults, at doses up to 2000 ng/insect. The six essential oils displaying synergistic effects in larvae inhibited the in vitro activities of cytochrome P450 monooxgenases and carboxylesterases in the low ppm range when tested in extracts from Aedes aegypti larvae. The data indicated that cytochrome P450 monooxgenases and carboxylesterase were probably targets for these natural synergists, and explained the synergistic effects observed. Thus, the mechanism of synergism was most likely inhibition of metabolism and not interacting target site effects.

59. THE IMPACT OF THE EMERGENCE OF WEST NILE VIRUS (WNV) ON THE GENETIC DIVERSITY OF THE AMERICAN CROW

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The objective of this study was to assess the impact of the emergence of West Nile virus (WNV) on the genetic diversity of the American crow (Corvus brachyrhynchos). This avian species has been the most negatively affected host species resulting in estimated population decline up to 60% since the arrival of the virus. Thus, it is hypothesized that the genetic diversity and

genetic structure of the crow has been modulated by WNV in a short timescale. Changes on mortality rate and seroprevalence to WNV in avian species were extensively reviewed as an indication of heterogeneity of host susceptibility across time, geographic areas, and host species. A consistent increment of the seroprevalence was detected in eight species, including C. brachyrhynchos, although changes on time were at different magnitudes. C. brachyrhynchos showed also a consistent decline in the mortality rate. Thirty two polymorphic microsatellite markers distributed in six multiplex panels were developed and characterized for genetic analysis. The temporal analysis detected a significantly lower allelic richness and heterozygosity after four years of WNV arrival. Reduction of allelic diversity was faster than heterozygosity, a measure of recent population bottleneck. The genetic diversity was rapidly recovered by the year 2002 showing evidence of a population expansion by immigrants. Finally, a consistent pattern of higher heterozygosity levels towards resistance to WNV was detected. However, these differences in infection status were not significant, neither by a local effect nor by overall genetic diversity. This study provides molecular evidence for a strong genetic impact induced by severe mortality events attributable to WNV since 1999 in the AC. These changes occurred in a short time after the introduction of the virus. Relatively slow changes in the seroprevalence and mortality rates by WNV may indicate the slow process of adaptation of the AC to this virulent pathogen. Thus, WNV may be exerting considerable selection force to drive genetic evolution of the crow population but in an ecologically relevant time scale. These results suggest a lack of specific genetic adaptation of the AC towards resistance to WNV may be due to a constant immigration of susceptible individuals.

60. LACK OF PLASMODIUM VIVAX INFECTIONS AND HIGH FREQUENCY OF THE ERYTHROID SILENT DUFFY ANTIGEN GENOTYPE IN HAITI

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Background: Malaria is a significant public health concern in Haiti where approximately 30,000 cases are reported annually with CDC estimates as high as 200,000. Malaria infections in Haiti are caused almost exclusively by Plasmodium falciparum, while a small number Plasmodium malariae and an even smaller number of putative Plasmodium vivax infections have been reported. The lack of confirmed P. vivax infections in Haiti could be due to the genetic background of native Haitians. Having descended from West African populations, many Haitians could be Duffy negative due to a single nucleotide polymorphism from thymine to cytosine in the GATA box in the promoter region of the Duffy antigen receptor for chemokines (DARC) gene. This mutation, encoded by the FYES allele, eliminates the expression of the Duffy antigen on erythrocytes, which reduces invasion by P. vivax. This study investigated the frequency of the FYES allele and P. vivax infections in malaria patients with the goal of uncovering factors for the lack of P. vivax infections reported in Haiti. **Methods:** DNA was extracted from dried blood spots collected from malaria patients at four locations in Haiti. The samples were analysed by polymerase chain reaction (PCR) for the presence of the P. vivax small ribosomal subunit gene. PCR, sequencing, and restriction enzyme digestion were used to detect the presence of the FYES allele. Matched samples were examined for both presence of P. vivax and the FYES allele. **Results:** No cases of P. vivax were detected in any of the samples (0/136). Of all samples tested for the FYES allele, 99.4% had the FYES allele (163/164). Of the

matched samples, 99% had the FYES allele (98/99). **Conclusions**: In this preliminary study, no cases of P. vivax were confirmed by PCR and 99% of the malaria patients tested carried the FYES allele. The high frequency of the FYES allele that silences erythroid expression of the Duffy antigen offers a biologically plausible explanation for the lack of P. vivax infections observed. These results provide sound insight on the host susceptibility of malaria patients for P. vivax infections that has never before been investigated in Haiti.

61. EVOLUTION OF VIRULENCE GENES OF VIBRIO CHOLERAE ISOLATED FROM CLINICAL AND ENVIRONMENTAL SAMPLES IN HAITI

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Cholera continues to be a major public health threat in countries where safe drinking water, adequate sanitation and hygiene are suboptimal. On October 21, 2010, cholera was first reported in the Artibonite region of Haiti which within weeks spread to other parts of the country, including the capital of Port-Au-Prince. Through October 20, 2012, 604,634 cholera cases have been reported with 7,436 deaths as reported by public health ministry of Haiti. Earlier studies confirmed that Haiti cholera was caused

by a clonal V. cholerae O1 El Tor strain carrying classical ctxB gene (an altered V. cholerae) with the possible Asian origin. To determine the possible evolution of virulence of circulating V cholerae strains in Haiti and to examine whether the strains have established environmental reservoirs in Haiti, we have established both clinical and environmental surveillance for toxigenic V. cholerae in the Gressier region of Haiti. Between April and August, 2012, we collected 39 stool samples from patients presenting cholera in a cholera treatment center (CTC) run by a non-governmental agency (NGO). Out of 39 stool samples, 29 (74.3%) were positive for V. cholerae O1 El Tor strain. Further molecular analysis was performed on 23 isolates, and out of that 23 isolates, eleven (47.8%) isolates acquired rstC gene in contrast to 2010 epidemic strains, suggesting that these strains have begun evolving from the clonal strain introduced in Haiti in 2010. We also collected 90 water samples from 18 fixed sites in Haiti, including four estuarine and 14 riverine sites. V. cholerae O1 El Tor strain was isolated from 5 of the 90 (5.6%) water samples, including 3 strains from three estuarine sites and 2 strains from river sites. One of the strains (env-9) isolated from an estuary site had lost gene encoding CTXϕ, (ctxA, ctxB, rstR and rstC). Interestingly, this strain also replaced its tcpA-El Tor gene with tcpA-classical gene as corroborated by PCR and sequencing. Our results demonstrate the further evolution of Haitian V. cholerae strains. Our consistent isolation of strains from multiple environmental sites suggests that environmental reservoirs are being established in Haiti (or at least that the opportunity for establishment of such reservoirs exists), and raise the specter of long-term endemic cholera in Haiti.

62. MOLECULAR EPIDEMIOLOGY OF AN OUTBREAK OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN A NEONATAL INTENSIVE CARE UNIT

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Background: Methicillin-resistant Staphylococcus aureus (MRSA) is a leading cause of healthcare-associated infections, and a significant contributor to increased healthcare costs. Additionally, community-associated strains, previously prevalent among individuals with non-healthcare related risk factors, have infiltrated the healthcare setting, raising new questions regarding the epidemiology of S. aureus. Current molecular-typing methods lack the discriminatory resolution to discern outbreaks from sporadic cases of these highly clonal bacteria. During a 12 month period, 34 cases of MRSA were identified among patients in a neonatal intensive care unit (NICU) of a Florida hospital. Of these, 17 isolates were identified by PFGE analysis as type USA300, a strain predominately recognized as "community associated." These 17 isolates were further investigated as the "outbreak strain" and presumed to be epidemiologically related. A myriad of interventions were implemented and the outbreak was mitigated; however, no clear etiology was established, hindering the ability to understand transmission and develop permanent control measures. This outbreak provides a unique opportunity to apply rapidly evolving molecular techniques. The utilization of enhanced molecular methods, especially whole genome sequencing, would provide the resolution required to differentiate between outbreak strains and sporadic strains reintroduced from the community. **Methods**: To elucidate the outbreak etiology, we retrospectively obtained the 17 USA300 isolates and corresponding epidemiological data. We applied spa-typing, which sequences the polymorphic X region of the protein A gene (spa), to provide the next level of molecular resolution. We then used Ridom StaphType v2.2.1 software to estimate the relatedness of spa-types. We subsequently plan to apply whole genome sequencing and phylogenetic analysis to further resolve the transmission dynamics of the outbreak. **Results**: We identified 12 isolates that were spa-type t008, two t118, two

t211, and one t5593. Based on calculated genetic distances, the t118 isolates were likely unrelated to the primary outbreak, and the t211 isolates may represent a sub-epidemic within the NICU. **Conclusion**: Our preliminary analysis demonstrated that enhanced molecular typing can discriminate pathogen strains during outbreaks which will further be resolved by subsequent whole genome sequencing. With new advances in bench-top rapid sequencing technologies, the ability to conduct these analyses real-time during outbreak investigations is becoming more feasible. Additionally, continued investigation of bacterial pathogens using phylogenetic analysis will allow us to understand the epidemiology of these pathogens in more detail. This will further our ability to understand their emergence and transmission dynamics as well as develop targeted control measures.

63. SPATIAL PATTERNS AND ECOLOGICAL FACTORS ASSOCIATED WITH LIVESTOCK ANTHRAX IN OSH, KYRGYZSTAN

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Anthrax, a zoonotic diseased caused by the Bacillus anthracis bacterium, has been an enduring public health problem in the central Asian country of Kyrgyzstan. Although the majority of anthrax infections in Kyrgyzstan are found inlivestock, particularly sheep and cattle, the country has a high population of at-risk individuals who practice animal husbandry, live on farms in close proximity to their herds, or butcher infected animals. In recent years, a number of human cases have been reported. Here we use a series of surveys taken from 1937-2005 to investigate the spatial and ecological patterns of anthrax across the country. The majority of reported outbreaks occurred in the Osh Rayon of southern Kyrgyzstan, with additional foci in the northwest and north central regions of the country. The objectives of this study were to define the ecological factors associated with areas of persistent outbreaks around Osh. We also aim to determine if

these factors can be used to discriminate and predict at-risk areas on the landscape. This study employed the tasseled-cap transformation (tcap) and time-specific Landsat satellite imagery to the asses the brightness, greenness, and wetness bands and zonal statistics to determine the common values within 100 meters of known outbreaks and in areas with no known outbreaks (control sites). Mann-Whitney U tests will be used to determine if there are significant differences between case and control sites. Using such differences, we will employ an algorithm to determine if such features are found in the remaining two foci in the north. Such analyses can be paired with spatio-temporal statistical analyses of outbreaks and time series analyses to determine if there is a specific seasonality to tcap values that may aid in predicting outbreaks in future high risk periods. These data can be used to inform control efforts in-country, such as vaccination campaigns and farmer education programs.

64. MAPPING OUTBREAKS, MODELING NICHES: GENETIC DIVERSITY, SNP DISCOVERY AND ECOLOGICAL MODELING OF BACILLUS ANTHRACIS IN AZERBAIJAN

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Anthrax is an acute zoonosis of domestic and wild herbivores, with secondary human cases, often from spillover associated with

slaughtering animals. The disease is caused by the spore-forming bacterium Bacillus anthracis, which has potential for persistence and reoccurrence in some soils, as well as long-distance transmission events. Though Azerbaijan has a long record of human and animal outbreaks, little data are available on the geography of anthrax or genetic relationships between local strains and global diversity. Villages reporting human and veterinary anthrax were each aggregated by decade to map hotspots of disease reporting from 1940–2000 using GIS. An exploratory kernel density estimation was used to map concentrations of disease. Thresholds of 75%, 90% and 95% were used to map areas of greatest anthrax concentration in each group by decade. A collection of strains was genotyped using the MLVA-25 and SNR-4 systems. All genotyped isolates were mapped in the GIS and used to construct an ecological niche model of the Azerbaijani B. anthracis sub-lineage. Azerbaijan had sustained hotspots in the south, central and northwest across the time period. These areas partially correspond to rayon-level zones of anthrax risk defined by the State Veterinary Service. Genotyping efforts assigned all available isolates into a single lineage, with MLVA-25 and SNR-4 identifying 3 genotypes. The niche model predictions broadly correspond to epidemiological hotspots. MLVA types were most closely related to neighboring Iran. Sequencing of the MLVA loci identified Azerbaijan specific SNPs, which may be useful for rapid identification of regional strains. These results identify surveillance priorities and add to our understanding the genetic relationships between Azerbaijan and other parts of the world.

65. BACILLUS ANTHRACIS DIVERSITY AND GEOGRAPHIC POTENTIAL IN NIGERIA: FURTHER SUPPORT OF A NOVEL AFRICAN LINEAGE

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Palm Bay, FL; **Vincent Pereten** - Institute of Veterinary Bacteriology, University of Berne, Berne Switzerland; **Angaya Maho** - Laboratoire de Recherches Vétérinaires et Zootechniques, N'Djaména; **Mikeljon Nikolich** - Walter Reed Army Institute of Research; **Martin Hugh-Jones** - Louisiana State University, Baton Rouge, LA; **Ted Hadfield** - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, MRI Global, Palm Bay, FL

Although anthrax is endemic in Nigeria, little is known about the genetic composition of B. anthracis strains or the geographic distribution in the country. Archived Bacillus anthracis isolates from cattle outbreaks in Nigeria collected between 1949-1966 were genotyped using a 25 marker multiple locus variable number tandem repeat analysis method. In addition, 9 isolates from cattle outbreaks (1996-2003) in the bordering country of Chad were also genotyped. Cluster analysis with previously published global genotype data indicate Nigerian and Chadian strains belong to a globally unique African lineage. Considering the temporal and geographic range of the Nigerian and Chadian samples and their genetic relatedness to previously published genotypes in Cameroon, our data suggests this unique lineage is ecologically established in this African region.

66. INFORMING ZOONOSIS SURVEILLANCE WITH ANIMAL MOVEMENT ECOLOGY: TRACKING ELK DURING THE MONTANA ANTHRAX RISK PERIOD

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Despite rare and sporadic reports of human anthrax in the US, the disease is common in wildlife in Texas and has been documented since the early part of the last century. Historically, it was speculated that the first anthrax cases in the US were white-tailed deer, Odocoilleus virginianus, in Louisiana. Anthrax in wildlife in northern states is poorly documented. Before 2008, there were

no records of anthrax in deer, bison, Bison bison, or elk, Cervus elaphus, in southwestern Montana. In 2008, a major epizootic occurred on a large (~300 sq.km.) ranch near the Gallatin River affecting all three species. At least 300 bison, 2 deer, and ~65 bull elk died over a four week period from July to August. This was the first confirmed report of anthrax in elk in the US and only the second report in the species across its wider range. Livestock anthrax is controlled through vaccine campaigns in the spring season ahead of the summer risk period. With the exception of limited use of livestock vaccine in bison that can be regularly herded and captured on the ranch, vaccination as a management strategy is untenable for wildlife. Because of this, accurate surveillance and carcass clean-up are the most efficacious control measures for the cervid populations. However, surveillance in expensive and requires significant personnel across such large, rural landscapes. At the same time, the transmission pathways are poorly understood in all of these species. Wildlife telemetry can provide a means of better understanding animal movement patterns during disease risk periods. These data can aid in determining the best use of personnel for summer-time carcass searches and inform our knowledge on species ecology. We initiated a GPS telemetry study of bull elk on the ranch from April – August 2010. The goal was to determine the home ranges of elk on this landscape, determine if the animals left the ranch property entering public lands, and to evaluate the home range size and habitat characteristics across the risk period. Using data from 9 elk, we determined that home range sizes decrease from early to late summer, suggesting infection likely occurs over a small portion of their range. Additionally, half of the elk left the ranch early and remained off the ranch through the study period. These data suggest that surveillance efforts should not be limited to the ranch in future summers and provides target areas on the ranch to monitor.

67. P. GINGIVALIS TRAFFICS INTO ER-RICH-AUTOPHAGOSOMES FOR SUCCESSFUL SURVIVAL IN GINGIVAL-EPITHELIAL-CELLS

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P. gingivalis is a host-adapted pathogen that can successfully survive and replicate in primary gingival-epithelial-cells (GECs) for extended periods of time, and later spread intercellularly. We recently reported that endoplasmic-reticulum (ER) network serves as a prominent subcellular-niche for live P. gingivalis in GECs. However, the precise characterization of the intracellular trafficking and fate of P. gingivalis in its target cell type, GECs, remain incomplete. Objectives: To study the intracellular trafficking of P. gingivalis in GECs. Methods: Primary GECs were infected with wild-type strain or FMN-green-fluorescent transformant-strain (PgFbFP) over 24-hours. High-resolution three-dimensional-transmission-electron-microscopy was utilized to determine the predominant location of intracellular P. gingivalis and reveal the double-membrane autophagosomal vacuoles in GECs. The induction of autophagy was studied by immunofluorescence-microscopy, western-blotting, and transfection of GECs with GFP-LC3 plasmid during P. gingivalis infection. The utilization of PgFbFP strain along with digitonin treatment and anti-P. gingivalis-antibody staining allowed quantification of the vacuolar versus cytoplasmic bacteria. Furthermore, an autophagy inhibitor, 3-methyladenine (3MA), was utilized to quantify the viability of intracellular bacteria in the antibiotic-protection-assay. Results: The serial sections of transmission-electron-micrographs and their tomographic reconstruction by IMOD-software demonstrated that majority of intracellular P. gingivalis rapidly target ER-rich regions where

autophagic vacuoles are formed. The ~60% of live P. gingivalis resided in vacuoles at 3-hour post-infection and it increased to ~80% at 24-hours. Western-blotting and fluorescence microscopy of infected-cells with anti-LC3 (autophagy-marker)-antibody show that P. gingivalis induces LC3-lipidation in time-dependent-manner. The ImageJ analysis of the data indicates that maximum induction of autophagy occurred at 6-hour post-infection. Fluorescence-microscopy displayed high-degree of stably-expressing GFP-LC3 accumulation and significant degree of co-localization between the intracellular-bacteria and LC3. The amount of viable P. gingivalis was considerably decreased upon 3MA treatment. Conclusion: The results reveal a novel mechanism of P. gingivalis for successful intracellular survival in GECs by harnessing autophagy. Supported by NIDCR-R01DE016593 and R01DE019444.

68. P. GINGIVALIS-NUCLEOSIDE-DIPHOSPHATE-KINASE CONTRIBUTES TO PERSISTENCE BY INHIBITING DANGER-SIGNAL-ATP INDUCED REACTIVE-OXYGEN

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Ligation of P2X7 receptors with extracellular ATP (eATP), results in production of intracellular reactive-oxygen-species (ROS) in macrophages. We showed recently that primary gingival epithelial cells (GECs) produce sustained, robust cellular-ROS upon stimulation by eATP dependent on P2X7-signaling coupled with NADPH-oxidase and mitochondrial-respiratory-chain. Furthermore, opportunistic pathogen P. gingivalis, up-regulated the antioxidant-glutathione response and inhibited eATP-induced cytosolic- and mitochondrial-ROS via P2X7-NADPH-oxidase interactome. Objectives: Recent studies reported P2X7-mediated ROS play a role in eliminating intracellular infections in macrophages. We showed that P. gingivalis diminishes P2X7-

activity by production of a Nucleoside-diphosphate-kinase (Ndk) and the ndk-deficient-strain lacks the ability to inhibit ROS induced by eATP during infection of GECs. Therefore, we studied the role of Ndk against eATP-mediated ROS generation and the functional effect of ROS on intracellular survival of organism in GECs. Methods: GECs, infected with wild-strain, or ndk-deficient-strain, complemented-strain w±/o eATP pre-treatment, were studied over 24-hours. Secretion of the Ndk from infected-GECs was analyzed in the GEC culture-supernatants, using ammonium-sulfate-precipitation and Western-blotting. A mass-spectrometry was performed for validation. ATPase-activity of the supernatants from infected-GECs was evaluated by a colorimetric-ATP-hydrolysis assay. Lactate-dehydrogenase-release assay was done in the supernatants to confirm the GEC viability and plasma-membrane integrity. Survival of the intracellular-bacteria upon eATP treatment was quantified by the antibiotic-protection-assay. Results: The ndk-deficient-strain was greatly affected by eATP treatment as ~40% of the bacteria could be recovered. Pre-treatment of the GECs with N-Acetyl-Cysteine, an inhibitor of ROS, significantly restored the intracellular survival of the ndk-deficient-strain. The analyses demonstrated that Ndk was actively secreted into the extracellular-milieu and up-regulated during the intracellular-infection. The secreted enzyme was functional with ~0.1 μM/sec ATPase-activity. The recombinant-Ndk treatment reduced eATP-induced ROS in a time-dependent mode in uninfected-GECs. Conclusion: Temporal secretion of the Ndk appears to serve as a central effector against ROS-mediated bacterial killing and contributes to persistence. Supported by NIDCR-R01DE016593 and R01DE019444.

69. CD8+ T Cells Dampen Brucella melitensis Infections Subsequent Mucosal Immunization With Live Brucella Vaccine

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Brucellosis remains a significant human health threat in many parts of the world. Humans mostly acquire infection primarily following mucosal exposure due to aerosols or ingestion of contaminated foods. Despite mucosal exposure, brucellosis is primarily a systemic disease. Thus, mucosal vaccination represents a useful alternative for the induction of protective immunity against disease. Previous work has shown that single oral or nasal vaccination with ΔznuA B. melitensis conferred significant protection from disseminated infection of spleens and lungs ($p<0.001$) relative to PBS-dosed mice. Moreover, the combination of nasal and oral immunizations conferred the best protection against wild-type challenge, as evidenced by 100% of the mice showing undetectable levels of brucellae. It is well recognized that T cells and IFN-□ are essential for the cell-mediated immune responses to Brucella. However, the role of CD4+ and CD8+ T cells in resistance to brucellosis still remains controversial. Evaluation of cytokine responses in mice vaccinated with ΔznuA B. melitensis and subsequently challenged with wild-type B. melitensis revealed that IFN-□-producing CD8+ T cells play a key role in controlling B. melitensis infections whereas IFN-□-producing CD4+ T cells had a lessor role in the clearance of infection. IFN-□-producing CD8+ T cells exceeded IFN-□-producing CD4+ T cells by >10-fold. To further confirm the above findings, wild-type, CD4-/-, and CD8-/- mice were nasally immunized with a single dose of ΔznuA B. melitensis, Rev-1, or PBS. Four wks after vaccination, mice were challenged nasally with 104 CFUs of wild-type B. melitensis 16M. Four wks post-challenge, lungs and spleens were evaluated for colonization. PBS-dosed CD8-/- mice showed exacerbated disease relative to similarly treated B6 and CD4-/- mice. Complete protection was obtained in ΔznuA B. melitensis-vaccinated B6 and CD4-/- mice, while some colonization remained in CD8-/- mice. These results implicate the importance of CD8+ T cells either in combination

with CD4+ T cells or alone for protection against Brucella infections.

70. Detection of Escherichia coli and Other Pathogens in the Drinking Water Fountains

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The objective of this study is to evaluate the potential risk associated with the presence of Escherichia coli and Salmonella spp., Shigella spp., Escherichia coli O157:H7, Clostridium perfringens, Staphylococcus aureus, and Campylobacter jejuni from public drinking water fountain sites in Gainesville, Florida. Over more years, drinking water samples will be monitored for the occurrence of the waterborne pathogens. Samples will be collected at elementary, middle, high schools, public areas, and the University of Florida from drinking water fountain sites. Some drinking water samples will be expected positive results for total coliforms and Escherichia coli or other pathogens from contaminated drinking water fountain sites. The positive results of drinking water samples will be compared monthly, seasonally, and annually from the drinking water fountain sites. This result will provide data to minimize a potential public health risks associated with drinking water fountains.

71. Risk Factors for Escherichia Coli Growth in Preprocessing Cultures of Musculoskeletal Allograft Tissue

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Background: Recent studies have characterized disparate risk factors associated with pre-processing cultures of musculoskeletal allograft tissues positive for Clostridium spp. and beta-hemolytic streptococci. Data from the Centers for Disease

Control and Prevention suggest that gram-negative microorganisms are responsible for a substantial number of allograft-associated infections in the United States. Therefore, we conducted this study to identify those risk factors associated with pre-processing leg-en-bloc tissue cultures positive for Escherichia coli. **Methods**: A case was defined as any donor with a leg-en-bloc preprocessing tissue culture that grew E. coli in the Regeneration Technologies Inc. (RTI) microbiology laboratory during January – May 2003 (study period). Twenty-four cases were ascertained through examination of RTI medical and laboratory records. Forty-nine controls were randomly selected donors with negative leg-en-bloc cultures during the study period. **Results**: Tissues were procured by 14 different recovery agencies. Of the 24 donors that met the case definition, 15 (63%) were male and the median age was 51 (range: 23-93) years. Cases and controls were matched on the following: age, sex; duration of recovery, time between death and refrigeration, time between death and recovery; occurrence of carcinomas, diabetes, heart, lung, kidney, or liver disease; trauma, invasive or surgical procedures at time of death; or receipt of cardiac resuscitation. No significant difference was found in contamination rates between donors who were refrigerated and those who were not. However, E. coli contamination of tissues was more likely to occur when dissection was conducted by fewer than 3 personnel (OR: 5.6, CI: 1.3-26, $p<0.01$), or when donor death occurred outside the hospital setting (OR: 4.3, CI: 1.3-15.5, $p<0.01$). Moreover, tissues were twice as likely to be contaminated during recovery within the hospital setting versus outside the hospital though this was not statistically significant (OR: 2.0, CI: 0.6-7.3, P=NS). **Conclusion**: Our data confirm that the risk factors associated with pre-processing cultures positive for E. coli are different from the risks associated with Clostridium species and group A streptococcus contamination. In addition, tissue recovered outside the hospital setting appears to be at higher risk of contamination compared with tissue recovered inside the hospital. Regulatory agencies need to take these issues into consideration when they formulate guidelines for recovery and processing of allograft tissue. Variables such as place of death

and autopsies cannot be controlled; this underscores the need for the allograft tissue industry to use processing methods that include a sterilization step.

72. EVIDENCE OF LOCAL CLUSTERING OF HUMAN ANTHRAX IN GEORGIA ASSOCIATED WITH ENVIRONMENTAL AND ANTHROPOGENIC FACTORS

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Anthrax is a soil-borne disease caused by the bacterium Bacillus anthracis and is considered one of a growing list of neglected zoonoses. In Georgia human anthrax remains a threat to public and veterinary health. The purpose of this study was to evaluate the baseline presence of the disease and identify environmental/anthropogenic factors associated with elevated reporting. A database of human anthrax in Georgia during the period 2000 to 2009 was constructed using geographic information systems (GIS) with case data recorded at the village location. The study period was divided into two equal 5 year periods. Cumulative incidences per 10,000 population were calculated each period (2000 to 2004) and (2005 to 2009). Spatial and aspatial correlations between anthrax incidence and the following variables were examined: human population density, cattle density, soil pH, average annual precipitation, and elevation. SaTScan was used to identify space and space-time clustering of anthrax. A logistic regression was used to model factors related to the presence of clusters of disease. Incidence maps indicated that there were a greater number of villages

reporting human anthrax during the period 2005 to 2009. Results showed positive correlations between incidence and cattle density and elevation, with negative correlations between precipitation and human population density. SaTScan identified significant spatial and space-time clustering. Logistic regression indicated clusters of disease were positive associations between clusters and cattle density. Anthrax is a disease that persists at relatively low levels in Georgia yet experiences some of the highest rates of the disease in the world. However, without adequate surveillance the true burden of the disease is most likely unknown. This study provides baseline estimations of disease in order to monitor deviations from normal related to outbreaks or a nefarious release.

73. Ecological Niche Modeling of Bacillus anthracis on Three Continents: Evidence for Genetic-Ecological Divergence

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Anthrax, caused by Bacillus anthracis, is a disease with important public health and national security implications. Understanding the geography of B. anthracis plays an important role in predicting areas having a high likelihood of pathogen persistence. Ecological niche models (ENMs) are useful for estimating spatial distributions of pathogens, which can be used to focus disease control efforts in high risk regions. Ideally, ENMs would be capable of estimating disease potential in areas with limited surveillance or epidemiological data by transferring models

developed on a 'native' landscape to novel landscapes. However, developing a globally applicable ENM for B. anthracis is complicated by limitations in our understanding of the interrelationship between the genetics of the pathogen and ecological conditions. We utilized the Genetic Algorithm for Rule-Set Production (GARP) to model the ecological niche of a globally successful Bacillus anthracis sublineage in the United States, Italy and Kazakhstan. Country-specific ecological-niche models were developed using native pathogen datasets and reciprocally transferred to the other countries to determine if pathogen presence could be accurately predicted on novel landscapes. Native models accurately predicted endemic areas within each country, but transferred models failed to predict known occurrences in the outside countries. Results indicate differing ecological associations for the B. anthracis populations within each country and may reflect niche specialization within the sublineage. While the effects of variable selection on model transferability and limitations of the genetic data should be considered in future studies, our findings emphasize the need for more comprehensive, comparative genomic data across landscapes and provide guidance for developing accurate ecological niche models for this pathogen. We recommend models should be developed on the native landscape, be informed by genetic data, and applied regionally. More complete genomic analysis will improve our understanding of the genetic-ecological dynamics of B. anthracis across these countries and may lead to more refined predictive models. Such models will be more successful in predicting outbreaks and better inform surveillance and proactive vaccination programs.

74. GLOBAL METABOLOMICS OF PATHOGEN-EXPOSED CAENORHABDITIS ELEGANS

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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 host to study bacterial pathogenesis and host immunity and response. Despite the wealth of information in genetics, cell biology, and developmental biology, metabolomic and chemical biology studies in C. elegans have only recently become active areas of research. Most notable has been the research on a large family of molecules called ascarosides, which act as regulators of development, mating attraction, aggregation, and dispersal. A primary interest of our research is the study of the exo- and endo-metabolomic responses of C. elegans under pathogenic stress. 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.

Isotopic Ratio Outlier Analysis (IROA) is a mass spectrometry based technique which allows the discrimination between molecules of biological origin and non-biological artifacts in two-group studies. The groups are randomly isotopically labeled with 13C at levels of enrichment of 95% or 5% for the control and experimental groups. The two groups are mixed together for uniform extraction, sample preparation, and LC-MS analysis. Because both samples are simultaneously analyzed, many of the errors created by normal sample-to-sample variance are controlled, most particularly ion suppression and losses due to sample preparation. The isotopomers that arise from the control and experimental groups are readily distinguished and form an easily recognizable pattern that can be used to identify true biological metabolites as well as to quantify relative amounts of each compound. Using IROA, we challenged C. elegans with P. aeruginosa, or a toxin produced by the pathogen, 1-hydroxyphenazine (1-HP), and analyzed the resulting changes in the global metabolome of the worm. We found significant changes

in several compounds, notably ascarosides, TCA cycle intermediates, and purine related compounds. Interestingly, we found a large increase of ascr#3 and a decrease in ascr#4 in toxin treated worms. These ascarosides are involved in male attraction and dauer formation (an alternative, stress-resistant developmental stage), suggesting the possibility that they could serve as a signal to repel other worms or induce dauer conditions under detrimental conditions leading to an increased survival of the population. In total, we found almost 900 features which changed by 2-fold or more, 102 of which are named by matching to the KEGG database.

75. CHARACTERIZATION OF IN VITRO ANTIMICROBIAL ACTIVITY OF VERTILMICIN ALONE AND IN COMBINATION WITH CEFTAZIDIME USING SEMI-MECHANIS

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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 of vertilmicin alone and in combination with ceftazidime in the clinic. **Method**: The in vitro antibacterial activity of vertilmicin alone 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. 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 and carried out using a manual dilution system with half-life of 2 h. The combined killing ability of vertilmicin and ceftazidime was assessed using constant time-kill study against Pseudomonas aeruginosa based on 0.25- to 4-fold

MIC of vertilmicin under five different concentrations of ceftazidime. Subsequently, two-compartment model was developed to fit the data of mono- and combination therapies simultaneously. The Loewe additivity was used as reference to evaluate the drug-drug interactive effect. **Result**: Vertilmicin alone displayed the concentration-dependent killing effect against the three bacterial strains. Surprisingly the elimination half-life of vertilmicin in the human body had a dramatic impact on its antimicrobial activities, which was further confirmed by the EC50 estimates from the semi-mechanistic PK/PD model. The combination of vertilmicin and ceftazidime against Pseudomonas aeruginosa showed significantly enhanced bactericidal capacity compared to either drug at the same level. The model that incorporates Loewe additivity and killing inhabitation was able to elucidate the time course of bacterial growth and killing kinetics after different treatments. Furthermore, the estimate showed that the overall drug-drug interaction between vertilmicin and ceftazidime was additive, but highly varied with respect to the combinations. The optimal dose regimen of vertilmicin in combination with ceftazidime could be easily identified using there-dimensional surface response. **Conclusion**: The result of PK/PD modeling demonstrated that vertilmicin has promising prospect for future clinical application if reasonable dosing strategy was taken into consideration. It also indicated that PK/PD modeling was capable of assisting dose selection for combination therapy of vertilmicin and ceftazidime to maximize the probabilities of positive clinical outcomes. Undoubtedly, PK/PD model is a powerful tool to evaluate the in vitro antibacterial activity of novel antibiotics, as well as to make key decisions in drug development.

76. HIV-1 GP120 ACTIVATES STAT1, 3, AND 5 AND MODIFIES APOPTOTIC GENE EXPRESSION AND PRO-INFLAMMATORY RESPONSES IN HUMAN MACROPHAGES

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HIV-1 infection of monocyte derived macrophages [MDMs] induces changes in expression of apoptosis related genes and primes the cell to become hypersensitive to subsequent inflammatory stimuli, such as LPS. In MDMs, soluble HIV-1 envelope protein, gp120, binds to CD4 and activates Signal Transducer and Activator of Transcription [STAT] proteins 1, 3 and 5, which are involved in immune activation, anti-viral responses and regulation of apoptosis. We hypothesize that gp120 induced activation of STAT1, 3 and/or 5 mediates changes in apoptosis related genes and induces a cellular activation state favorable for the virus. To investigate our hypothesis, MDMs or PMA-differentiated monocytic THP-1 cells were incubated with soluble gp120 and lysed over time for collection of total protein or RNA. STAT phosphorylation was observed by western blots, and expression of pro-apoptotic genes BAD and BID, and anti-apoptotic genes Bcl-2 and Bcl-xL were assessed by qRT-PCR. Soluble gp120 induced rapid phosphorylation of all three STAT proteins. BAD [pro-apoptotic] expression peaked [1.8-fold] after one hour of gp120 incubation compared to untreated cells but returned to baseline by 2 hours. The expression of BID [pro-apoptotic] and Bcl-2 [anti-apoptotic] were similar to baseline at 1 and 2 hours of gp120 incubation but increased at 6 hours [3.6-fold and 4.1-fold, respectively]. The expression of Bcl-xL [anti-apoptotic] initially decreased at 1 and 2 hours of gp120 incubation [12% and 32%, respectively] but was followed by a 2-fold increase at 6 hours. Next, using priming by HIV-1 infection as a control, we examined priming of PMA-differentiated THP-1 cells by soluble gp120 or anti-CD4. As expected, HIV-1 infection induced a priming phenotype characterized by an increase in LPS-stimulated pro-inflammatory cytokine secretion compared to uninfected cells. In contrast, 18 hrs of pre-incubation with gp120

[2.5 μg/ml] or anti-CD4 [50 ng/ml] significantly decreased LPS-induced cytokine production. As both anti- and pro-apoptotic genes are induced by gp120 in macrophages, further experiments assessing apoptosis of gp120 treated cells are required to determine if the changes in gene expression are biologically relevant. While priming of macrophages is not dependent on gp120-CD4 interaction, gp120 induced suppression of LPS-stimulated cytokine production may contribute to a cellular activation state favorable for early events in the viral life cycle.

77. TRANSMISSION ECOLOGY OF SIN NOMBRE HANTAVIRUS IN NATURALLY INFECTED DEERMICE IN OUTDOOR ENCLOSURES

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Sin Nombre virus (SNV), hosted by the North American deermouse causes hantavirus pulmonary syndrome (HPS) in humans. SNV transmission studies using experimentally infected deermice in the laboratory have failed to demonstrate transmission. Our objective was to construct an outdoor enclosure system and study transmission of SNV in deermice in their natural environment. During the summers of 2007-2008, we studied the effects of virological, immunological, and behavioral factors on SNV transmission among naturally infected deermice in replicated outdoor enclosures in Montana. Donor mice with the highest viral RNA levels infected susceptible mice. Mice acquiring infection within enclosures maintained viral RNA in blood throughout the experiment and blood RNA levels varied up to 100-fold, even in individuals infected with identical strains. There may be a threshold level of virus necessary for infected hosts to infect susceptible deermice. Outdoor enclosures are an

effective system for studying SNV transmission in hosts and may be adapted to other disease systems.

78. PROTECTIVE CD8 T CELL MEMORY TO A VIRAL PATHOGEN REQUIRES TRANS COSIGNALING BETWEEN HVEM AND BTLA

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Defining the molecular interactions required to program activated CD8 T cells to survive and become memory cells may allow us to understand how to augment anti-viral immunity. HVEM (herpes virus entry mediator) is a member of the tumor necrosis factor receptor (TNFR) family that interacts with ligands in the TNF family, LIGHT and Lymphotoxin-α, and in the Ig family, B and T lymphocyte attenuator (BTLA) and CD160. The Ig family members initiate inhibitory signaling when engaged with HVEM, but may also activate survival gene expression. Using a model of vaccinia virus infection, we made the unexpected finding that deficiency in HVEM or BTLA profoundly impaired effector CD8 T cell survival and development of protective immune memory. Mixed adoptive transfer experiments indicated that BTLA expressed in CD8α+ dendritic cells functions as a trans-activating ligand that delivers positive co-signals through HVEM expressed in T cells. Our data demonstrate a critical role of HVEM-BTLA bidirectional cosignaling system in antiviral defenses by driving the differentiation of memory CD8 T cells.

79. Serological Evidence of Human Infection with Porcine Reproductive and Respiratory Syndrome Virus

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Background: Porcine reproductive and respiratory syndrome virus (PPRSV) causes severe disease in pigs and has a worldwide distribution. We tested archived serum samples from swine-exposed agricultural workers for serological evidence of PRRSV infection. **Methods**: In this study we selected 612 archived serum samples collected from swine-exposed agricultural workers during 2004-06 in Iowa and compared their antibody response with 44 archived serum samples from human controls who reported no swine exposure. We adapted veterinary diagnostics for the comparison: an immunoperoxidase monolayer assay (IPMA) and a confirmatory immunofluoresence assay (IFA). A North American PPRSV was used for the assays. Each serum was tested in at least three separate IPMA tests. **Results**: Of the 612 samples, 29 (5%) were tested positive through IPMA. There was no statistically significant difference between the number of positive among swine-exposed and non-swine-exposed. However, among four swine-exposed workers, there was a four-fold increase in the antibody titer in the sera between 2005 and 2006, suggesting exposure between the collection periods. We tested 87 serum samples with the confirmatory IFA and identified evidence

of PPRSV antibody in nine. Seven of 29 IPMA-positives were also positive by IFA assay. However the agreement statistics between the two assays were not high (Kappa coefficient =0.25; 95% CI: 0.06-0.44). **Discussion**: These data suggests evidence of previous PPRSV or a PPRSV-like infection among the subjects. Further studies are requires to determine population level prevalence of the disease, modes of transmission, and specificity of the diagnostics.

80. MEASURING INTER-ANNUAL DYNAMICS OF THE TRANS-CAUCASIAN LOW-LAND PLAGUE FOCUS IN AZERBAIJAN USING HISTORICAL MAPS AND STAMP

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Plague, caused by Yersinia pestis, is a zoonosis with enzootic cycles in rodents. In Azerbaijan, the Trans-Caucasian Lowland-Foothills focus spans much the arid plains across the central portion of the country where the Libyan Gird, Meriones libycus, is the primary mammalian reservoir for plague. Historically, annual surveillance for Y. pestis was associated with zoological expeditions across known colonies of M. libycus within this focus. As part of these surveillance efforts, the Republican Anti-Plague Station collated annual sampling data and produced accurate, hand-drawn maps delineating areas of gird abundance based on five categories (very low, low, average, high, and very high). Each category is associated with a defined population range. Maps were published in annual yearbooks summarizing surveillance, control, and diagnostic activities. For this study, we used a GIS to heads-up digitize yearbook maps defining M. libycus abundance across the focus from 1972-1985. We used the spatial-temporal

analysis of moving polygons (STAMP) analysis tool to evaluate inter-annual changes of abundance across the landscape. STAMP quantifies changes in abundance categories by measuring polygon stability, expansion, and contraction between consecutive time periods. We tested for inter-annual changes across the study period. Within categories, we evaluated long-term stability as the proportion of the stable polygons that persisted and for how long. Some portion of the focus was defined by at least one of the five categories in each year, with most stable areas persisting for fewer than five consecutive years. Polygons of low abundance had several polygons across the focus that persisted for five or more years. High and very high abundance polygons were restricted to the east. M. libycus is still active in this focus and continued surveillance is essential. These analyses provide important insights into plague focus dynamics and provide a baseline for guiding modern surveillance for this important pathogen and host.

81. THE ASSOCIATION BETWEEN EXECUTIVE FUNCTION, RISKY INJECTION PRACTICES AND HEPATITIS C VIRUS AMONG INJECTION DRUG USERS

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Aim: To measure associations between impaired executive functioning (e.g., planning and problem solving ability) as measured by the Tower of London (TOL) and outcomes of risky injection practices, and Hepatitis C virus (HCV) among injection drug users (IDU). **Hypothesis**: Individuals with impaired executive function as indicated by the TOL will have greater odds of sharing injection equipment, backloading, and HCV than those with intact executive function. **Methods**: Data from the Neuro-

HIV Epidemiologic Study, a study of neurobehavioral risk factors and infectious disease among injection and non-injection drug users in Baltimore, was restricted to those who had ever injected in their lifetime (n=458). Impaired executive function was defined as a TOL standardized total excess moves score below the 10th percentile. Injection practices were dichotomized and included ever having shared needles, cookers, cotton, rinse water and ever having backloaded in one's lifetime. The outcome of HCV was binary. Logistic regression was performed to assess the association between impairment on the TOL and each of the risky injection behaviors and HCV infection. Mediation analyses were conducted to assess the extent to which the association between TOL and HCV were attenuated by the hypothesized injection intermediates. **Results**: Impairment on the TOL was associated with greater odds of ever having shared a cooker (OR: 1.99, 95% CI: 1.16-3.44), or cotton (OR: 1.92, 95% CI: 1.15- 3.22), and ever having backloaded (OR: 1.69, 95% CI: 1.00- 2.83). Impaired individuals had 1.97 (95% CI: 1.05- 3.73) times the odds of HCV infection compared to intact individuals. Mediation analyses suggested that the association between TOL and HCV was mediated by risky injection practices. **Conclusion**: Interventions designed to reduce infectious disease among IDU should aim to improve planning and problem solving abilities to reduce risky injection practices.

82. THE AP1 TRANSCRIPTION FACTOR BATF3 DIFFERENTIALLY REGULATES VIRUS SPECIFIC CYTOTOXIC AND CD4 T FOLLICULAR HELPER CELLS

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Vaccinia virus (VACV) elicits a robust CD8 and CD4 T follicular helper (TFH) cell response that play an important role in host resistance. To date, there is little information on the dendritic cell subsets that are essential to generate large pools of virus-specific effector T cells. To address this, we examined vaccinia virus infection of Batf3-/- mice, which selectively lack only lymphoid resident CD8α+ DCs and related peripheral CD103+ DCs. We found that Batf3-/- mice were extremely susceptible to VACV infection, exhibiting rapid weight loss and death within 9 days. CD8α+ DCs were the principal DC subset that promotes anti-viral CD8 T cell expansion and survival. Unexpectedly, deficiency in CD8α+ DCs had little or no effect on TFH cell activation and germinal center reactions. These results reveal that during an acute virus infection distinct DC subsets are specialized for priming of CD8 and TFH cells.

83. THE TNFR FAMILY MEMBER OX40 CONTROLS T FOLLICULAR HELPER CELL SURVIVAL AND FUNCTION

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T follicular helper (TFH) cells are the CD4+ T helper cells that provide cognate help to B cells for high-affinity antibody production in germinal centers (GC). Defining the signals that control effective TFH responses has broad implications for human vaccine design and in the management of adverse immune reactions. We show that fully differentiated TFH cells (CxCR5+BCL6+) express the TNFR family member OX40 (also known as CD134) and that OX40-dependent signals directly shape the magnitude and quality of the response to viral antigens. OX40 deficiency in TFH cells profoundly impaired the acquisition

of GC B cell phenotype, plasma cell generation, and virus-specific neutralizing Ab responses. Sustained interactions between OX40 and its ligand, OX40L, beyond the time of initial encounter with antigen were required for maintenance of the TFH cell phenotype and GC reaction. Our data provide new insight into the nature of molecules required for TFH cells to direct GC B cell responses and suggest that targeting the OX40/OX40L pathway may be useful in promoting and sustaining anti-viral immunity.

84. Δ9-TETRAHYDROCANNABINOL [THC] REDUCES INFLAMMATION AND HIV-1 INFECTION OF TARGET CELLS

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Use of cannabis is reported to be as high as 81% among HIV-1-infected adolescents. The major psychoactive constituent of cannabis, Δ9-tetrahydrocannabinol [THC], mediates anti-inflammatory effects through interaction with cannabinoid 2 [CB2] receptors on immune cells. As most studies of THC and immunity have been conducted in rodents, the role of THC in modulating human immunity and HIV-1 immune pathogenesis remains unclear. The impact of cannabis use on immune activation was investigated by a study combining in vivo and ex

vivo approaches. Expression of THC receptors CB2, CB1, and GPR55 by human monocytes, monocyte derived macrophages, and undifferentiated or PMA differentiated THP-1, HL-60 and U937 monocytic cell lines was evaluated by nested reverse transcriptase PCR. CB2 mRNA levels decreased 40% with monocyte differentiation, while mRNA levels for CB1 increased 40-fold or remain unchanged for GPR55. Modulation of inflammation was evaluated by pretreatment of cells with a range of THC doses followed by immune activation via different pathways. THC treatment reduced: 1)TNF-induced ICAM-1 protein levels in undifferentiated THP-1 cells and monocytes [$p<0.05$]; 2) IL-6 levels produced by LPS-activated peripheral blood mononuclear cell [PBMC] [$p<0.001$] and monocyte cultures [$p<0.001$]; and 3) IFNβ-stimulated CXCL10 production by PBMCs [$p<0.001$] and monocyte-derived macrophages [$p<0.001$]. Intracellular flow cytometry revealed that the predominant cellular source of CXCL10 and IL-6 among PBMCs was the monocytes. Acute THC treatment of monocyte-derived macrophages or PBMCs had no effect on HIV-1 replication. However, chronic THC treatment of monocytes during differentiation into monocyte-derived macrophages or PBMCs during activation significantly inhibited HIV-1 replication as measured by p24 levels in cell supernatants. Levels of HIV-1 receptors, CD4, CXCR4, and CCR5 were not affected by THC or ETOH vehicle control during chronic treatments. Finally, a panel of biomarkers of immune activation associated with neurocognitive impairment or overall morbidity/mortality was measured in plasma samples from a cohort of 78 HIV-1-infected and 35 uninfected subjects. In the HIV-1-infected group 35.8% tested positive for cannabinoids compared to 37.1% in the control group. For the vast majority of biomarkers, cannabis use failed to correlate with immune activation in either group. Significantly decreased levels of sCD27 were observed in cannabinoid-positive subjects compared to cannabinoid-negative subjects in the uninfected group [$p=0.014$]. THC reduces inflammation and HIV-1 infection of target cells. However, futher investigation into the mechanism of the inhibitory affects of THC is required. Funded by NIH R01DA031017

85. HIGH THROUGH-PUT ASPIRATOR AND BIOASSAY SYSTEM FOR THE EVAL. OF RESIDUAL PESTICIDES APPLIED TO NAT. AND ARTIFICIAL SUBSTRATES

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The advancement and testing of residual insecticides on insects is an ongoing mission because of continuous changes in insecticide tolerance. To assay residual insecticides efficiently and rapidly it is necessary for a bioassay to consist of streamlined procedures and equipment specifically intended to gently handle test organisms. We have developed a high through-put bioassay system built around the utilization of an aspirator (Wynn gun) that is constructed of readily obtainable materials and can be modified for a range of insects. Our system has saved countless man-hours due to swifter preparation, implementation, and lower control mortality by comparison with systems where forceps are used for insect transfer. In order to quickly put into service new insecticides and their application techniques, the implementation of this high through-put bioassay system and equipment should be given priority since it will greatly reduce the time spent in bioassay testing.

86. SOUTH SUDAN HEALTH ASSESSMENT: OUTCOMES AND DEVELOPMENT TRENDS

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Introduction: Global health threats impact a variety of social phenomena including economics, political stability, trade, tourism, access to goods and services and demographic shifts. Health threats transition over time and space making some challenges more prominent as countries redefine borders, governments, and development priorities, as is the case with the newly independent Republic of South Sudan. Detailed and systematic quantitative and qualitative analysis of health problems is crucial for South Sudan as the government designs health interventions and national budgets to address the paradigm shift of relief aid to development aid in the emerging health sector. While Demographic Health Surveys (DHS) gather useful national and regional health and development data, new countries such as South Sudan lack region-specific data and rely on information generated from previous surveys like the 2006 Sudan Household Health Survey. This project took a novel approach to fill this gap by utilizing GIS methods to disaggregate data and make comparisons in South Sudan. **Methods**: The 2006 Household Health Survey data was obtained from the South Sudan National Statistics Bureau Data for four major health outcomes. Data on maternal mortality, undernutrition, malaria and diarrheal disease were extracted and combined with supplementary GIS layers to create regional profiles. Against these health outcomes, developmental trends of gender equality and women's empowerment, education, human population movements and conflict and food security and agriculture were examined. Conceptual frameworks were developed to address linkages between the national development trends and health outcomes through underlying determinants and risk factors. **Results**: Women in South Sudan had a 3.85 times greater risk for maternal mortality than those in Sudan. The risk of

undernutrition in South Sudan was high as 33% of people face food deprivation and 31% of children under five were estimated to be moderately underweight. Diarrheal death contributed to 12% of the total mortality in children under five with malaria being the lead cause. Analysis of the data showed significant variation in health outcomes across the different states and between South Sudan and Sudan illustrating zones of high priority with critical intervention and development needs. **Conclusion**: South Sudan must address education and capacity building, gender equality and empowerment, sustainable agriculture and food practices, and human population movements and demographic shifts resulting from previous and current conflict to improve the national health infrastructure. The study results may be used to improve future data collection methods and strategically allocate and prioritize resources per state.

87. IN-VITRO USES OF MICRODIALYSIS TO MEASURE UNBOUND DRUG CONCENTRATIONS

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Microdialysis is a minimally-invasive sampling technique which enables measurement of unbound drug concentrations and this technique has been extensively used in humans and animals including some novel usages such as plasma protein binding determinations, cytokine/biomarker sampling from tissues and delivery of drugs. The in vitro microdialysis may be used for the determination of unbound concentrations in isolated tissues, plasma, cell culture systems and bacterial culture without affecting the test system. This technique is a relatively new technique employed for the determination of plasma protein binding, which was largely determined by ultrafiltration

technique due to its high throughput. However, ultrafiltration carries several disadvantages and may not be used in circumstances, e.g. determination of unbound concentration in cell culture or bacterial cultures. While centrifugal force used in ultrafiltration may rupture cells, determination of unbound concentration in highly pathogenic bacterial cultures may be practically challenging using ultrafiltration. Additionally, the sample volume required by ultrafiltration is a major limitation in many situations. In our lab, we have used microdialysis technique in different situations where use of ultrafiltration was difficult. In our current work we present use of microdialysis in different circumstances and compare it with the ultrafiltration technique, if possible. In-vitro microdialysis technique was applied to measure the unbound concentration of a selected antibiotic agent. The experiments were performed using a microdialysis probes CMA63 and the infusion rate was optimized to exhibit satisfactory probe recovery. Probe recovery was estimated using extraction efficiency technique. The entire set up was maintained at 37±1 °C during the sampling and recovery experiment. We have successfully used the microdialysis technique in different situations where ultrafiltration was not a viable technique. One of the major limitations of microdialysis technique is that the drug should be dialyzable.

88. THE EFFECTS OF SILVER NANOPARTICLE ADMINISTRATION ON INTESTINAL MICROBIOTA COMPOSITION IN C57/B7BL6 MICE

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Silver nanoparticles are currently being explored for their known antimicrobial properties. Several studies have been conducted using silver nanoparticles to investigate their effect on different organs such as the liver, brain, and kidneys. However, to date, few studies have analyzed their effects on the composition and diversity of gut microbiota. Here we investigate in a mouse model the hypothesis that administering silver nanoparticles will alter the microbiota composition. For this study, we used 10 healthy male C57/B7bl6 mice separated into a control group (N=5) and treatment group (N=5). Both groups of mice were allowed to acclimate to their environment for a week prior to start of treatment. Two stool pellets were collected from each mouse at baseline (0 hrs) and at 24 hour intervals, up to 72 hours, following administration of treatment. The treatment group received a daily dose of 11mg/kg of silver nanoparticles suspended in a colloidal solution and administered via oral gavage. The control group received an equivalent volume of colloidal solution. At 72 hours the mice were sacrificed and cecum and small intestinal contents were collected. Microbial community DNA was extracted from the fecal matter and used for DGGE analysis and 16S rRNA sequencing to determine diversity and composition. DGGE analysis did not show statistically significant differences in diversity of stool and cecum samples. By contrast the small intestine DGGE analysis did show a statistically significant difference in the Simpson diversity index between the cases and controls. For a more detailed analysis we performed 16S rRNA 454 sequencing. These results support our hypothesis that changes in gut microbiota composition occurred after treatment with silver nanoparticles. Effects of these microbiota changes on health are currently unknown.

89. VOLTAGE-SENSITIVE POTASSIUM CHANNELS EXPRESSED BY HORMONE TREATMENT IN MOSQUITO CELL LINES

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At present, in vitro screens with insect nervous tissue often employs primary cultures that are time-consuming to establish and typically survive only a few weeks, leading to major obstacles associated with insecticide discovery. The goal of this research was to evaluate the presence of insecticide target proteins from undifferentiated insect cell lines, which could lead to new high throughput screening methods and a way to mass produce insect proteins for basic research. This study used cultures of Sua1B cells with the application of 20-hydroxyecdysone (20-HE), an insect molting hormone, to initiate expression of insecticide target proteins, such as ion channels and neurotransmitter receptors, and to evaluate insecticidal compound effects. Electrophysiological studies, using whole cell patch clamp techniques, have shown the presence of KIR inwardly rectifying and Kv2 delayed-rectifier potassium channels expressed in as little as four hours after treatment with 20-HE. The expressed currents had current-voltage relationships diagnostic or these channels, and were inhibited with an IC50= 3 mM of tetraethylammonium (TEA), a well established potassium channel blocker. Another potassium channel blocker, 4-aminopyridine, showed inhibition of cell growth when applied in combination with 20-HE in Sua1B cells. The direct presence of ion channels and receptors in these cells will accelerate high throughput screening for new insecticides, and make screening more economical.

90. EMERGING PATHOGENS AS A TOOL FOR TEACHER PROFESSIONAL DEVELOPMENT

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A recent push for STEM (Science, Technology, Engineering, and Mathematics) education at all levels has increased the need for content-based professional development for teachers. The Center for Precollegiate Education and Training (CPET) has long offered such professional development by partnering with University of Florida (UF) researchers to bring cutting edge science content into secondary school classrooms. One such program is funded with a grant from the Howard Hughes Medical Institute (HHMI), with a focus on emerging pathogens. With such a theme, CPET has collaborated with the Emerging Pathogens Institute (EPI) to develop a year-long program for middle and high school science teachers, the Interdisciplinary Center for Ongoing Research/Education (ICORE) Partnership Program. By utilizing a 2 week summer institute of seminars, wet labs, lab visits, and pedagogical assistance, these educators take the interdisciplinary nature of EPI back to their classrooms through innovative learning modules. As such, ICORE has three major goals: (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. Over the five years of ICORE, the program has reached nearly 150 teachers and over 16,000 of their students through modules developed during the program, as well as our equipment borrowing program and classroom and campus visits for wet labs with CPET laboratory teaching specialists. This program has also led to the development of several curricula on emerging pathogens, including one on Vibrios and another on Dengue fever. Teachers have indicated through pre and post surveys and focus groups that ICORE was the most meaningful professional development program that they had ever participated in, and that the collaboration with university faculty was key in the program's success. As the 5-year HHMI grant comes to a close, we discuss future directions for secondary science teacher professional development through

CPET, and how UF researchers can partner with CPET programs for their broader impacts.

91. CHARACTERIZING CLIMATOLOGICAL HEAT WAVE RISK IN FLORIDA USING EXTREME VALUE ANALYSIS

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Maximum daily temperatures from the second half of the 20th century are examined using a high resolution dataset of 833 grid cells across the state of Florida. An Extreme Value Analysis approach is used to model characteristics including the frequency, magnitude, duration, and timing of periods or heat waves where daily maximum and minimum temperatures are above 95th percentile thresholds. Variability in heat wave characteristics are examined across the state to give an indication of where heat waves with certain characteristics may be more likely to occur. Changes in heat wave characteristics through time are also examined. The temperature record is divided into two equal time periods and changes to heat wave characteristics are analyzed between the two periods allowing for an exploration of changes in heat wave risk through time that gives an indication of trends in future heat wave risk. Preliminary results indicate that there is considerable spatial variability in heat wave characteristics across the state and that heat waves are becoming more intense throughout much of the state.

92. SEMIPARAMETRIC RELATIVE-RISK REGRESSION FOR INFECTIOUS DISEASE DATA

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We introduce semiparametric relative-risk regression models for infectious disease data based on contact intervals, where the

contact interval from person i to person j is the time between the onset of infectiousness in i and infectious contact from i to j. The hazard of infectious contact from i to j is the product of a baseline hazard function and a relative risk function of a covariate vector X and a coefficient vector β. When who-infects-whom is observed, the Cox partial likelihood is a profile likelihood for β maximized over all possible baseline hazard functions. When who-infects-whom is not observed, we use an expectation-maximization (EM) algorithm to maximize the profile likelihood for β integrated over all possible combinations of who-infected-whom. This extends the most important class of regression models in survival analysis to infectious disease epidemiology.

93. Novel Mode of Action of Synthetic Mosquitocides for Control of Disease Vector Mosquitoes, Aedes aegypti and Anopheles gambiae

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Development of resistance within disease vectors has created the need for the development of insecticides with novel modes of action. With this in mind, we have developed a larval bioassay that bypasses the cuticular barrier to assess compounds that are known potassium channel blockers. The exposure of head-less Aedes aegypti larvae over a 5-hour period has shown that when probed, the larvae in control solutions still show strong contractile movement, while drug exposure causes the larvae to have sluggish to no movement at all. Many of the known potassium channel blockers have LC50 values of ca. 100 ppm. Tetraethylammonium chloride and 4-aminopyridine however

have significantly lower LC50 values (9.69 ppm 95% fiducial limits 1.76-26.79 ppm and 3.13 ppm 95% fiducial limits 0.521-7.35 ppm, respectively). In addition to the headless larval assay, a 24-hour larval bioassay that utilized intact larvae was run using experimental compounds. The compounds showed moderate toxicity to fourth instar Aedes larva with PRC725 having an LC50 value of 40 ppm, PRC728 at 141 ppm, and β-Thujaplicin at 154ppm (95% fiducial limits 36-45 ppm, 121-164 ppm, and 135-174 ppm respectively). Spastic paralysis was observed with compound PRC725 on both the Aedes and Anopheles gambiae larvae. In addition to spastic paralysis, a blackening of the anopheline larvae was seen. This effect is thought to be due to oxidation of the catechol PRC725 by phenol oxidase. Electrophysiology was also performed on Aedes larvae resulting in terms of muscle contraction and depolarization responses for 4-aminopyridine and PRC728. Contraction data recorded with an isometric force transducer showed that 1 mM 4-aminopyridine and 800 μM PRC728 caused an increase in larval body wall muscle tension, while reducing the force of electrically evoked contractions. The depolarization data corroborates the contraction findings by showing that there is a large depolarization shift at 1mM PRC728, as measured with an intracellular microelectrode. While the compounds screened thus far may not have a high enough toxicity to become viable insecticides for practical use, the data show that potassium channel directed compounds are a possible target for future novel insecticides.

94. GIANT CELL MENINGOENCEPHALOMYELITIS IN A PREGNANT ANDALUSIAN MARE

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Multiple institutions across the United States have encountered cases of a unique granulomatous meningoencephalomyelitis in horses. However, this is the first formal report of such a case where investigations have resulted in identification of a potential etiology. A pregnant mare was euthanized after rapidly developing a fever and neurologic signs, including progressive ataxia and intermittent obtundation. Analysis of the CSF indicated a severe neutrophilic pleocytosis comprised of 502 WBC/µL (27% neutrophils, 62% lymphocytes, and 11% macrophages) and 47 RBC/µL. Leukophagia and erythrophagia were frequently observed. Histopathology revealed a lymphohistiocytic leptomeningitis and encephalitis, most prominently within the dorsal cortex, hippocampus, and cerebellum, which extended into the cervical spinal cord. Affected areas exhibited multifocal lymphohistiocytic perivascular inflammation that was often admixed with multinucleate giant cells (MNGCs) and occasionally surrounded by areas of hemorrhage and necrosis. Multinucleated giant cells were most numerous within the hippocampus and cerebellum, containing up to 30 nuclei, although areas with MNGCs were not always associated with perivascular cuffing. No organisms were identified within MNGC-rich areas. Testing for rabies, arboviruses, flaviviruses, equine infectious anemia, and equine herpes virus was negative. Viral cultures and testing was expanded to included novel species of Orthoreoviruses and Herpesviruses, all genera of the Paramyxoviridae family, and

equine influenza virus. The brain tissue was cultured in numerous cell lines including Vero, MDCK, and LLCMK2 cells, resulting in cytopathic affects characterized by karyomegaly, enlarged nucleoli, and syncytia. Negative staining electron microscopy of spent media from LLC-MK2 cells resulted in the detection of 80 nm diameter virus-like particles.

95. EXPANDING SEX AND GENDER DIFFERENCES RESEARCH AND EDUCATION AT THE UNIVERSITY OF FLORIDA

Hannah Norton - Health Science Center Library, University of Florida; **Linda Butson** - University of Florida, Health Science Center Library; **Mary Edwards** - Health Science Center Library, University of Florida; **Nancy Schaefer** - Health Science Center Library, University of Florida; **Michele Tennant** - Health Science Center Library, UF Genetics Institute, University of Florida

Throughout much of history, the majority of medical research and thinking has been based on studies of the human male. Anatomy and physiology have focused on obvious differences in reproductive structures, but evolving molecular and genetic technologies are increasingly enabling researchers to detect biochemical and other biological differences across body systems. Over the past decade, researchers and clinicians, including those at the University of Florida, have begun publishing study results and clinical experiences related to sex differences in symptom presentation, reaction to therapy, and prognoses. The University of Florida Health Science Center Library (HSCL), in collaboration with the National Institutes of Health's Office of Research on Women's Health and the National Library of Medicine, has made a commitment to disseminating information on research in sex and gender differences in health, facilitating the growth of basic research in this area, and helping develop a diverse clinical workforce able to recognize these differences and to apply this knowledge in clinical care. In support of these goals, the HSCL is currently undertaking a seven-part outreach effort related to sex and gender differences research. Librarians are introducing students to this field and its resources, with the expectation that such an introduction may lead some to focus on sex differences

research, and others to be aware of the importance of sex differences in other areas of research and patient care. Early career faculty are also being introduced to these issues and resources, with the similar expectation that this may influence their choices of research focus and may raise awareness of the importance of sex differences in patient care. These outreach efforts include student training within existing coursework and orientations for undergraduate, medical, and PhD biosciences students and faculty training and professional development. Additional efforts include collection building and increasing document access in the areas of women's health and sex and gender differences through open access publishing funding.

96. Fecal and Bloodborne Pathogen Community Assemblage of an Invasive Alien Species: Feral Swine in the Southeastern U.S.A.

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Feral swine were first introduced to the United States in the 1400s by European explorers but have recently spread to more than 38 states and increased in population size to exceed 5 million individuals. Both the World Conservation Union and the Invasive Species Specialist Group have identified feral swine as

the “world’s worst invasive alien species”. Additionally, feral swine have been identified as posing serious threats to human, domestic animal and wildlife health as well as the environment because of their ability to harbor a wide range of communicable pathogens. We used a state-of-the-art pathogen detection microarray to monitor the presence of >6000 unique viral, bacterial, and fungal microbes in the blood and feces of 20 feral swine collected at 4 locations across the southeastern United States.

97. UNDERSTANDING AND PRIORITIZING CLIMATE CHANGE ADAPTATION NEEDS FOR FOOD SECURITY IN THE ANDES

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Climate change will affect the crop production systems of the Andes in many ways. Recent studies based on local meteorological networks have evidenced a significant warming

after 1979 (0.32 -0.34°C/decade). Climate change models for the Andes indicate continued warming of the troposphere throughout the 21st century, increased precipitation in the wet season (when potatoes are grown in the Andean highlands), and decreased precipitation in the dry season. Because the past and projected changes in temperature and precipitation will likely pose challenges for food production systems, especially in the highlands, a comprehensive study was initiated by the International Potato Center (CIP) and University of Florida (UF) to quantify the impact of climate change, the risks to potato based agricultural systems and the adaptation needs for food security in the Andes. To reach these objectives, an interdisciplinary research approach is needed. At UF, four graduate students are carrying out this research, one in each of four disciplines: crop simulation modeling, soil and water science, plant pathology and agricultural economics. Central to this research is the development of a potato simulation model by Raymundo and Asseng in collaboration with R. Quiroz (CIP). The simulated potato biomass (and that of rotation crops) will be included in a process-based carbon simulation model by Gavilan and Grunwald together with B. De Bievre and R. Quiroz (CIP). The potato simulation model will be extended with a submodel for late blight, the most important potato disease in the Andes and worldwide, by Shakya, Goss, van Bruggen and J. Kroschel (CIP). Economic components will be combined with the other models by Spagna, Stern and G. Hareau (CIP). Different climate change scenarios will be simulated using the IMPACT model framework to assess potential interactions and impacts of climate change on potato production and food security. Local level studies will follow to understand micro economic implications of risk, variability and extreme events on food systems in the Andes.

98. DETERMINATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY BY A RAPID DIAGNOSTIC TEST METHOD IN HAITI

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G6PD deficiency is a hereditary enzyme abnormality in which individuals with the disorder are more susceptible to oxidative stress due to medications or certain infections. The identification of this deficiency is of clinical importance especially in malaria endemic regions where severely deficient individuals are at risk of developing hemolytic anemia when treated with the gametocytocidal drug, primaquine (PQ). This information is particularly relevant to Haiti, where the national treatment policy for malaria was recently changed to include PQ with its previous monotherapy chloroquine (CQ), yet there is no information on the prevalence of G6PD deficiency in this country. The present study aimed to estimate the prevalence of G6PD deficiency in febrile patients attending the outpatient department in Hospital Sainte Croix in Leogane, Haiti, as part of a larger investigation into the epidemiology of malaria in Haiti. We hypothesized that the G6PD deficiency rates in Haiti are similar to the estimated 20% as found in West Africa, due to Haiti's West African lineage. In this preliminary study, febrile patients were tested for G6PD deficiency by a rapid test kit. The rapid test kit measures the change in enzyme activity between normal and deficient blood by a spectrophotometric calibration method that enables the determination of G6PD status. A total of 55 patients (25 males and 30 females) were tested out of which 10.9% (6/55) were found to be severely deficient in the G6PD enzyme. Furthermore, 5.5% (3/55) of the patients were found to have an intermediate level of G6PD enzyme activity. No difference in enzyme activity was observed between males and females, but any statistical inferences were limited by our small sample size. Our preliminary

data suggest that the G6PD deficiency prevalence in Haiti is approximately 16%, which may be very similar to rates observed in West Africa. However, due to the small sample size in our study we recommend expanded studies on G6PD deficiency in Haiti. Haiti's standard practice of presumptively treating febrile patients for malaria may expose non-infected patients to the potential hemolytic effects of PQ, thus more data is needed on G6PD deficiency rates in both the general and febrile population. Screening patients for G6PD deficiency may be appropriate and necessary in the coming era of primaquine treatment in Haiti.

99. COMMUNITY BASED NEEDS ASSESSMENT SOUTH SUDAN

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Emerging from half a century of war and neglect, South Sudan, the world's newest nation, faces an uncertain future filled with extreme challenges and opportunities. Despite possessing vast natural resources, development indicators rank South Sudan among the poorest and most dangerous countries in the world. Facing depleted government funds, volatile security issues, a sparse infrastructure and limited capacity for delivering public services, the Government of South Sudan and its development partners are tasked with building a healthy and functioning state. To aid in development planning there is a great need for information on enduring social and economic constraints and resources in communities throughout South Sudan. Contributing research should simultaneously address community needs and assets while taking into account political and ethnic factors at the heart of the regions instability. The following research, which took place between April and July, 2012 in the remote village of Maar, Pakeer Payam (district), Twic East County, Jonglei State, South Sudan, explores community assets and needs in a remote community in South Sudan, including a map of local clan structures, a state initiated needs assessment and enduring economic practices.

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