EPI *Research Day*

UF | Emerging Pathogens Institute
UNIVERSITY *of* FLORIDA

LETTER FROM THE DIRECTOR

February 10, 2011

Welcome to the fourth annual EPI Research Day! This day is notably exciting because it marks a year since we began operating in our new building, a space that has allowed us to better facilitate interdisciplinary collaborations in the field of emerging pathogens and infectious diseases. As you peruse the research at today's event, it is my hope that it will give you a feel for the wide range of emerging pathogens-related research conducted by EPI members and their collaborators. I would like to say a particular word of thanks to University of Florida investigators from outside of Gainesville and to our partners at the Florida Department of Health.

We are honored to have two outstanding speakers for our afternoon session. Dr. Frederick M. Ausubel is a professor of genetics in the Department of Molecular Biology at Massachusetts General Hospital and in the Department of Genetics at Harvard Medical School.
Dr. Lonnie King is dean of the College of Veterinary Medicine at Ohio State University, and serves as a member of the EPI External Scientific Advisory Committee. I greatly appreciate their contribution to the day's activities.

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.

J. Glenn Morris, Jr., M.D., M.P.H. & T.M.
Emerging Pathogens Institute, Director and Professor of Medicine
University of Florida

RESEARCH DAY 2010
SCHEDULE OF EVENTS

9:00 AM - 10:00 AM	Coffee & Breakfast *(EPI Rm. 150)*; poster set up *(EPI Front Lawn)*
10:00 AM - 1:00 PM	Poster Session *(presenters, please stand by your posters)*
NOON - 12:45 PM	Lunch *(EPI Rm. 150)*
1:00 - 1:15 PM	*(Introductions by Dr. J. Glenn Morris, Director, EPI)* Welcome by **Dr. Win Phillips**, UF Vice President for Research
1:15 PM - 3:30 PM	Keynote Speakers

EPI is proud to present keynote speeches by

Dr. Frederick M. Ausubel
Professor of Genetics
Department of Molecular Biology, Massachusetts General Hospital &
the Department of Genetics, Harvard Medical School

"Identification of Novel Antimicrobial Compounds that Target Bacterial Virulence or Host Immunity" *(1:15 PM - 2:15 PM, Cancer Genetics Research Auditorium)*

Dr. Lonnie King
D.V.M., M.S., M.P.A., Diplomate A.C.V.P.M.
Dean of the College of Veterinary Medicine, Ohio State University

"Emerging Infectious Diseases: A Mandate for One Health"
(2:15 PM - 3:15 PM, Cancer Genetics Research Auditorium)

01. Genetic and Physiologic Basis of the persistence of *Vibrio cholerae* in the Filter Sterilized Lake Water

Marzana Ahmed[1], J. Glenn Morris, Jr[1]., and Afsar Ali[1]
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Toxigenic *Vibrio cholerae* is an important cause of epidemics in cholera endemic countries where sanitary conditions are not optimal. Humans acquire the infection by ingestion of food and water contaminated with the bacterium. While the primary reservoir for the microorganism is the aquatic environment, we have found in studies in Bangladesh that isolation rates from aquatic sources tend to be low, even in the setting of epidemic disease. We hypothesize that upon entrance into fresh water only a subset of cells are able to adapt to the resultant low-nutrient environmental conditions and persist for extended periods of time. To test our hypothesis, we inoculated individual colonies of *V. cholerae* strain N16961 into flasks containing filter sterilized fresh water (microcosms). Often microcosms established, long-term persistence of culturable *V. cholerae* (in excess of 230 days) was seen in four (40%); the direct transfer of *V. cholerae* cells from surviving microcosms to a fresh microcosm resulted in a similar rate of persistence. While supplement of available carbon (sucrose 2%, glucose 1%) in microcosms was unable to promote the persistence of *V. cholerae*, phosphate suppplementation (0.5, 1.0 and 1.5mM) resulted in persistence of *V. cholerae* for at least 53 days. *phoB/R* and *pst* mutants, in which there is inhibition of phosphate metabolism under low phosphate conditions, persisted in phosphate supplemented microcosms, but not in standard low-nutrient microcosms, underscoring the major role of phosphate in the persistence of *V. cholerae* in nutrient poor environments. In summary, when placed in nutrient-poor fresh water conditions, the majority of *V. cholerae* cells die off, with surviving cells appearing to shift to a non-carbon based life style; however, persistence is prolonged with increasing phosphorus concentrations. Our findings have clear implications for the understanding of the ecology of *V. cholerae* and potentially of other food- and waterborne human pathogens.

02. Development and Implementation of an antimicrobial barrier wound dressing optimized for prophylactic use

Albina Mikhaylova, Ph.D., Quick-Med Technologies (QMT), 2. Bernd Liesenfeld, Ph.D., QMT, 3. William Toreki, Ph.D., QMT, 4. David Moore, B.Sc., QMT, 5. Jillian Vella, B.Sc., QMT, 6. Lisa Youngblood, ASN, University of Florida, 7. Robert Nappo, MSN, University of Florida, 8. Janet Popp, BSN, University of Florida, 9. Gregory Schultz, Ph.D., University of Florida

Antimicrobial dressings are becoming increasingly attractive to aid in the prevention of nosocomial infections, partly based on increasing pressures on healthcare systems to drive down costs. Currently, the most prevalent antimicrobial dressings in the marketplace are silver based. These function well in controlling microbes and are a very good option for some types of wounds, but may be too aggressive and too expensive for many prophylactic applications. We describe the product development and testing done to bring an antimicrobial barrier dressing optimized for prophylactic use to market. The emphasis is made on the underlying principle of antimicrobial efficacy for the technology, efficacy and safety testing performed. We also illustrate the regulatory submission process to receive market clearance from FDA, which was achieved through a deNovo process (for products that have no direct predicate device) as opposed to the originally planned 510(k) (for devices that have a direct predicate cleared by FDA). Production development was performed concurrently with regulatory clearance, allowing a quick progress to market (as the BIOGUARD dressing line sold by Derma Sciences). Initial clinical observations at Shands have been very positive. The Shands Burn Unit has documented the ability of the dressings to suppress bacterial growth when used on patients with heavily exudative wounds where traditional gauze dressings foul rapidly (within 12-24h). Caregivers from the Burn unit believe that the reduction in bacteria on the dressings could lead to a decrease in the contamination of open wounds, as compared to standard dressings. Additional benefits of using antimicrobial bandages may include reduced wound odor, frequency of dressing changes, and spread of bacteria from fouled dressings to the patient and clinical personnel.

03. Quinacrine impairs pathogenicity in *Francisella tularensis*

Algevis Wrench, Chris Gardner, Claudio Gonzalez, and Graciela Lorca
[Department of Microbiology and Cell Science, University of Florida]

In the highly infectious intracellular pathogen *Francisella tularensis*, up-regulation of virulence genes is mediated through MglA-SspA interactions with the RNA polymerase (RNAP). In addition, the DNA binding proteins PigR and PmrA make contact with the MglA/SspA/RNAP complex to positively regulate the expression of genes clustered in the F*rancisella* pathogenicity island (FPI). We hypothesized that small molecules can be used to modify the MglA/SspA interaction, affecting their ability to induce transcription of the FPI genes. To identify interacting small molecules, differential scanning fluorometry was used to screen MglA and SspA against chemicals in the Prestwick library. The efficacy of the compounds was tested *in vivo* using a bacterial two-hybrid system. The amino acid residues involved in the interactions were evaluated using site directed mutagenesis and differential scanning calorimetry (DSC). The effect of the small molecules in *F. tularensis* was evaluated by measuring the mRNA levels of FPI genes and the intramacrophage survival using cell lines. Quinacrine was identified as a thermal stabilizing compound for MglA and SspA of *F. tularensis* SCHU S4. This compound was able to modify MglA/SspA interactions, as shown by a 38.3 % decrease in β-galactosidase activity when compared to the control. Based on DSC, both Tyr63 of MglA and Glu101 of SspA are necessary for the binding of quinacrine to the MglA/SspA complex. Quinacrine decreased the expression of *iglA*, *iglD*, *pdpA* and *pdpD* by 2.1; 1.5; 2.2 and 5 folds, respectively. In addition, intramacrophage survival of *F. novicida* decreased by 2.5-log units in the presence of quinacrine. Using a combination of high-throughput screening, biochemical and functional assays, a small molecule capable of modifying the interactions between MglA and SspA was identified. Consequently, quinacrine specifically interferes with the ability of *Francisella tularensis* to activate gene expression, resulting in decreased intracellular viability, and ultimately neutralizing the infection.

04. Establishment of Critical Operating Standards for Chlorine Dioxide in Disinfection of Dump Tank and Flume Water for Fresh Tomatoes

Angela M. Valadez[1], Michelle D. Danyluk[1], and Trevor Suslow[2]
[1] Department of Food Science and Human Nutrition, Citrus Research and Education Center, University of Florida, Lake Alfred, FL [2] Plant Science Department, University of California, Davis, CA]

Postharvest water quality management represents one of the few unit operations that approach a true *critical* control point for fresh produce. For fresh tomatoes, the dump and flume tank water sanitation has been the focus from industry and public health regulatory for several years due to the recognized potential for introduction and widespread cross-contamination of human pathogens from incoming fruit and environmental sources that may occur. Renewed interest and promising recent model system work with chlorine dioxide (ClO_2) exist for fresh tomatoes, however very little is known about the management of this sanitizer in dump tank and flume water. The objectives was to develop scientifically-based critical operating standards for ClO_2 use in dump tank and flume tank waters in Florida packing houses for regular round, mature-green tomatoes (MG) by measuring: bacterial loads of incoming and final washed fruit, fruit pulp temperature, water temperature, pH, ClO_2 residual levels, turbidity, conductivity, oxidation-reduction potential (ORP), and bacterial loads of treated water over a full packing shift on four selected seasonal dates within commercial tomato packing operations in Florida. During the first packing shift, water operation zones D and AS, the beginning of each flume, and RF1 and RF2, the end of each flume were comparable. Tomato pulp temperatures of both top and bottom of the gondola were comparable and were below the temperature of the tomato pulp after the ClO_2 wash. The pH of the water operation zones ranged from 7.4-8.0. The turbidity, conductivity, and ORP of water operation zones D and RF1 were comparable, the 1st flume, as were AS and RF2, in 2nd flume. Mesophiles and coliform bacteria were isolated on both tomatoes and in all the water operation zones. *E. coli* was isolated at random points from water operation zones D, RF1, and AS throughout the sample day. Ultimately, this study will develop scientifically-based critical operating standards for chlorine dioxide use in dump tank and flume tank waters directly applicable for the fresh tomato industry.

05. Evolution of an RNA virus in predictable and unpredictable temperature environments

Barry W. Alto[1, 2], Nadya M. Morales[2], and Paul E. Turner[2]

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The differing ability for organisms to respond to environmental changes can dictate their future evolutionary success. All biological entities occasionally experience environmental change. It is implicitly assumed that a constant habitat will tend to select for specialization: evolution of adaptive traits that foster survival and reproduction under nonvariable conditions. It is also assumed that these specialist traits will tend to trade-off with performance in alternative habitats. It is expected that specialists will perform poorly when growing under conditions in which they have not been selected. Generalists, by contrast, can thrive under broader conditions because they have experienced selection in multiple habitats. Adaptation to multiple environments occurs through experiencing environmental variation. However, environmental variation may manifest itself in a number of ways, both spatially and temporally. Further, another component of adaptation to variable environments is whether environmental variation is predictable or unpredictable. Adaptations to unpredictable environments are likely to constrain performance and rate of evolution relative to predictable environments. The differing ability for viruses to respond to environmental changes can dictate their future evolutionary success, an important public health concern for emerging RNA viruses. Experimental evolution of RNA viruses is a powerful approach for conducting research on the evolutionary consequences of virus adaptation under environmental change. These model systems feature high mutation rates, short generation times, large population sizes, and indefinite freezer storage allowing direct comparisons between an ancestor and its evolved descendants. We examined the consequences of adaptation to constant and variable temperature environments using vesicular stomatitis virus (VSV). VSV is an RNA virus that infects insects and mammals. Replicate populations of a wild type VSV clone were allowed to evolve for 100 generations at constant temperatures (29ºC or 37ºC) and in environments where temperature fluctuated in a predictable or random way between 29ºC and 37ºC. Contrary to predictions, viruses selected for specialization were not associated with significant fitness trade-offs in alternative environments. Selection for generalization was associated with the highest and lowest fitness gains for predictable and unpredictable environmental changes, respectively.

06. Characterization of the Emerging Killer Cold Virus Adenovirus type 14a

Benjamin D. Anderson, Kelli L. Barr, Ph.D, Gregory C. Gray, M.D., M.P.H

The last decade has seen the emergence of a newly mutated serotype of Adenovirus, the prevalence of which has significantly increased across the United States. Studies have shown that this new strain of Adenovirus, typed 14a, causes more severe symptoms in patients, such as acute febrile respiratory illness (FRI), gastroenteritis and pneumonia. In addition, mortality from this new strain has also increased primarily in children and the elderly. Molecular analysis has successfully isolated the mutation, presenting as a two amino acid deletion on the fiber knot gene of the virus. No studies have yet been conducted to characterize the fitness of Adenovirus 14a to determine the causes in increase in virulence. In this study, we performed assays designed to determine the viral growth, plaque size and competitive fitness of Adenovirus 14a, designing our experiments to compare it with the prototype Adenovirus 14p strain. For virus growth we used a standard plaque assay to develop a growth curve, assessing type 14a and 14p virus titers at time of infection, and every 24 hours up to 168 hours (T_0, $T_1 \ldots T_7$). Plaque size was also determined using the same plaque assay for virus growth. Competition was analyzed by co-infection and quantification of the relative ratios of DNA in both strains using PCR and enzyme digestion techniques.

Samples from both growth and competitive fitness assays are currently being analyzed to determine final virus titers and DNA concentration of both mutant and prototype Adenovirus serotypes. If Adenovirus 14a succeeds over the prototype 14p strain in both growth and competitive fitness, then further studies should be conducted to determine the mechanism in which the 2 amino acid deletion conveys this increased fitness in human populations.

07. Establishing a University of Florida-DoD-GEIS Field Laboratory for Infectious Disease Research in Haiti

Bernard Okech[1,2], Dana Focks[1,2], Ali Asfar[1,2], Matthew Montgomery[3], Gary G. Clark[4], Edsel Redden[1], Andrew Kane[1,2], Michael Perri[1], Glenn Morris[2], Gregory Gray[1,2][1. Department of Environmental and Global Health, University of Florida, Gainesville 32610; 2. Emerging Pathogens Institute, University of Florida, Gainesville 32610; 3. Navy Environmental Preventive Medicine Unit 2, 1887Powhatan Street, Norfolk, VA 23511-3394; 4. Mosquito and Fly Research Unit, Center for Agricultural and Medical Entomology, United State Department of Agriculture, Gainesville, FL]

We are developing an infectious disease laboratory in Haiti to support University of Florida's research in infectious diseases and to provide infrastructure for public health disease surveillance for DoD-GEIS and the Haitian Ministry of Public Health and Sanitation. The field laboratory will also be used for preparation of samples and specimens before shipment to UF for further in-depth studies. The laboratory would support collaborations between the UF scientists, DoD and others interested in disease surveillance, research, and intervention work in support of the Haitian Ministry of Health and Sanitation. The laboratory is located in Gressier, 20 miles west of Port-Au-Prince in a gated and guarded compound of FISH Ministries Haiti (an NGO). FISH Ministries Haiti has been working in Haiti for many years and collaborates closely with the University of Florida. FISH Ministries offered land to build the laboratory and there is an adjacent guest house where UF researchers and collaborators could stay and work. A bigger guest house with modern accommodations, multiple bathrooms, a kitchen, and stable electrical power through generator support is almost complete. This facility will serve as headquarters for University of Florida, Department of Defense and other collaborating scientists and will be available to other research groups interested in public health in the region.

08. Molecular Analysis of *Plasmodium falciparum* Drug Resistance in Haiti

Bernard A. Okech[1,3], Jeannette Constante[4], Robert Cook[2], David Walmer[4], Jean Yves Saint Victor[4] [1) Department of Environmental and Global Health, University of Florida, Gainesville 32610; 2) Department of Epidemiology, University of Florida; 3) Emerging Pathogens Institute, University of Florida; 4) Family Health Ministries, Blanchard Clinic, Haiti]

Malaria is a major cause of morbidity and mortality in Haiti. Management of malaria is being hampered by drug resistant *Plasmodium falciparum*. Although the drug resistant phenotype of malaria is well established in many areas around the world, Haiti is reportedly free of chloroquine resistant *Plasmodium falciparum* even with the continued use of chloroqiune in many clinical settings in Haiti. The reasons why CQ resistance has not spread to Haiti are not clear. In this research, the prevalence of resistance phenotypes of *Plasmodium falciparum* parasites will be determined in three sites in Haiti. *P. falciparum* samples will be collected from infected patients and used for: 1) Genotyping for drug resistance using 4 molecular markers, and 2) I*n-vitro* culture for drug sensitivity testing. In addition, infected malaria patients will be followed every 7 days for 28 days after treatment to monitor treatment outcomes.

09. Ecological Studies of Dengue Mosquito Vectors in Haiti

Bernard Okech[1,2], Jean Pierre Miller[3], Jean Pierre Benier[3], Herold Guillame[3], Matthew Montgomery[4], Dana Focks[1,2]
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Dengue is endemic in Haiti and is major public health problem. While it is minimally important to native Haitians as they have acquired immunity, to non-immune visitors, dengue fever and the potentially deadly dengue hemorrhagic fever and dengue shock syndrome are a major problem. All the 4 dengue serotypes (DEN1, DEN2, DEN3, DEN4) occur in Haiti. There is no active surveillance of dengue transmission making it very difficult to estimate potential number of cases nor is there active dengue vector control in Haiti. Extremely high rates of dengue transmission have been found. With no dengue vaccine available, controlling the dengue mosquitoes remains the most effective preventive measure against transmission of dengue. Our research aims to collect detailed information about potential dengue mosquito species, breeding container types and productivities and insecticide resistance status that would facilitate control measures for dengue mosquitoes in Haiti. This research will be conducted in 2 sites; Gressier (Rural) and Terre Noirre, Port Au Prince (Peri-Urban) . Larval demographic surveys will be conducted to calculate the **Breteau index** (index of the number of containers with immature stages per 100 houses surveyed), the **house index** (the number of houses per 100 houses containing immature stages), the **container index** (the number of containers with immature stages per100 containers with water), and **pupae/person index** (the number of pupae collected/human population in a sector). In addition, oviposition traps will be set up to calculate the proportion of indoor, outdoor and total ovitraps positive for Aedes eggs, after 7 days of trap exposure. Pupal demographic surveys will also be conducted in 400 houses per location. In addition, adult mosquitoes (biting and gravid) will be colletced from the houses and tested for insecticide susceptibility and dengue infection. Data will be used in models that would permit the development of a targeted source reduction strategy for dengue vectors in Haiti.

10. Stay-Fresh Medical Textiles to interrupt pathogen transfer in healthcare settings

Bernd Liesenfeld, Ph.D., Quick-Med Technologies (QMT); William Toreki, Ph.D., QMT; Albina Mikhaylova, Ph.D., QMT; David Moore, B.Sc., QMT; Susan Leander, M.Sc., QMT; Christopher Batich, Ph.D., University of Florida

The disruption of pathogen transfer on absorbent surfaces is a major research goal for many applications. Healthcare settings in particular feature patients with a reduced resistance to bacterial infection. There are many technologies offered to achieve this goal, but most of these have significant shortcomings for use in durable textiles: cationic biocides are blinded by anionic detergents, and leachable biocides added to textiles can deplete in laundering. In response to these challenges, we have developed an antimicrobial based on peroxides sequestered in an inert metal oxide binder. Treated textiles (we call this treatment *Stay-Fresh*™) were tested to demonstrate durable and highly effective microbial control. Treated cotton textiles were laundered 'hot' for 25 to 75 cycles (normal consumer laundering conditions and detergents). Antimicrobial efficacy was validated per industry standard methods against bacteria (AATCC method 100) and fungi (ASTM G21 and D3273, AATCC 30). Antimicrobial testing showed this material to be effective against a wide range of pathogens, including resistant strains, showing >99.99% kill against MRSA and VRE strains. The material also remains effective in the presence of bodily fluids, and is able to withstand multiple inoculation challenges. Analytical techniques were applied to confirm persistent peroxide activity and retention of binder phase in the fabrics. SEM and EDS analysis confirmed the treatment-induced morphological and chemical changes on the surface and in the body of the fibers.

11. Incidence and awareness of ciguatera fish poisoning in St. Thomas, U.S. Virgin Islands

E Radke[1], L Grattan[2], S Roberts[2], M Abbott[1], and JG Morris[1]
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Introduction Ciguatera fish poisoning (CFP) is a significant public health concern for persons in tropical and sub-tropical areas. We performed a telephone survey to estimate the incidence of CFP in St. Thomas, describe those affected, and gauge public awareness. *Methods* Home and cellular phone numbers were randomly selected from a database of residential telephone exchanges. Incidence was estimated with emergency department (ED) data from the local hospital and survey data on the proportion of people who visited the ED during their most recent CFP episode. Chi-square tests were used to compare participants with history of CFP illness to those without. *Results* 407 households were contacted and willing to participate. Eighty-seven participants (21%) recalled ever having fish poisoning. Of these, 36% reported visiting the ED. Based on a four year average, the incidence was estimated at 3.6 per 1000 population (95% confidence interval=2.8-5.2). Factors associated with CFP were education, fish consumption, and being born in the Caribbean. A majority of participants believed that certain types of fish are poisonous, but no species was mentioned by more than 20%. A large proportion (41%) incorrectly believed they could tell if a fish is poisonous. *Conclusion* The annual incidence estimate is lower than older estimates in St. Thomas. Future surveys will use a more comparable measure to examine the trend over time. Despite at least 25% of households having someone affected by CFP, there were large gaps of knowledge that indicate the need for further public education. This telephone survey provides information about the status of CFP in the U.S. Virgin Islands and provides a base for planned future surveys on the island.

12. Antimicrobial effect of sodium metasilicate marinades on *Salmonella enterica* serovar Typhimurium and psychrotrophs in ready-to-cook skinless and boneless chicken breast meat stored at 4 ± 1°C

Chander Shekhar Sharma[1], Sally K. Williams[2], Gary E. Rodrick[1]
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The objectives of this study were to determine the antimicrobial effects of sodium metasilicate (SMS) marinades against *Salmonella* Typhimurium and psychrotrophic organisms in fresh marinated ready to cook skinless and boneless chicken breast meat, and to ascertain the effects of the treatments on pH. The chicken breasts were inoculated with *S.* Typhimurium (ATCC 14028), marinated in solutions containing either 0% SMS and no inoculum (negative control), 0% SMS and inoculum (positive control), 1% or 2% SMS, packaged and stored at 4 ± 1°C. All samples were analyzed in duplicate after 0, 1, 3, 5 and 7 days of storage for recovery of *S.* Typhimurium, psychrotrophic organisms and pH measurements. Chicken breasts marinated with 1% and 2 % SMS had lower ($P < 0.05$) *Salmonella* counts when compared to the positive control at 3 d storage and through 7 d. Chicken breasts treated with 1% and 2% SMS resulted in 0.83 to 0.91 and 1.04 to 1.16 log cfu/g reductions of *S.* Typhimurium, respectively, after 3 days through 7 days of storage as compared to positive controls. The psychrotrophic counts were similar ($P > 0.05$) for all treatments. The pH values for 1% and 2% SMS treatments were higher ($P < 0.05$) when compared to the controls. This study revealed that SMS could function to control the pathogen *S.* Typhimurium, but had no effect on reducing the spoilage microflora when it was used in the marinade.

13. Temporal silencing of two *Culex pipiens quinquefasciatus* (Diptera: Culicidae) mosquito genes: WNV activates the Toll and IMD pathways

Chelsea T. Smartt and Sheri L. Anderson [University of Florida, Florida Medical Entomology Laboratory - Vero Beach, Florida]

Our studies on gene expression responses in midgut tissues of *Culex pipiens quinquefasciatus* infected with West Nile virus (WNV) revealed two genes, one encoding a leucine rich repeat-like protein (LRR) and the other a gram-negative bacteria binding-like protein (GNBBP), whose expression was altered after infection, indicative of involvement in pathogenesis. To elucidate the function of these genes in WNV infection, we injected double-stranded (ds) RNA representing the LRR-like gene, CQ G12A2, and the GNBBP-like gene, CQ G1A1, into *Cx. p. quinquefasciatus*. Knockdown of the message representing the LRR-like gene decreased expression in midgut tissue over time through 4 days (d) after feeding on an uninfected blood meal. Midguts from the same dsRNA injected female mosquitoes fed a WNV containing blood meal showed a remarkable decrease in LRR-like gene expression at all time points compared to mosquitoes fed uninfected meals, with a small spike in expression at 1 d post-infection (dpi). The LRR-like gene appeared to have a decrease in expression over time in mosquitoes subjected to double knockdown of the LRR-like gene and the GNBBP-like gene when fed an uninfected meal. The presence of WNV suppressed, but did not knock out, the expression of the LRR-like gene in all times except at 7 dpi, where the expression suddenly increased. The expression of the LRR-like gene in mosquitoes injected with dsRNA from the GNBBP-like gene or the GFP control was similar to the expression in non-injected control mosquitoes. Injection of dsRNA representing the GNBBP-like gene, CQ G1A1, knocked down the expression in midgut tissue 16 h -1 d after feeding on an uninfected blood source, however the presence of WNV appeared to influence stronger suppression of GNBBP gene expression after 16 hpi. Expression of the GNBBP-like gene in double injected mosquitoes fed an uninfected blood meal was knocked down gradually, with cycles of intermittent gene expression and suppression. Upon WNV exposure, the double injected mosquitoes showed a similar pattern of GNBBP expression, but the cycle occurred earlier. Interestingly, the GNBBP-like gene showed remarkably low levels of expression in mosquitoes injected with dsRNA from the LRR-like gene at 16 h -1 d, exposure to WNV further suppressed its expression. Results from this project will contribute to our understanding of the physiological process and molecular interactions affected in the midgut after infection with WNV.

14. A mathematical model of citrus huanglongbing

Christinah Chiyaka, Emerging Pathogens Institute, University of Florida Gainesville, FL; Ariena H.C. VanBruggen, Emerging Pathogens Institute and Department of Plant Pathology, IFAS; University of Florida; Susan E. Halbert, Florida Department of Agriculture and Consumer Services, Division of Plant Industry Gainesville, FL; Burton H. Singer Emerging Pathogens Institute christinah@epi.ufl.edu

A mathematical model of the transmission dynamics of huanglongbing (HLB) between its psyllid vector and citrus host has been developed to understand and assess the mechanisms involved in pathogen acquisition from vector and transfer to host plants. Citrus flush is considered as the basic variable because that is where most of the vector activity occurs. The spread of the pathogen is modeled from flush to flush within a tree. Using parameters from literature, simulations show dynamics in vector and host populations as the tree approaches complete infection. Results from numerical analysis show that the effectiveness of spraying psyllids using insecticides in curbing the spread of HLB depends on the time of initial spraying, frequency and efficacy of the insecticides. When rouging is carried out, only symptomatic trees testing positive for Liberibacter asiaticus are removed, while infectious non-symptomatic trees remain. It has been deduced that the removal of symptomatic flush will not eliminate the disease from an infected tree. The model will be extended to include migration of psyllids from tree to tree and analyzed to understand the dynamics of HLB infection within a groove.

15. Modulation of Intracellular Reactive-Oxygen-Species in Primary Gingival Epithelial Cells by *Porphyromonas* Infection

C. CHOI1. J. DEGUZMAN1, D.M. OJCIUS2, and O. YILMAZ1, [1University of Florida, Gainesville, FL, 2University of California, Merced, Merced, CA]

Porphyromonas gingivalis, an opportunistic pathogen, can successfully invade and persist in gingival epithelial cells (GECs). The infection inhibits GEC death by impacting mitochondrial-apoptotic pathways and suppressing $P2X_7$-ATP coupled signaling. *P. gingivalis* secretes an effector, nucleoside diphosphate kinase (Ndk), which consumes the "danger signal" extracellular ATP (eATP) released from infected or stressed cells and diminishes $P2X_7$ activity. $P2X_7$ receptor has lately emerged as a critical mediator for generation of cellular ROS and oxidative-stress in addition to triggering immune response and cell death, and controlling intracellular infections upon ligation with eATP. **Objectives:** Study of kinetics of ROS w/ and w/o ATP treatment as an indicator of oxidative-stress during infection of GECs by *P. gingivalis* and examination of roles of NADPH-oxidase and mitochondrial oxidative-stress pathways. **Methods:** Levels of cytosolic and mitochondrial ROS (mROS) production in primary GECs were measured by flow-cytofluorimetry and fluorescent imaging using CM-H_2DCFDA and MitoSox upon infection by *P. gingivalis* or its *Ndk*-deficient strain after stimulation with eATP during 24hr. Mitochondrial-uncoupling UCP_2 expression levels were measured by real-time Q-PCR. **Results:** Infection by wild-*P. gingivalis* provoked substantial but transient increase in global ROS and mROS levels declining at 3hr p.i. similar to the levels in untreated-control cells. Amounts of ROS produced from cells treated with eATP prior to infection were significantly reduced over the course of infection. The *Ndk*-deficient mutant lacked the ability to block the eATP-mediated ROS production. Only wild-*P. gingivalis* modulated the eATP-induced UCP_2 upregulation to basal levels at 24 hr. Diphenyleneiodoniumchloride, a specific inhibitor of NADPH-oxidase substantially inhibited eATP-mediated cytosolic ROS and mROS production. This suggests NADPH-oxidase is partially responsible for generation of ROS via eATP-$P2X_7$ activation, and NADPH-oxidase-induced cytosolic ROS can synergistically promote mROS. **Conclusion:** The results provide novel insights on how *P. gingivalis* can maintain persistence through physiological regulation of GEC metabolism. Supported by NIDCR-R01DE016593, R01DE019444.

16. A Spontaneous Variant of Yersinia Pestis with an Elevated Mutation Rate

Chythanya Rajanna, Henry Gibbons, Gary Ouellette, Mohammed Rashid, Lauren McNew, Charles Hong, Tamara Revazishvili, Lela Bakanidze, Paata Imnadze, Evan Skowronski, and Alexander Sulakvelidze

Clonal pathogens such as *Yersinia pestis* that lack significant horizontal gene transfer generate genetic diversity by a combination of gene amplifications and point mutations, whose frequency determines the "evolvability" of a pathogen. The balance between evolvability and the maintenance of genomic integrity is critical for long-term survival, but studies in other bacteria have demonstrated that strains exhibiting elevated mutation rates can emerge both in the wild, in vivo, and in vitro. A strain of *Y. pestis* (G1670) isolated in the Republic of Georgia was found to exhibit diverse colony morphologies on agar plates. Whole-genome sequence analysis of a colony-purified variant revealed that the strain had acquired a point mutation in the *mutS* gene resulting in the substitution of a conserved leucine at position 689 with arginine (*mutS(L689R)*). Because the mutation occurs at a highly conserved residue within the P-loop of the ATPase domain of MutS, we predicted that the strain had acquired a mutator phenotype. The lineage was sequenced without colony purification, revealing significantly greater genomic diversity within the population than colony-purified strain. In addition, a mixture of wild-type and *mutS(L689R)* alleles was observed, with the *mutS(L689R)* allele predominating. To test whether the *mutS(L689R)* allele exhibited a mutator phenotype, variants expressing wild-type (G1670E) and mutant (G1670A) alleles were separated and tested directly for mutation frequency to rifampicin resistance. Strains expressing the *mutS(L689R)* allele exhibited a >250-fold increase in baseline mutation frequency relative to the wild-type controls. A single variant of the wild-type strain was sequenced to determine whether the mutation emerged prior to or following extended in vitro passage. A *ΔmutS* variant of the *Y. pestis* EV76 live vaccine strain (*pgm*-, pPCP1-) was constructed and transformed with plasmids

expressing the wild-type or the *mutS(L689R)* allele, and mutation rates were compared to those of native EV76 and EV76(*ΔmutS*) strains containing empty vector controls. Importantly, since no other lesions in DNA repair genes were observed in whole-genome sequences of G1670 variants, the mutator phenotype in 1670A arose from a point mutation that has the potential of reversion to the wildtype allele. This report is the first documented evidence that, as in other Gram-negative pathogens, a mutator phenotype can occur in *Y. pestis*. This observation has profound implications for our understanding of the evolvability of a Category A pathogen.

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17. Profile of the 2010/2011 Alachua County School-located Influenza Vaccination Program

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Background: School-located influenza vaccination programs (SLIVs) are increasingly recognized as a key component of community-based efforts to control annual influenza epidemics. Schools are effective virus exchange systems and children are "super spreaders," shedding more of the virus for longer periods of time than adults. Computer modeling suggest that immunizing 70% of school children will protect an entire community from the flu. *Methods:* In 2006, the Health Department, working in collaboration with the School System and the University of Florida, began exploration of a non-mandatory community-wide SBIIP, with the goal of achieving high levels of immunization of the ~22,000 public and private pre-K through grade 8 students in the county. In 2009/2010 the program was repeated and this report provides a description of the procedures developed to achieve the goal, the barriers that were encountered and solutions to problems that occurred during the implementation of the program. Data suggests that the crude immunization rate was approximately 55%.In 2010/2011 the program began integrating the program into the high schools. *Results:* The immunization rate in the public elementary and middle school children remained roughly the same in both years (48% in 10/11 vs. 50% in 09/10 and 36% in 10/11 vs. 36% in 09/10, respectively). 16% of high school students participated in the program, higher than the national average of 9.1%. Over half of the children immunized (53%) were White Non-Hispanic, 23% African American, and 20% marked other. 58% of the participants had private insurance, 24% were on Medicaid, and 8% had no form of medical insurance.

18. Mosquito movement patterns and the development of barrier trapping strategies for mosquito control

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Establishing barriers of traps to protect people in specific areas from mosquitoes is of increasing interest. Developing these methods would allow protection in key sites while reducing reliance on pesticides. The salt marsh mosquito, *Aedes taeniorhynchus* is a serious pest in coastal areas of Florida. We developed a model focused on mosquito movement and wind influences to identify the trapping strategy that best protected a target area in a public garden. One goal of the study was to determine if a simplified model could accurately predict which strategies would be most successful; therefore the landscape was relatively simple. Sensitivity analyses were used to assess

the effect of mosquito movement, attractiveness of traps and wind on the efficacy of trapping strategies. The two most effective strategies were chosen for use in a field study using CDC light traps. Each chosen strategy and no traps were alternated weekly, with a sentinel trap and landing rates measured in the center of the area. The relative efficacy of the two trapping strategies was compared to the outcome of the model. The results of the model, field studies and the ability of the model to predict efficacy of trapping strategies will be discussed.

19. Enzyme Kinetic Analysis of Allosteric Solvent Effects When Screening Mosquito-Selective Carbamates and the Malaria Vector, *Anopheles gambiae*.

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To control the malaria vector, *Anopheles gambiae* (*Ag*), we have developed several phenyl substituted *N*-methylcarbamates that display a high degree of selectivity (≥1000-fold) over human acetylcholinesterase (AChE). Selectivity data was originally obtained using EtOH as the solvent during the screening process. Re-screening of these carbamates in the presence of 0.1% DMSO (*v/v*), the standard solvent for high throughput screening, resulted in higher IC_{50}s for *Ag*AChE, thus reducing the *Ag*AChE-selectivity by at least 10-fold. However, the presence of DMSO did not antagonize the inhibition of human, *Drosophila melanogaster,* or *Musca domestica* AChE. No antagonism of inhibition was observed toward any species studied (including *Ag*AChE) with non-selective carbamates or when EtOH was used as a solvent. Enzyme kinetic analyses were performed to determine the interaction of 0.1% DMSO (*v/v*) toward the carbamoylation reaction of *Ag*AChE. The bimolecular rate constant (Ki) decreased at increasing DMSO concentrations, similar to the *in vitro* inhibition results. In the presence of 0.1% DMSO (*v/v*), the Ki of the experimental carbamates exposed to *Ag*AChE decreased by approximately 3 fold when compared to 10^{-5}% DMSO. This effect was not observed for non-selective carbamates (bendiocarb) or for human AChE. Secondly, 0.1% DMSO (*v/v*) displayed a small (but statistically significant) increase toward Vmax and Km of the enzyme substrate acetylthiocholine (ACTh), indicating little influence on the binding/hydrolysis of the substrate.
Molecular models will be presented to provide a potential explanation for the observed antagonism of inhibition under the presence of DMSO. Implications for high throughput screening of insecticides will also be discussed.

20. Climate and Health in Florida: Changes in risks of annual maximum temperatures in the latter half of the Twentieth Century

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Spatial patterns of changes in the probability, or risks, of annual maximum temperatures over Florida during the second half of the 20th century are examined using a high resolution daily maximum temperature dataset of 833 grid cells. An Annual Maximum Series (AMS) coupled with Extreme Value Theory approach is applied to analyze changes in probabilities of annual maximum temperatures with a focus on the highest third tercile of all annual maximum temperatures during the time period. Three parameters are estimated from the data contained within each grid cell 1) location parameter which is closely related to mean and median, 2) scale parameter which is closely related to variance, and 3) shape parameter which is closely related to skew. The data are then divided into the periods 1949-1974 and 1975-2000 and changes in each of the parameters are mapped. Considerable spatial variability with respect to changes in parameters is found across the state. Much of the state exhibits a decline in both the value of the location and scale parameters with the exception of the southern portion and areas on the Gulf coast in the Panhandle and peninsular Florida. Almost all of the state shows an increase in skew. The “worst” combination of directions of parameter changes in terms of increased risks of high annual maximums would be an increase in location and scale, combined with a increase in skew. The "worst" case scenario is observed in south and west central Florida.

21. Bacterial Genome Finishing Using Optical Mapping

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Our core laboratory has several ongoing bacterial genome projects presenting a variety of challenges to genome assembly and closure. Several factors contribute to these challenges; including sequence repeats versus read length, intrinsic sequencing errors, and dynamic genome rearrangements. Together these factors complicate genome closure when using shotgun DNA sequencing alone. The genome finisher may experience difficulty validating their assembly in the absence of a physical map. To address this problem, we adopted whole-genome optical mapping as a tool to validate bacterial genome assemblies. OpGen, Inc. (Gaithersburg, Maryland) prepared the optical maps used in this project. Briefly, an optical map is a complete genome restriction map deduced from a number of partial restriction maps. Optical maps are generated by spreading carefully extracted genomic DNA onto a treated glass surface containing many narrow channels, followed by digestion *in situ* with restriction enzymes. About 50–100 contiguous restriction fragments with sizes approaching up to one-third of the whole genome are selected and optically measured. The overlapping partial optical contigs are combined by alignment software to produce a contiguous whole genome restriction map. The contiguous optical map can be aligned and compared with the *in silico* restriction map determined for the partially complete whole-genome assembly. We successfully used optical mapping for guiding the closure of four closely related bacterial genomes. The optical map allowed us to identify assembly errors not possible using shotgun DNA sequencing data alone. Thus, we conclude that, in order to ensure the accuracy of a finished bacterial genome, optical mapping is an important tool to validate *de-novo* assemblies generated by next-generation DNA sequencing.

22. Voltage-Sensitive Ion Channel Screening in Patch Clamped *Anopheles gambiae* Sua-1B cells

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Screening insect ion channels and receptors for new insecticides requires new experimental platforms. Previous studies in our laboratory found that *Anopheles gambiae* Sua-1B cells showed cytotoxicity when exposed to a mixture of the sodium channel toxins, veratridine (a plant toxin) and fenvalerate (a synthetic pyrethroid). In the present work, we performed electrophysiological analysis of Sua-1B cells, to see if we could detect cells expressing voltage-sensitive sodium channels using the patch clamp method. Cells were maintained at a holding potential of -80 mV, and stepped up to +70 mV in 10 mV increments. Steps were of 100 msec duration. Out of 15 recordings, 6 were made with Cs+ (K^+ current inhibitor) in the intracellular solution and 9 with normal K^+ physiological solution. No currents consistent with voltage-activated Na^+ channels were observed. However, a slowly activating outward current was observed in these cells, especially at depolarized potentials. In Cs^+ containing intracellular solution, 5 out of 6 cells displayed this current with mono-exponential activation kinetics, and 1 was bi-exponential. In K^+ containing intracellular solution, 7 out of 9 cells displayed mono-exponential kinetics when voltage stepped, and 2 were bi-exponential. Thus, we did not find statistically significant differences between evoked currents with single exponential kinetics recorded with Cs^+ or K^+ intracellular solution. We also observed tail currents in each recording. "Tail" means that a residual voltage-gated current is observed at the end of depolarizing voltage steps, upon sudden removal of the depolarization of the membrane. Tail currents do not exist in normal physiological conditions; however, they can be useful for determining kinetic characteristics of channel currents. Every tail current we observed had fast and slow (bi-exponential) phases. Interestingly, 12 out of 15 evoked currents had mono-exponential kinetics, but their "tails" were bi-exponential. The identity of these currents remains to be determined, but in voltage or whole-cell patch clamp recordings, a voltage step to 0 mV activates L-type Ca^{++} current: at the end of voltage step a Ca^{++} current of larger amplitude and short duration is always recorded. Other possible candidates are calcium-activated chloride conductance, a calcium-activated non-selective cation conductance, or a Na+-Ca^{++} exchange pump current. Future studies will determine whether this current is sensitive to established chloride channel-directed insecticides. Additional studies will assess the ability of veratridine and fenvalerate mixtures to stimulate tetrodotoxin-sensitive inward currents by activating "electrically silent" sodium channels in Sua-1B cells, similar to previous work in NIE-115 neuroblastoma.

23. The Florida Keys spiny lobster fishery, environmental change, and the lethal lobster virus PaV1

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The Caribbean spiny lobster (*Panulirus argus*) is one of the most valuable fisheries in the Caribbean. In 2001, the fishery in Florida experienced a major decline in landings of approximately 30% from which it has not recovered. This decline was coincident with discovery of a lethal viral pathogen, PaV1, found infecting juvenile lobsters in Florida. *P. argus* has a complex life history and is exploited throughout its range – two factors that have made it difficult to determine the cause of the decline. Here we describe the first assessment of PaV1 within the fished segment of the population. We used PCR analysis to measure PaV1 prevalence from lobsters caught in commercial traps throughout the Florida Keys. We also tested the effect of diseased lobsters within traps on trap attractiveness to other lobsters and its effect on the transmission of PaV1 to other trapped lobsters. We found a mean prevalence of 11% in the fished population with PCR+ lobsters as large as 95 mm carapace length (76 mm is legal). We also found that traps harboring an infected lobster caught significantly fewer lobsters than traps containing healthy lobsters. Furthermore, healthy lobsters confined in traps with diseased lobsters became infected with PaV1 more frequently than those confined with other healthy lobsters. This study demonstrates the indirect and subtle effects that pathogens can have on fishery function through altered animal behavior and the unintended consequences of fishery practices on pathogen epidemiology. Whether PaV1 has recently emerged or recently risen to detectable prevalence is unclear. However, for other pathogens, disease prevalence or infection intensity have been linked to physiological stress driven by environmental change. To determine if environmental change may be driving PaV1 infection dynamics we tested the effects of hyper (25 psu) and hypo (45 psu) salinity and variable temperature (20°C, 26°C, and 31°C) conditions on PaV1 susceptibility and intensity. Early benthic juvenile lobsters have been shown to be the most sensitive benthic stage to environmental alterations, so we focused primarily on them. We found salinity to have no measurable effect, but lobsters held at 26° and 31°C had significantly higher infection intensity than those at 20°C. Climate change is having a dramatic effect on many ecosystems and these studies show that increasing ocean temperatures could enhance the effects of PaV1 on Caribbean spiny lobsters.
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24. Emergence of a plant pathogen via hybridization of the Irish famine pathogen, *Phytophthora infestans*, and an undescribed related species

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Emerging plant pathogens threaten natural ecosystems, food security, and commercial interests. Plant pathogens emerge via several mechanisms, including host range expansions and host jumps, which have often been associated with introductions to new geographic regions. Another mechanism for emergence is by hybridization between species or individuals. Hybridization between a recently introduced exotic pathogen and a resident pathogen may allow rapid evolution and adaptation to new hosts or environments, because hybridization introduces genetic

variation that has already been "tested by selection" in the resident parental species. The continuing global movement of plant pathogens may be creating opportunities for new and virulent hybrid pathogens to arise. We investigated the genetic origin of *Phytophthora andina*, an increasingly common pathogen of tomato tree, pear melon, and naranjilla in Colombia and Ecuador. We cloned four nuclear loci to obtain haplotypes and inferred the phylogenetic relationships of these alleles in relation to the potato late blight pathogen *P. infestans* and related species. Sequencing of cloned PCR products revealed two distinct haplotypes for each locus in *P. andina*, such that each isolate has one allele derived from a *P. infestans* parent and a second divergent allele from an undescribed species that is closely related but distinct from *P. infestans, P. mirabilis*, and *P. ipomoeae*. We also observed sequence polymorphism among *P. andina* isolates at three of the four loci, much of which segregates between previously described *P. andina* clonal lineages that may have different host ranges.

25. Suppressor of cytokine signaling-1 contributes to peripheral regulatory T cell stability in an inflammatory environment.

Erin L. Collins, Lindsey D. Jager, Rea Dabelic, Ken Lau, Mohammed I. Haider, Howard M. Johnson, and Joseph Larkin III

Suppressor of cytokine signaling-1 deficient mice (SOCS1-/-) die of a T cell mediated inflammatory, autoimmune disease by 3 weeks of age. The inflammation mediated by excessive interferon γ signaling and leukocyte infiltration results in the destruction of many vital organs. Significantly, numerous mouse models of inflammatory autoimmune disease have been associated with a deficiency in Foxp3+ regulatory T cells. Indeed, $SOCS1^{-/-}$ mice possessed a reduction in peripheral Tregs, despite enhanced thymic development. The adoptive transfer $SOCS1^{+/+}$ Tregs or $CD4^{+}$ T lymphocytes mediated an increased, yet limited, survival in the $SOCS1^{-/-}$ mice. However, the adoptive transfer of $CD4^{+}$ T lymphocytes in conjunction with SOCS1-KIR, a mimetic peptide sufficient to partially restore SOCS1 function, resulted in 30% of the mice living beyond 5 weeks. Moreover, the combined treatment mediated a decrease in leukocyte infiltration into vital organs, and a decrease in effector CD4+ cells. Additionally, the decrease in inflammation was associated with an increase in the peripheral Foxp3+ regulatory T cell population. Collectively, these results suggest that the SOCS1-KIR/CD4+ T cell combined treatment synergistically promoted long-term survival of the SOCS1-/- mice by restoring peripheral Tregs. Furthermore, these data propose that SOCS1 contributes to peripheral Treg stability under inflammatory conditions.

26. Exploring Emerging Pathogens: Three years of education outreach

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As science literacy in the US falls to record lows, the importance of bringing engaging and relevant science education to our K-12 classrooms becomes greater. With funding from the Howard Hughes Medical Institute, the Center for Precollegiate Education and Training (CPET) and the Emerging Pathogens Institute (EPI) at the University of Florida have collaborated to offer a year-long teacher biotechnology education program focused on the theme of new and emerging diseases. The goals of the *Interdisciplinary Center for Ongoing Research/Education (ICORE) Partnership Program* are threefold: (1) to engage teachers in current research being conducted at the University of Florida and empower them with the most up-to-date biotechnology techniques; (2) to promote science as a potential career choice; and (3) to increase public awareness of emerging diseases and their control by integrating the science of emerging pathogens into K-12 classrooms. To assess the impacts of the ICORE program on teacher professional development and teaching practices, we used a combination of pre- and post-program

surveys and questionnaires. Specifically, we were interested in participant expectations of and satisfaction with the program and effects of the program on teacher confidence and interest in science teaching. We found that teacher responses to the ICORE program were overwhelmingly positive. Participating teachers stated that they attended the program in order to learn about current issues in science and develop new activities for their classrooms. The majority of participants said attending the workshop had increased their knowledge of current issues in scientific research and that they had gained a greater understanding of the practical uses of science, math, and technology. Teachers also indicated that they became familiar with new materials and equipment that they could use in their classrooms. All of the respondents agreed that the ICORE program had increased their enthusiasm for teaching and had inspired them to think about new ways to improve their teaching. We discuss the future of the ICORE program and how UF scientists can continue to be involved in this successful research outreach experience for teachers.

27. Toxic effects of some essential oils to *Aedes aegypti* larvae

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Mosquito larvae are an important target for pesticides used in mosquito control, because adult female mosquitoes lay their eggs in standing water, and the egg, larva, and pupa stages are aquatic, which makes it easy to control mosquitoes in their water habit. The conventional larvicides for mosquito control have many disadvantages, such as long half-life in the environment, high toxicity to non-target organisms, including mammals, birds, fishes, and beneficial invertebrates, etc. Some natural products from plants and microbes have insecticidal and repellent effects against different pest insects, and they are considered to be alternatives of synthesized insecticides, because they show low adverse effects on mammals, and they are also friendly to the environment. In this study, we tested toxicities of 13 types of essential oils extracted from different aromatic plants (camphor, thyme, amyris, lemon, cedar wood, frankincense, dill, myrtle, juniper, black pepper, verbena, helichrysum, and sandalwood) against third-instar larvae of *Aedes aegypti*. At first, we screened the mortality of all these essential oils at concentration of 100 ppm after 24 hours treatment to the larvae. Essential oils with high toxicity (24-hour mortality higher than 50%) including thyme oil, lemon oil, cedar wood oil, frankincense oil, verbena oil, helichrysum oil, and sandalwood oil were selected, and the lethal concentration 50 (LC_{50}) values of these oils against third-instar larvae of *Aedes aegypti* were evaluated by using treatment with serial concentrations of these oils.

28. Internalization and movement of *Salmonella* in tomato

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Tomato has been a vehicle for *Salmonella enterica* outbreaks. *Salmonella* can move inside some plant species, survive from the 3-leaf-stage through fruit ripening on tomato, and move into *Arabidopsis thaliana* and lettuce. However, mechanisms of internalization and movement of *Salmonella* in tomato have not been investigated in detail. The purpose of this project is to investigate the probability of internalization of *Salmonella* Typhimurium into tomato plants via leaves as affected by bacterial fimbriae and microbial diversity in the plant. In this project, 126 tomato plants grown in conventional or organic soils were inoculated three times with GFP-labeled *Salmonella* Typhimurium strains, MAE110 (with fimbriae) or MAE119 (without fimbriae) by dipping leaves in suspensions of 10^9 CFU/ml. Inoculated and adjacent surface-sterilized leaves were tested for *Salmonella* 1, 3, 5, 7, 14 and 21 days after inoculation. Red ripe fruits were surface sterilized and *Salmonella* was recovered by placing the pulp surface of dissected tomatoes onto LB agar with kanamycin. Microbial communities in soils and plants were investigated 2 weeks before and after inoculation. Leave sampling data revealed that populations of both *Salmonella* strains in

surface sterilized leaves decreased during 2 weeks after inoculation but remained unchanged in week 3. Levels of MAE110 were significantly higher ($P < 0.05$) than those of strain MAE119 from day 3 after inoculation. Significantly more *Salmonella* survived in plants grown in conventional than in organic soil ($P < 0.05$). *Salmonella* was detected in noninoculated leaves of 8 tomato plants (5 grown in conventional soil), as well as in pulp of 9 tomatoes harvested from one plant grown in conventional soil inoculated with strain MAE119. There was a negative correlation between bacterial diversity in plants and the rate of internalization and transmission. All of the results indicated that *Salmonella* can enter tomato plants through leaves, move inside and contaminate fruits. . Fimbriae may benefit the colonization of *Salmonella* on/in tomato plants. This is the first time that a negative correlation is reported between bacterial diversity inside tomato plants and invasion by *Salmonella enterica*.

29. Responses of *C. elegans* to Pathogenic Challenge Using NMR Statistical Analysis and Motion Tracking Software

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The nematode Caenorhabditis elegans releases and responds to many different chemicals necessary for regulating important behaviors such as mating attraction, dauer formation, aggregation, and recognition and differentiation of food and pathogens. The nematode can sense bacterial populations through small-molecule messengers such as acyl-homoserine lactones, and the nematodes are able to interfere with certain bacterial quorum sensing systems. Additionally, C. elegans has a complex olfactory system which allows it to avoid detrimental conditions such as areas of high population density, high osmolarity, and pathogenic bacteria. Moreover, this system, which is affected by released pheromones, exhibits behavioral plasticity that allows the nematode to learn and adapt, for example, to avoid an odorant associated with harmful conditions. This study seeks to determine changes in C. elegans behavior and its exometabolome, the set of small molecules released into the environment by the worms, in the presence of a bacterial food source (Escherichia coli) versus a pathogen (Pseudomonas aeruginosa). We collected exudates from a synchronous population of C. elegans under bacterial-challenged and nonchallenged conditions, acquired 2D NMR spectra, and used a novel method developed in our lab for 2D NMR alignment and pattern recognition (Robinette et al., 2011) to identify NMR peaks correlated with worm responses. We also are developing high-throughput tracking software written in MATLAB to quantitate C. elegans responses to the bacterially-challenged exudates to identify potential alarm responses. We are developing a parallel camera system to record 6 assays simultaneously, and the software to track individual nematodes, quantify reversal frequency and calculate average speed for a set of nematodes on an agar plate. This system will allow us to understand the complex responses to varying environmental conditions. [1. Edison AS. *Current opinion in neurobiology*. 2009;19(4):378-88.; 2. Beale E, et al. *Applied and environmental microbiology*. 2006;72(7):5135-7.; 3. Kaplan F, et al. *Journal of chemical ecology*. 2009;35(8):878-92. ; 4. Schulenburg H, Ewbank JJ. *Molecular microbiology*. 2007;66(3):563-70. ; 5. Yamada K, et al. *Science*. 2010;329(5999):1647-1650.; 6. Robinette SL, et al. *In Press, Analytical Chemistry* (2011)]

30. Enhancing Multi-Disciplinary Collaboration with VIVO

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Membership in a cross-disciplinary research center like the Emerging Pathogens Institute contributes greatly to interdisciplinary collaboration because it enables communication among researchers. Imagine, then, the many benefits of a multidisciplinary digital network where scholars could easily browse the research and professional interests of all faculty at the university in order to find potential collaborators. VIVO is an online researcher profile system designed to showcase faculty members' research interests, publications, and grants in order to enable and encourage collaboration, particularly multi-disciplinary collaboration. Initially developed at Cornell University, VIVO is being expanded for national use by the University of Florida and six partner institutions through a $12.2 million grant from the National Institutes of Health. In addition to displaying detailed researcher profiles, VIVO includes visualizations of existing collaborative networks and faceted searching capabilities that allow easy navigation of the wealth of information available. Although it is designed to enable networking of researchers, VIVO is different from other social and business networking platforms in the ways it obtains its data. VIVO harvests existing public data from authoritative sources, both at the university (e.g. Division of Sponsored Research grants data) and beyond (e.g. PubMed), and ties them to individual profiles. Researchers are then able to edit their profiles and add further information, but the bulk of the work of populating a profile is done through automatically incorporating existing authoritative data. By highlighting particular use case scenarios, we aim to introduce members of the Emerging Pathogens Institute to the features and benefits of VIVO and encourage their use of this tool.

31. Epidemiology of soybean rust (*Phakopsora pachyrhizi*) in soybean (*Glycine max*) sentinel plots in Florida.

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Since its discovery in the southeastern United States in 2004, soybean rust (SBR) has been variable from year to year. Caused by *Phakopsora pachyrhizi,* SBR epidemics in Florida are important to understand as they may serve as an inoculum source for other areas of the country. This study examined the disease onset date, incidence, and severity of SBR in relation to prevailing weather data, growth stage, and maturity group (MG3, MG5, MG7) in soybean plots (225 square meters) across north Florida from 2005 through 2008. On average, MGV and MGVII became infected before MGIII soybeans and generally plots did not become infected until growth stage R4 (full pod) or later. Precipitation was the principle factor affecting disease progress; where disease increased rapidly after rain events and was suppressed during dry periods. On average, plots became infected 30 days earlier in 2008 than 2005. In 2008, there was a significant increase in disease incidence and severity associated with the occurrence of Tropical Storm Fay, which deposited up to 380 mm of water in areas of north Florida. Results from this study may lead to a better understanding of the impact of weather on the epidemiology of this pathosystem.

32. Isolation of the Causal Agents of Citrus Black Spot and Laurel Wilt for Epidemiological Studies

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Citrus black spot (CBS) and laurel wilt are two emerging fungal plant diseases in Florida. The first is caused by *Guignardia citricarpa* and is mainly spread through rain and wind-dispersed ascospores; the role of conidia is not clear. The second is caused by *Raffaelea lauricola* transmitted by the ambrosia beetle *Xyleborus glabratus*. Not much is known yet about the epidemiological parameters facilitating regional spread in Florida and neighboring states. The long-term goal of this project is to develop a spatial model for regional spread of these diseases. The immediate objective was to isolate the fungal strains causing these diseases, so that epidemiological studies can be carried out in the near future. Mature Valencia oranges and leaves with black spot lesions as well as asymptomatic leaves, twigs and immature fruits were collected in Immokalee for the isolation of *G. citricarpa* on various media. Discolored sapwood of wilted swampbay in the Austin Cary Memorial Forest and of a wilted avocado tree within the UF Gainesville campus was collected for the isolation of *R.lauricola* on the Ophiostoma Selective Medium (OSM). The fungal isolates were identified by colony morphology as well as species specific primers in the ITS region. Corky Root Medium (CRM) was the best medium for isolating *G. citricarpa*, whereas Acidified Potato Dextrose Agar (APDA) was the best medium for isolating *G. mangiferae*, a closely related endophyte. *G. citricarpa* was only isolated from the lesions on fruit, whereas *G. mangiferae* was isolated from lesions on fruit as well as from infected young twigs. Neither *G. citricarpa* nor *G. mangiferae* were successfully isolated from asymptomatic immature fruit and leaves or from symptomatic leaves. *R. lauricola* isolates were obtained from swampbay as well as the avocado tree. The identities of the fungal isolates were further confirmed by PCR. Koch's postulates are currently being carried out to verify the pathogenicity of these isolates.

33. Danger-Signal "Adenosine" Stimulates Growth of *Porphyromonas* in Primary Gingival Epithelial Cells

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Porphyromonas gingivalis is a host-adapted pathogen that can successfully replicate and persist in gingival epithelial cells (GECs). *P. gingivalis* infection inhibits ATP-induced GEC death by modulating purinergic $P2X_7$-ATP receptor signaling. Purinergic and adenosine receptors - called "danger signal receptors" – have emerged as key determinants for regulating apoptosis and controlling persistent infections. We found that primary GECs express the four adenosine-receptors: stimulatory A2a and A2b and inhibitory A1 and A3. **Objectives:** To examine the potential importance of these novel receptors in modulating intracellular infection, specifically to determine whether A2a ligation and associated signaling affect intracellular life of *P. gingivalis*. **Methods:** Expression of adenosine-receptors in GECs analyzed by RT-PCR. The intracellular levels of infection were measured by conventional antibiotic protection assay and fluorescence microscopy combined with NIH-ImageJ analysis. Briefly, primary GECs were stimulated with non-specific adenosine-receptor-agonist, NECA, or A2a-specific-agonist, CGS-21680, or A2a-specific-antagonist, SCH-58261 following 3hr p.i. by *P. gingivalis* for a total of 8, 12 and 24hrs of infection. To further confirm the functionality of A2a receptor in GECs, the cytosolic cAMP levels were measured using combinations of agonists or antagonist by a cAMP ELISA-based assay. **Results:** GECs express A1, A2a, A2b and A3 at high levels. Treatment with the broad-spectrum-agonist, NECA, had little effect on the infection. Whereas, an A2a-agonist, CGS-21680, induced a large effect on the levels of infection (~2.5-fold increase) compared to unstimulated-infected controls. Stimulation by A2a-antagonist, SCH-58261, significantly inhibited A2a-agonist

induced proliferation of *P. gingivalis.* A2a-agonist treatment evoked substantial increase in the concentration of cytosolic cAMP indicating activation of A2a-receptor and adenylyl-cyclase signalling. **Conclusion:** GECs express functional A2a receptors that are likely to be important mediators for modulating intracellular infection by *P. gingivalis*. Furthermore, this novel gingival danger signal receptor could be critical in controlling other chronic infections in the oral mucosa. Supported by NIDCR-R01DE016593-04S1 and R01DE019444.

34. Mycobacterial *hsp65* Sequence Database

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Background Mycobacteria include a large number of pathogens both obligate (e.g. *M. tuberculosis* and *M. leprae*) and opportunistic (e.g. *M. avium*, *M. kansasii*, etc). Identification to species level is important for diagnoses and treatments. The exponential increase of the number of mycobacterial species/subspecies (currently about 150) makes identification challenging, especially with culture-based biochemical tests. Molecular identification methods based on PCR and nucleotide sequencing shorten the detection time and improve the accuracy, but quality-controlled sequence databases with broad coverage are required. Here, we reported a development of a sequence database of widely used *hsp65* locus. *Methods* The *hsp65* sequences of 139 mycobacterial reference species/subspecies (including 136 type strains) were collected from GenBank. Identical sequences from the same species/subspecies were merged into a single database sequence entry. The sequences were trimmed to the 401 bp, corresponding to nucleotide position 165-565 of *M. tuberculosis* H37Rv *hsp65* gene. A BLAST service to search this database was created. A maximum likelihood phylogeny was also constructed using PhyML 3.0 (1).
Results The BLAST server of *hsp65* database can be accessed at http://msis.mycobacteria.info. Query sequence is accepted in FASTA format and searched against the database using BLASTN program. Output results will show 20 best hits to suggest their taxonomic categories at species level. The pairwise alignment and the percentage of identical match are also shown. The *hsp65* phylogeny covers 93% of the *Mycobacterium* genus. Slowly growing mycobacteria and rapidly growing mycobacteria are clearly separated except *M. arupense*, *M. hiberniae*, *M. nonchromogenicum*, and *M. triviale*. *Conclusions* Our web-accessible sequence database of *hsp65* locus from 139 reference species/subspecies can serve as a reference for identifying *Mycobacterium* species. The sequences were also used to construct a phylogeny for *Mycobacterium* genus. 1. Guindon, S., et al. 2010 Syst Biol 59:307-321.

35. Effect of SNARE Knockdown on *Leishmania* Parasitophorous Vacuole Maturation

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Leishmaniasis is caused by the intracellular protozoan parasite of the genus *Leishmania*. Leishmania affects over 12 million people in 88 countries worldwide. The biogenesis of the *Leishmania* parasitophorus vacuole (PV) involves interactions with various host cell compartments. Although there is some evidence on the growth and maturation of the PV, much remains to be elucidated. In this study, we investigate the role of N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins that mediate the fusion of ER vesicles on the growth of the PV. Knockdown of the SNARE molecules sec22b and syntaxin-5 by siRNA in the host cell, resulted in reduced PV growth as well as a reduction in the number of infected cells. The overexpression of a dominant-negative form of sec22b (^tm-Sec22b) also resulted in reduced PV growth. In order to further evaluate the disruption of ER-SNAREs on *Leishmania* infection we employed the recently described drug, Retro-2, which inhibits Syntaxin-5 function, to assess the effect of inhibiting ER/PV interactions *in vivo*. Mice treated with Retro-2 immediately after infection or 3 weeks post-infection had reduced footpad swelling compared to control mice. These studies suggest that a strategy to inhibit PV maturation by targeting components of the vesicle fusion machinery could be a feasible approach to cure leishmaniasis. Further studies of SNARE function in infected cells may provide insight into PV biogenesis and parasite survival within host cells.

36. PaV1 Detection by the Caribbean Spiny Lobster and its Effect on Population Spatial Structure

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PaV1 (*Panulirus argus* virus 1) is a lethal contact-transmitted pathogen that infects the Caribbean spiny lobster *Panulirus argus*. Juvenile lobsters are more susceptible to infection, which causes tissue degradation, lethargy, and mortality. However, *P. argus* has the ability to reduce infection risk by avoiding shelters inhabited by infected lobsters. Based on its role in many other aspects of lobster ecology such as conspecific attraction and mate searching, we hypothesized that olfaction was the most likely mechanism by which lobsters detect PaV1. We used a series of y-maze experiments to test this hypothesis and determine the source of the olfactory cue. Shelter avoidance behavior also has the potential to alter the population spatial structure, and based on the type of cue, could be affected by local hydrodynamics. To investigate this we manipulated shelter for wild populations in both high and low flow environments. Y-maze results showed that diseased lobster avoidance is driven by olfaction via urine release, and moreover, the olfactory cue alone was equivalent in effectiveness to having a diseased lobster present and visible. When given a choice between sheltering with diseased or healthy conspecifics, lobsters rarely sheltered with diseased individuals. Shelter manipulation experiments showed that PaV1 has the ability to alter the spatial structure of natural populations by causing local emigration and redistribution of juveniles.

37. Survey of Diseases of Ornamental Plants Processed at Statewide Diagnostic Clinics in Florida

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New plant diseases are emerging regularly, especially in Florida, where plant materials are introduced on a daily basis. Florida is home to many nurseries and greenhouses for ornamental plants, which are shipped throughout the United States and other countries. A survey was conducted to determine the prevalence of current diseases and of newly emerging diseases on ornamental plants in Florida. Data were retrieved from two statewide databases containing the disease diagnoses of plant samples processed by the diagnostic laboratories at the University of Florida Plant Disease Clinics and the Florida Department of Agriculture and Consumer Services Division of Plant Industry for the 3 year time periods of 2008, 2009, and 2010. The most common diseases found were those caused by *Pythium*, *Phytophthora*, *Fusarium*, *Colletotrichum*, and *Rhizoctonia* species. Two emerging and economically important pathogens, *Phytophthora ramorum* and *Raffaelea lauricola*, were diagnosed in some plant samples submitted during this time period. Ramorum blight, caused by *Phytophthora ramorum*, was diagnosed in 14 ornamental plant samples in 2008. During that year, the prevalence of ramorum blight on samples submitted for testing and subsequently diagnosed with *P. ramorum* was 5.6%. The host plants that tested positive with *P. ramorum* were *Camellia*, *Loropetalum*, *Rhododendron*, and *Viburnum* species. *Phytophthora ramorum* was not detected in any plant samples collected during 2009 and 2010. *Raffaelea lauricola*, the agent of laurel wilt, was confirmed as the cause of disease on 33 *Persea* sp. ornamental plants during 2009 and 2010. The prevalence of laurel wilt among *Persea* sp. ornamentals submitted for laurel wilt testing was 41.77%. The information gained during this survey will be utilized by federal and state regulatory officials and by university personnel to regulate and manage emerging diseases that have an impact on Florida agriculture and trade. Information from these databases also will be used for the formulation of a risk model for the introduction of *Phytophthora* species on ornamental plants and their spread in production and trade chains.

38. Dispersal of *Salmonella* on tomato plants by rain splash

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Salmonella has increasingly been associated with tomato outbreaks, and has been traced back to production areas. Mechanisms of *Salmonella* dispersal in the field have not been characterized. The objective of this research was to assess the dispersal of *Salmonella* by rain onto tomato plants as affected by rain intensity, duration, and mulch type. GFP-labeled kanamycin-resistant *Salmonella* Typhimurium dispensed on the surface of plastic mulch or soil on a watch glass at 10^8 CFU/cm^2 were used as the point source in the center of a rain simulator. Forty-centimeter-tall tomato plants with and without plastic mulch were placed around the point source and rain intensities ranging from 60 to 120 mm/h were applied for 5, 10, 20, and 30 min. The GFP-labeled *Salmonella* cells were recovered from plants at various distances from the point source by making leaf imprints on LB agar with kanamycin or by washing off the cells from leaf surfaces with peptone water. Dispersal of *Salmonella* followed a negative exponential model with a half distance of 9.2 cm at an average rain intensity of 90 mm/h. Recovery of *Salmonella* from plants ranged from 5 CFU/plant after a rain episode of 60 mm/h for 5 min to 10^3 CFU/plant after a rain of 120 mm/h for 30 min. Short rain cycles (less than 10 min total) produced no significant differences in the dispersal of *Salmonella* on plants with and without mulch. Conversely, plastic mulch significantly increased ($P < 0.05$) by 2 logs the amount of *Salmonella* recovered from plants in rain cycles longer than 10 min. *Salmonella* may be dispersed by rain to contaminate tomato plants in the field, especially during longer rain cycles and when plastic mulch is used. Additional research is planned to compare different mulch surfaces to limit potential contamination of *Salmonella* on tomatoes.

39. Cholera in Haiti

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The current cholera epidemic in Haiti began in October, 2010, and has spread throughout the country resulting in more than 190,000 cases and about 4000 deaths. Haiti has not had reported cases of cholera since 1960; although cholera spread throughout Latin American in the 1990s. Controversy surrounding the source of the Haitian epidemic continues and to examine this question we analyzed strains of *Vibrio cholerae* from Haiti.
Stool samples from nineteen patients with severe diarrhea were provided by staff at St. Mark's Hospital, Artibonite Department, Haiti, to UF investigators on November 9, 2010. Stool samples were directly planted on thiosulfate citrate bile-salts sucrose (TCBS) agar and also inoculated into alkaline peptone water for enrichment. Yellow colonies were identified and biotyped by standard biochemical tests. PCR was done for presence of *omp*W, and classical versus El Tor versions of *ctxB*, *tcpA*, and *rstR*. Up to 20 isolates from each patient were typed by Variable-Number Tandem-Repeat (VNTR) analysis to examine the relationship with other global *V. cholerae* isolates and to determine the amount of genetic diversity within Haitian cholera strains. Altered *V. cholerae* O1 El Tor Ogawa strains with a classical *ctxB* gene were isolated from 16 of 19 patients. (VNTR) typing of 187 *V. cholerae* isolates from stool samples (n=13) showed limited diversity, consistent with a point source for the epidemic. Ten VNTR sequence types (ST's) were identified; all were within a single clonal complex, with each ST differing from the

others by a single allele. The majority of the diversity was in the two loci on the second chromosome with 3 and 5 alleles as compared for 1,1, or 2 alleles for the 3 loci on the large chromosome. The diversity among 187 colony picks is significantly less than seen in a similar study in Bangladesh, suggesting that the Haiti strains are more clonal. None of the Haitian STs has been reported in any prior studies. The most closely related isolates are altered *V. cholerae* from Bangladesh and India. Although current strains are clonal, we anticipate that continued passage of these strains in the Haitian population and possibly in environmental reservoirs will result in increasing diversity. VNTR analysis provides excellent discrimination and will be an important molecular tool for tracking the transmission and survival of *V. cholerae* in Haiti. Development of an improved international VNTR database would also allow studies on the global spread of cholera. jajohnson@pathology.ufl.edu

40. A Curriculum Integrating Emerging Pathogens Topics into High Schools

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The *Biotechnology in the Classroom Curriculum* is a Laboratory Manual and complementary Activity Guide developed to assist science teachers participating in the year-long Interdisciplinary Center for Ongoing Research/ Education Partnership Program (ICORE). The ICORE professional development program, funded by the Howard Hughes Medical Institute, engages Florida teachers in a two-week summer institute and results in a the incorporation of biotechnology concepts and techniques into the high school science curriculum based on authentic research experiences at the University of Florida. The *Biotechnology in the Classroom Curriculum* focuses on tomato spotted wilt virus (TSWV), a model plant pathogen system that is relatively easy to study in the classroom and relevant to many of Florida's agriculturally-based communities. TSWV is the HIV of the plant world and facilitates connections between plant and human health. The centerpiece of the TSWV module as it is performed on the University campus is the invaluable expertise and perspective researchers from the Emerging Pathogens Institute provide. To convey the knowledge of many scientists, varied strategies are used to translate new and emerging scientific knowledge into classrooms and communities. A jigsaw activity is used to determine the epidemiology of TSWV and discuss global and economic impacts. This activity simulates the interactions and knowledge different scientists and stakeholders contribute to controlling TSWV and highlights science careers. Working as teams, participants also observe peanut plants for evidence of disease and perform a simulated and actual immunoassay to determine TSWV infection. Participants then investigate ways to prevent or minimize the impact of TSWV and look to genetically modified peanut seeds as a solution. The laboratory manual steps users through DNA extraction from peanut seed, polymerase chain reaction, and gel electrophoresis. As an extension, a bioinformatics lesson confirms the PCR result. In the past three years, the curriculum has been used increasingly in Florida high school classrooms. The response has been quite positive with measureable outcomes in learning and excitement in science. Additional modules related to emerging pathogens are in development to help disseminate current scientific advances and to partner with teachers to improve science teaching and learning and career awareness in this interdisciplinary field reflective of the interest of Florida students and teachers.

41. Anthrax Lethal and Edema Toxins Do Not Directly Affect Human Platelet Function

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Blood vessel leakage and hemorrhage are prominent clinical manifestations of systemic anthrax, and a defect in platelet function has been proposed as an explanation for anthrax-associated mediastinal and pleural hemorrhage. Here, we examined the direct effects of *Bacillus anthracis'* lethal and edema toxins (LT or ET) on human platelets. We have found that anthrax LT fails to cleave its anticipated target, MEK1 and anthrax ET fails to increase intracellular cyclic adenosine monophosphate (cAMP) in human platelets. We have also found neither LT nor ET directly inhibit platelet activation or bind to human platelets. These results are explained by an absence of the two anthrax receptors on human platelets. This is the first report indicating no direct effects of anthrax toxins on human platelets. We conclude the hemorrhagic clinical manifestations of systemic anthrax cannot be attributed to the direct actions of anthrax ET or LT on human platelets.

42. Technical Advances in Field Studies: Recovery of Live Virus from a Synthetic Matrix After Storage at Ambient Temperature.

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A major impediment to performing virological field studies in developing nations is the lack of freezers as well as the expense and difficulty of shipping frozen samples. Traditionally, biological samples could be shipped dried on filter paper; however, viruses were often destroyed in the process. A recently developed product, ViveST™, provides the ability to store up to 1mL of viable biological specimen on a synthetic matrix at ambient temperature for use in specimen storage and transportation. The matrix is housed on the screw-cap of a tube such that the sample is self-contained during storage and shipping. This product has been validated for use with plasma and after reconstitution, successful molecular testing. Human adenovirus is a non-enveloped DNA virus and dengue virus is an enveloped RNA virus. The biology and morphology of these viruses are generic and concepts generated using these viruses can be applied to other viruses with similar morphology or physiology. In this report, we evaluate the performance of the ViveST device in recovering live virus from serum and cell culture supernatants that have been stored at ambient temperature in the synthetic matrix. In this work, we used the ViveST technology to store 500 ul aliquots of human adenovirus and dengue virus in fetal bovine serum and cell culture media. Virus was recovered from the devices on a weekly basis for 4 weeks and titered via plaque assay. Results of the viral titers indicate that significant quantities of virus can be recovered from serum that has been stored for up to 4 weeks though virus that was recovered from cell culture supernatants was viable for only 2 weeks. These results indicate that the ViveST device may be a simpler, more cost-effective alternative for storage and transport of live virus.

43. Implications of gut bacteria /dendritic cells interactions in development of Th17 lymphocytes and Type 1 Diabetes resistance

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TH17 cells have typically been regarded as an agent of autoimmunity. However, recent literature has suggested that Type 1 Diabetes (T1D) onset is negatively correlated to the presence of TH17 cells. Consistent with this notion, we observed that total lymph nodes isolated from NOD mice (which spontaneously develop T1D) produced lower levels of IL17 *in vitro* compared to lymphocytes isolated from diabetes resistant NOR or C57BL/6 strains of mice. TH17 cells are responsible for extracellular pathogen defense and are most prevalent in the digestive tract. Increasing evidence shows a direct link between gut bacteria and the proper development of TH17 cells. Germ-free derived NOD mice display an accelerated onset of T1D development. Oral feedings of *Lactobacillus johnsonii* (LjN6.2), a bacterial strain isolated from the gut of a diabetes resistant rodent, has been shown to mediate T1D resistance and a TH17 bias in diabetes prone rodents. We used LjN6.2 to stimulate both diabetes prone and resistant strains, measuring cytokine production through ELISAs and cell populations via flow cytometry. When equal numbers of T cells and antigen presenting cells (APCs) were incubated with LjN6.2, it was observed that NOD lymphocytes were capable of producing abundant IL17. Literature has previously established that the APCs of NOD mice are defective in both number and function. Therefore, we next analyzed whether the IL17 production of the lymphocytes was due to restored APC function by LjN6.2. NOD APCs responded to LjN6.2 by producing extensive IL6. Like NOR and C57BL/6 mice, NOR APCs were capable of upregulating CD80 and CD86 (markers of activation necessary for costimulation of T cells) in response to incubation with LjN6.2 However, only NOD APCs display a marked upregulation of MHC I (involved with antigen presentation). Because of the potent effects LjN6.2 exhibited on APCs, we next tested whether a footpad injected vaccine of LjN6.2 pulsed bone marrow derived dendritic cells into NOD mice could cause a TH17 bias *in vitro*. Indeed, splenocytes isolated from treated NOD mice showed increased production of IL6 and IL17 with or without antiCD3 treatment. These studies present a possible role for gut microbiota in biasing T lymphocyte effector functions away from autoimmune diseases like T1D through dendritic cell modulation.

44. Mosquito cell lines as an economical platform for discovery of new insecticides to control malaria

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The need for insecticide compounds with novel modes of action is becoming more urgent due to increasing issues with insecticide resistance and the decrease of conventional insecticides on the market. At present, *in vitro* work 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 multiple insecticide target proteins from undifferentiated insect cell lines, which could lead to new high throughput screening methods and a way to mass produce insect material for basic research. This study used cultures of Sua1B cells and evaluated insecticidal compounds to initiate expression of target proteins specific to ion channels and neurotransmitter receptors. Sua1B cells showed no effect of veratridine alone, but when applied in combination with the pyrethroid, fenvalerate, there was inhibition in cell growth that was fit by a two-site binding

model. A potent effect of IC_{50} = 147 nM, was shown for the high affinity site and a lower potency at IC_{50} = 58 μM was shown for the second site. This effect was inhibited in the presence of one micromolar tetrodotoxin, a specific sodium channel blocker. 4-aminopyridine, a potassium channel blocker, showed inhibition of cell growth when applied in combination with veratridine 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.

45. HSV-1 Strains KOS and 17*syn*+ Exhibit Striking Differences in Histone Modifications and Transcription During Latency in Mouse Dorsal Root Ganglia

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Despite high sequence similarity, there are many phenotypic differences between HSV-1 strains 17*syn*+ and KOS, including virulence, establishment of latency, and reactivation. LAT promoter deletion mutants in these strains identified contradictory effects of LAT on lytic transcript abundance and chromatin profiles of the latent genomes. It was unclear, however, if these differences were due to experimental model (rabbit vs. mouse) or anatomical site of latency (DRG vs. TG). To understand the factor(s) involved in transcriptional control of HSV-1 genes between these phenotypically dissimilar strains, we analyzed viral transcript abundance and chromatin deposition on latent viral genomes within mouse DRG. Six-week-old ND4 Swiss mice were infected with 500 pfu of 17*syn*+ or KOS/M. At 28 days post-infection, mice were sacrificed and DRG were removed and processed for ChIP or for RNA isolation. In agreement with previous data, the tested lytic genes of both 17*syn*+ and KOS were enriched in triMe H3K27 (indicating transcriptionally inactive heterochromatin), while the LAT region was enriched in diMe H3K4 (indicating transcriptionally active euchromatin) relative to lytic genes during latency. However, the diMe H3K4 results highlighted an important difference between KOS and 17*syn*+. The LAT enhancer was most enriched in diMe H3K4 in strain KOS while the LAT promoter region was most enriched in diMe H3K4 in strain 17*syn*+. This suggests differences in transcriptional regulation of the LAT region between viral strains. In addition, we found that the greatest difference in latent transcript abundance originated in the LAT region, with 100-fold fewer LAT transcripts per viral genome in KOS than 17*syn*+. These results demonstrate that there are strain-specific differences in the regulation of latent gene expression between 17*syn*+ and KOS. The differences in LAT regulation may explain the opposing effects of LAT promoter deletion in these two strains and could have implications on strain-dependent differences in reactivation.

46. Alcohol Consumption among Women with HIV - Qualitative Findings

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Background: Alcohol consumption is associated with poorer medication adherence, riskier sexual behaviors and poorer health outcomes in women with HIV. Yet, little is known about why these women drink. The objectives of this qualitative study were to identify reasons HIV-infected women drink alcohol, their perceived consequences of drinking, and their willingness to cut down drinking if an intervention was available. **Methodology:** We conducted 4 focus group discussions among HIV-infected women; in Jacksonville, FL (n= 6), Chicago (n= 6, n=7), and Washington DC (n=5). Trained facilitators asked open ended questions during recorded 1-1.5 hr sessions, followed by transcript analysis for thematic categorizations and identification of representative quotations. **Results:** Participants identified the main reasons associated with drinking as: *Psychological factors* such as stress, depression,

guilt, shame, anger, physical and emotional pain, and pleasure; *Peer and family influence; Awareness of being HIV Positive*; *Escape from personal and family problems*; *Addiction;* P*erceived social norms*; and S*ense of false courage.* Some women also reported drinking to cope with loss of loved ones, remain in denial of problems, and to deal with an inferiority complex and failure. Women identified these key consequences as a result of drinking: *physical and sexual abuse; accidents, loss of family and friends network, loss of employment; health problems*; *criminal activity*; *poor decision-making;* and *lapses in medication intake*, including HIV medications.
Some participants expressed that they were willing to cut down their drinking if appropriate help was accessible to them in order to improve their health outcomes and family relationships. **Conclusion**: Our focus group sessions illustrate complex psychological, biological, environmental and physical challenges that influence drinking behavior in HIV-infected women. Some women are willing to cut down their drinking if appropriate help is available to them. Identifying and understanding these unique motivations for and perceived consequences of harmful alcohol consumption are important steps in designing alcohol interventions tailored for HIV-infected women.

47. Detection of naturally occurring resistant variants in NS3 protease region of hepatitis C virus through 454 deep sequencing

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Hepatitis C virus (HCV) encodes a nonstructural protein (NS3) protease required for virus replication. This protease is currently one of the preferential targets for new therapies and several small molecules compounds that inhibit this protease have been developed in recent years and used in clinical trials. Due to the error prone viral polymerase, HCV circulates in a mixture of viral sequences that differ slightly from each other and is referred to as quaisespecies. There is indication that naturally occurring resistant variants may already be present at low frequencies in a predominantly wild-type viral population. Thus, determining the baseline occurrence of resistant variants to the new drugs may have important implications on the response rates possibly requiring that patients be screened for drug resistance. Using 454/Roche pyrosequencing, we examined ~45,000 high-quality NS3 sequences from 11 cross-sectional samples of subjects infected with HCV and ~690,000 high-quality NS3 sequences from 61 longitudinal samples of 13 HCV-infected subjects undergoing liver transplant. The abundance and the dynamics of drug resistance mutations were quantified using a custom analysis pipeline. Low level resistance mutations to the NS3 protease that are not detectable by cloning or population sequencing were detected using pyrosequencing. Therefore our methods should provide a framework for addressing the impact of low abundance resistance variations on the treatment outcome of infected subjects treated with small molecular inhibitors.

48. *Salmonella* SdiA Lacks Recognition of N-acyl Homoserine Lactone Signals in Bacterial Soft Rot Wounds of Produce

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Salmonella spp. are not known to produce N-acyl homoserine lactones (AHLs), but the genome of *Salmonella enterica* contains *sdiA*, which encodes an AHL receptor. The presence of the AHL receptor is hypothesized to play a role in inter-species communication with AHL-producing bacterium in the plant environment. Under *in vitro* conditions, the *sdiA*-dependent resolution of a *srgE* recombinase-based in vivo expression technology (RIVET) reporter was observed to detect the AHL signals of *Pectobacterium carotovorum* SR38, a species known to cause soft-rot in plants. The purpose of the research was to study the recognition and response of *sdiA* to AHL signals within various produce commodities. The *in planta* gene expression was quantified using *srgE-tnpR* and *sdiA-tnpR* RIVET reporters in tomatoes (green and red), bell peppers (green and red), carrots, and green onions. A low incidence of resolution for the RIVET reporters was observed in red tomatoes and there was no resolution of the *sdiA- tnpR r*eporter in the green tomatoes, bell peppers (green and red), carrots, or green onions. The results of the *in planta* studies suggest a lack of *sdiA* expression within the plant environment, thus preventing the *Salmonella* spp. from recognizing the AHL signals of other bacterium.

49. Effects of Antimicrobial Peptides on Growth and Survival of *Vibrio spp*

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Vibrio vulnificus is an estuarine bacterium responsible for 95% of all seafood related fatalities in the U.S., the majority of which arise from the consumption of raw or undercooked oysters (Feldhusen, 2000). These bacteria are natural inhabitants of oysters and have proven difficult to remove using post-harvest processing (PHP) methods while still keeping the animal alive. Treatments such as depuration and refrigeration preserve the integrity of the shellfish but are ineffective in killing *V. vulnificus* (Tamplin and Capers, 1992; Motes et al., 1998). Other methods, such as low temperature freezing, irradiation and pasteurization are effective in removing *V. vulnificus* from oysters but kill the animal and render it unusable in the raw oyster market (Jakabi et al., 2003; Liu and Su, 2009; Kural and Chen, 2008). In recent years, the use of antimicrobial peptides (AMPs) has been employed by the food industry to inhibit and/or remove pathogens from foods. The human AMP hepcidin is structurally similar to bacteriocins that inhibit growth of an assortment of gram-positive and gram-negative bacteria (Krause et al., 2000; Park et al., 2001). Studies evaluating the effectiveness of hepcidin against *V. vulnificus* showed the bacterium was susceptible to the AMP at various inocula 10^3-10^5 CFU/mL, but only at high hepcidin concentrations (50, 75 and 100μM). Based on these results, the food safe AMP Nisin was also evaluated for its ability to kill and/or inhibit growth of *V. vulnificus*, *V. parahaemolyticus* and *V. cholerae*. Results showed that when used alone Nisin had no effect on growth or survival of these bacteria. However, when it was applied following certain chemical or physical stressors it was able to reduce in vitro population concentrations for all three species. These synergistic effects offer promising potential for the application of a hurdle-type postharvest processing treatment for oysters.

50. An Outcome-Based Comparative Risk Assessment of Microbiological Hazards in Foods

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Risk-based food safety is built upon the premise that data-driven analysis should inform efficient targeting and allocation of resources to maximize reductions in foodborne illness risk. The first step towards risk-based prioritization is identifying the greatest problems: *which pathogens in which foods cause the greatest impact on public health?* We focus on pathogen-food combinations because food safety interventions are usually targeted at specific pathogens in specific foods. To address this question, we developed a novel risk ranking model that, (a) combines recently published CDC estimates of the disease burden of 14 foodborne pathogens with information on health states excluded from their estimates, including rates of physician visits, long-term chronic sequelae of acute infection (e.g. kidney failure, paralysis), and latent impacts to developing fetuses (e.g. miscarriage, neonatal death, lifelong mental impairment), (b) utilizes disease symptoms, severities, and likelihoods to estimate two integrated measures of public health burden for each pathogen – monetary valuation in dollars and Quality Adjusted Life Years (QALYs) – that provide more comprehensive and comparable metrics of disease burden than summary incidence statistics, and (c) attributes foodborne diseases associated with each pathogen across 12 comprehensive food categories based on analysis of 11 years of foodborne outbreak data and an extensive in-house expert elicitation study. Thus, we estimate the annual public health impact due to 168 pathogen-food pairs in 5 metrics (overall illnesses, hospitalizations, deaths, dollars, and QALYs). We rank pathogen-food pairs and, by summing within foods across pathogens, we estimate and rank the disease burden associated with each food category. We conducted sensitivity analysis around key parameters such as incidence estimates, valuation of premature mortality, and assumptions used in outbreak attribution. We estimate annual public health impacts of over $14 billion and 60,000 QALY loss due to 14 pathogens, with over 90% due to five microorganisms: *Salmonella*, *Campylobacter*, *Toxoplasma gondii*, *Listeria monocytogenes*, and Norovirus. Rankings of the pathogen-food combinations are dominated by these 5 pathogens in a wide variety of food categories including poultry, produce, dairy products (soft cheeses), pork, eggs, beef, and complex foods (non-meat multi-ingredient dishes, such as processed and prepared foods). Our findings suggest the importance of including chronic conditions in disease burden estimates, the importance of impacts to developing fetuses as part of overall societal impacts, and the need for better data on the attribution of illness to foods, as well as the need for pathogen- and food-specific efforts.

51. Developing a More Coordinated and Integrated Approach to Food Safety Information: Findings from Denmark, the Netherlands, and the United Kingdom

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A science- and risk-based food safety system is built upon a foundation of data, and indeed, copious data are collected by government, industry, and academia. This food safety information infrastructure is complex and fragmented, however, with few resources for connecting disparate datasets and numerous obstacles to data sharing and availability. A more coordinated, integrated approach to collecting, managing, analyzing and communicating food safety information is needed. Other nations have wrestled with these challenges and implemented reforms accordingly. In particular, the European Union (EU) has gone through major reforms over the past decade. To identify opportunities and learn lessons about improving the U.S. system, we looked at EU actions and conducted case studies of Denmark, the Netherlands, and the United Kingdom. We examined the relevant literature and traveled to all three countries to interview experts and government officials in key agencies. We held follow-up phone interviews, as well as interviews with experts in the U.S. and other countries. We found that consolidation and centralization of authority have improved information flows, that annual reports on surveillance of pathogens in

humans, animals, and food have been critical for consistent information sharing and communication, and identified coordinated approaches to surveillance programs for animals, food, and feed, as well as integrated approaches to attributing illnesses to foods. We found that data collection and analysis is often done in stand-alone scientific institutes separated from regulatory agencies, and that regulatory agencies partially coordinate research programs. Risk analysis, transparency, and public participation are codified as key principles, and traceability requirements and data reporting are extensive. Our findings, while based on limited case studies, support institutional reform in the U.S., including greater integration of food safety regulatory agencies, as well as the creation of a stand-alone federal institute for food safety risk analysis that would combine currently disconnected analytical groups within FDA, FSIS, and CDC. We also recommend: (1) cross-agency annual reports of foodborne pathogen surveillance in humans, animals, food, and feed; (2) a national surveillance plan for farm-to-fork surveillance of domestic and imported food; (3) an integrated approach to food attribution analysis; (4) coordination of food safety through a long-term strategic vision and by integrating risk regulators into research prioritization; (5) transparency policies that increase publication of data and analyses used in decision making; and (6) strengthening and harmonization of trace-back and trace-forward requirements and data for outbreak response.

52. Application of a multiplex quantitative PCR (BAX) assay for simultaneous enumeration of *Vibrio* species in oysters

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Background *Vibrio vulnificus* (Vv), *V. parahaemolyticus* (Vp) and *V. cholerae* (Vc) are responsible for over 75% of seafood-borne bacterial infections. Simultaneous enumeration of pathogenic *Vibrios* is needed for the evaluation of post harvest processing (PHP) of oysters and to determine their relative distribution in the environment. This study evaluated the application a quantitative multiplex real-time PCR (BAX) assay for direct and most probable number (MPN) enumeration of these bacteria in oysters. **Methods** Growth and survival of the individual species were evaluated in broth culture (Luria Burtani with 1% NaCl, LBN) and live oysters by plate count and by multiplex QPCR. Competitive growth was also evaluated by QPCR following simultaneous inoculation of the three species. Environmental samples (n=3) were collected from three sites in Apalachicola Bay, FL, and MPN of the *Vibrio* species was evaluated using standard microbiological methods and by QPCR-MPN. **Results** Close agreement was observed between QPCR and plate count for Vv, Vp, and Vc (R2=0.89, 0.96, and 0.97, respectively) *in vitro*. The growth of the three species in tri-culture also showed excellent correlation with individually inoculated cultures (R2=0.96, 0.92, and 0.97, respectively), indicating minimal effects of competitive growth. Similarly, no significant differences were noted by QPCR in recovery from individually or simultaneously inoculated oysters. QPCR-MPN increased recovery compared to standard assay for Vc but not for Vv. The BAM and QPCR MPN assays also showed close agreement for recovery of Vv and Vp (R2=0.94 and 0.91) from environmental samples. Recovery of Vp was highest at the site with the highest salinity (21.9 ppt), while recovery of Vv was highest at the site with the lowest salinity (9.3 ppt). **Conclusions** These studies demonstrated that the QPCR MPN assay is a viable alternative to standard methods for the simultaneous evaluation of the three species in PHP oysters. Data from environmental samples demostrated the potential of this assay to determine environmental parameters that may promote the growth and survival of the different species.

53. Mycobacterium tuberculosis

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Mycobacterium tuberculosis, the bacterium that causes tuberculosis (TB), remains one of the most frequent and important infectious diseases causing morbidity and death. One third of the world's population is infected with *Mycobacterium tuberculosis*, and approximately 1.7 million people per year die from TB. It is especially difficult to control because of human immunodeficiency virus/TB co-infection and the emergence of virtually untreatable extensively drug resistant strains of TB. Consequently, there is a dire need for new drug therapies. Rv2224c (hip1) is a putative *Mycobacterium tuberculosis* serine protease or esterase that has been shown to be required for bacterial

survival in mice. Disruption of the Rv2224c gene leads to prolonged survival of infected mice and highly reduced lung pathology. We report on the subcloning of the Rv2224c gene into a GST fusion vector, expression and refolding of the 53 kDa gene product, and purification of hip1. Our efforts will lead to the exact identification of hip1 catalytic activity, the screening of small molecule inhibitors, and the X-ray crystal structure determination of this important TB drug target.

54. Potential spread of Huanglongbing through soil

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Huanglongbing (HLB) emerged in Florida in 2005 and has spread rapidly throughout the State. The bacterium associated with this disease (*Candidatus* Liberibacter asiaticus, Las) has not been isolated consistently and Koch's postulates have not been fulfilled. Las is a symbiont of the psyllid *Diaphorina citri*, which transmits the pathogen to citrus. Other potential avenues of transmission and spread of Las have not been investigated yet. When HLB infected trees are removed, young trees are often planted in the same planting hole. Replant disease is a common phenomenon for other fruit trees. In 2009-2010, we tested if Las could be transmitted through soil. One year after planting 120 mandarin seedlings in soil collected underneath HLB positive citrus trees from two groves, Las qPCR tests were positive for two symptomatic seedlings. Asymptomatic trees in soil from HLB positive groves, in autoclaved soil and in potting mix tested negative in Las qPCR tests. These results could mean that nonpathogenic *C*. Liberibacter species are common in soil and endosphere but test positive with qPCR (and that the symptoms were nonspecific) or that pathogenic Las is transmitted through soil besides psyllids. The experiment on soil transmission will be repeated; nematodes, protozoa and root residues will be isolated from soil and tested for Las by qPCR. Nematode and protozoa species that test positive will be used in transmission tests.

55. A Two-Patch Metapopulation Model for *Plasmodium falciparum* Malaria

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Mathematical models developed for studying malaria dynamics often focus on a single, homogeneous population. However, human movement connects environments with potentially different malaria transmission characteristics. To address the role of human movement and spatial heterogeneity in malaria transmission and malaria control, we consider a simple malaria metapopulation model incorporating two regions, or patches, connected by human movement, with different degrees of malaria transmission in each patch. Using our two-patch model, we calculated and analyzed the basic reproduction number, R_0, an epidemiologically important threshold quantity that indicates whether malaria will persist or go extinct in a population. Although R_0 depends on the rates of human movement, we show that R_0 is always bounded between the two quantities R_{01} and R_{02} – the reproduction numbers for the two patches if isolated. If without migration, the disease is endemic in one patch but not in the other, then the addition of human migration can cause the disease to persist in both patches. This result indicates that regions with low malaria transmission should have an interest in helping to control or eliminate malaria in regions with higher malaria endemicity if human movement connects them. Performing a sensitivity analysis of R_0 to various parameters in the two-patch model allowed us to determine, under different parameterizations of the model, which patch will be the better target for control measures, and within that patch, what type of control measure should be implemented. We found that if the extrinsic incubation period is shorter than the average mosquito lifespan, the control measures should be targeted towards reducing the mosquito biting rate. On the other hand, if the extrinsic

incubation period is longer than the average mosquito lifespan, control measures targeting the mosquito death rate will be more effective. For patches with similar mosquito biting rates, control measures that reduce biting rate should be applied to the patch with the higher immigration rate. If the immigration rates of the two patches are similar, then the control measures should be applied to the patch with the higher biting rate. Under more complicated scenarios, simulations can be used to determine which patch will be most effective to target. While human movement between regions poses challenges to malaria control and elimination, if estimates of relevant parameters in the model are known, including migration rates, our results can help inform which region to target and what type of control measure to implement for the greatest success.

56. All That Glitters Is Not Gold: Molecular Genetic Follow-Up to Genomic Sequencing of *Vibrio vulnificus*

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Vibrio vulnificus is the leading cause of reported death from infections related to consumption of seafood in the United States. Affected predisposed individuals die rapidly from sepsis. Otherwise healthy people can experience severe wound infection, which can lead to sepsis and death. There are two genetic profiles of *V. vulnificus* that exhibit differential isolation and virulence characteristics. Profile 1 strains are predominantly isolated from the environment, whereas profile 2 strains are predominant in clinical settings. In a mouse model of disease following skin infection, profile 2 strains are generally more adept at causing systemic infection and death compared with profile 1 strains. However, there are exceptions in both directions.

We recently sequenced the genomes of four *V. vulnificus* strains using SOLiD technology and compared the sequence reads with the two complete and annotated *V. vulnificus* genomic sequences. We identified 80 genes that were specific to profile 2 strains and 61 genes that were common to a highly virulent profile 1 strain and the profile 2 strains, suggesting that they are virulence genes. Some of the profile 2-specific genes encoded GGDEF proteins, Flp pili, and glutathione synthetase. Genes in common between the virulent profile 1 strain and profile 2 strains included those encoding utilization of mannitol, utilization and synthesis of sialic acid, and a large genomic island. In this study, we used allelic exchange mutagenesis and transposon mutagenesis to examine mutations in some of these candidate virulence genes for their effects on virulence.

Of the four genes/loci examined so far, only one was identified as necessary for virulence. Deletion of *mtlA*, required for utilization of mannitol, had no significant effect on skin or systemic infection. Deletion of *nanA*, required for the assimilation of exogenous sialic acid, had no effect on either local or systemic infection. Examining the presence or absence of Genomic Island 12 among a larger set of *V. vulnificus* strains revealed that several fully virulent strains did not encode this genetic element, casting doubt on its role in virulence. In contrast, a previously isolated and characterized Tn*PhoA* insertion mutation in an operon encoding Flp pili was severely attenuating for systemic, but not local infection.

Molecular pathogenesis studies are ongoing to examine additional candidate virulence genes. These results demonstrate that genomic sequencing and molecular epidemiology only create pools of virulence genes that must be followed with directed mutagenesis and examination of pathogenesis.

57. Improving Tuberculosis Infection Control in Mexico

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Background: Improved Tuberculosis (TB) infection control (IC) is one of the major objectives of the WHO Global Response Plan to Extensively and Multi-drug Resistant Tuberculosis. However, the National TB Program (NTP) and the majority of healthcare facilities in Mexico, do not have TB IC policies and procedures. This pilot project sought to expand existing IC practices, build capacity and strengthen the Mexican National IC Plan.
Intervention: In August 2009, we launched a pilot TB IC training project in Mexico. Three states with varying burdens of TB were chosen. One hospital and one primary health center were identified in each. The pilot consisted of TB IC facility assessments followed by a three day interactive course finalized by the development of a TBIC plan tailored to the each setting. **Results:** A total of 146 participants were trained Six facilities participated and five facilitators were trained. Participants reported high levels of satisfaction with training, materials and facilitators. The average TBIC knowledge of participants has increased significantly (+29.56% and +16.95%). TBIC plans have been developed for five of the six facilities. All three states developed a TBIC plan. One hospital had 24 active tuberculosis cases among health care workers in 2009, but only3 confirmed by microscopy. The rate of occupational TB is 600 per 100,000, 50 times higher than community rate. -12.7 per 100,000. The highest rates were among nurses in the emergency department. There was poor microbiology laboratory infrastructure that contributed to the lack of confirmed diagnoses. Numerous administrative changes were made rapidly during the trainings, including the implementation of surveillance programs for occupational TB cases with tuberculin skin testing.
Major limitations of the project were the lack of previously collected surveillance data and the short duration, so we were unable to assess the impact of our interventions. **Conclusions:** The incidence of healthcare associated TB in HCWs was very high in one of the facilities. The hands-on trainings led to rapidly implemented administrative changes that were very inexpensive but potentially highly effective. The Mexico NTP, encouraged by the success of this pilot project, is planning to expand the training program to additional states and facilities. An educational tool-kit will be the foundation for the expansion of the training. Political commitment from the administrators of the facility, the healthcare providers and the local, state and national TB programs is necessary for TB IC plan implementation. Expert technical assistance is recommended.

58. A Fluorescence Method for Screening Compounds Designed to Selectively Control the Mosquito Vector of Malaria, *Anopheles gambiae*

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Malaria remains a major global health challenge for millions of people, and effective methods of controlling malaria vector presents an urgent need. Currently available insecticides cannot be used effectively due to emerging resistance, as well as their toxicity to humans. We are striving to develop insecticides via inhibition of acetylcholinesterase (AChE) that would be lethal to mosquito, yet safe for humans. Here we present a fluorescence method of measuring interactions of human recombinant AChE with newly designed compounds. This method takes advantage of the presence of a peripheral site (P-site) and an acylation site (A-site) on AChE and binding of the fluorophore Thioflavin T (TFT) to the P-site. The fluorescence of TFT bound to AChE at the P-site can be reduced by competitive displacement of P-site ligands, and is also partially quenched by the binding of acylation site ligands in ternary complexes. This latter effect suggests deformation of the catalytic gorge by ligands bound to A-site. Our results show that fluorescence of TFT rises in a dose dependent manner, when it binds to AChE in the absence of an inhibitor, up to a maximum at 10 μM. The binding constant (Kd) is ca. However, A-site ligands 3-*tert*-butyl-phenylmethylcarbamate (PRC 331) and 9-amino-1,2,3,4-tetrahydroacridine (Tacrine) form ternary complexes with AChE and TFT, and the fluorescence is quenched in dose-dependent fashion, with EC_{50} = 43 nM

and 300 nM, respectively. Moreover, *d*-tubocurare, a P-site ligand, also quenches fluorescence of TFT, albeit at higher concentrations (EC_{50} = 247 uM). Kinetic studies will be used to distinguish direct versus allosteric quenching of TFT fluorescence. These findings will be used to measure interaction with AChE by various inhibitors for the design of new active ingredients in mosquito control systems, leveraging the known I70Y difference in the P-site sequence between insect (I70) and human (Y70).

59. Testing spatiotemporal hypothesis of bacterial disease epidemics using Bayesian phylogeography

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The rise of emerging infectious diseases constitutes a major global public health and economic problem. Understanding the reciprocal roles of transmission and the environment is critical for developing effective intervention strategies. The spatial spread of pathogen genomes can be modeled within a Bayesian framework known as "phylogeography" which incorporates both genetic and geographical data, thereby allowing *a priori* hypothesis of spatial association to be tested. The complementary field of geographic information systems (GIS) enables posited associations between ecological and landscape variables and disease incidence to be tested. Unifying these approaches would allow genomic, epidemiological, and geographic data to be analyzed in concert. Thus far, formal phylogeographic methods have not yet been applied to the molecular epidemiology of bacterial pathogens because the limited genetic diversity of datasets based on individual genes usually results in poor phylogenetic resolution. *Staphylococcus aureus* is a common cause of infections that has undergone rapid global spread over recent decades. We investigated a whole genome SNP dataset of healthcare-associated Methicillin resistant *S. aureus* sequence type 239 (HA-MRSA ST239) strains using Markov spatial models that incorporate geographical sampling distributions. The reconstructed timescale indicated a temporal origin of this strain shortly after the introduction of Methicillin, followed by global pandemic spread. The estimate of the temporal origin was robust to the molecular clock, coalescent prior, full/intergenic/synonymous SNP inclusion, and correction for excluded invariant site patterns. Finally, phylogeographic analyses statistically supported the role of human movement in the global dissemination of HA-MRSA ST239, although it was unable to conclusively resolve the location of the root. This study demonstrates that bacterial genomes can indeed contain sufficient evolutionary information to elucidate the temporal and spatial dynamics of transmission. Future applications of this approach to other bacterial strains may provide valuable epidemiological insights that may justify the cost of genome-wide typing.

60. Cholera in Florida, 2010-2011

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Since late 2010, an epidemic due to toxigenic *Vibrio cholerae* 01, serotype Ogawa, has affected Haiti, with over 200,000 reported cases and 4,000 deaths. A smaller outbreak followed in the Dominican Republic (DR). We expected introduced cases, as there were during the Latin American pandemic of the early 1990s, but little or no transmission in Florida.

As of late January, 2011, nine confirmed and probable cases of cholera have been reported to the Florida Department of Health (DOH) in persons with recent travel to Haiti. Six were hospitalized. Several suspected cases are under investigation. Additional persons with appropriate travel histories and clinical illnesses have tested negative for *Vibrio cholerae*. CDC is assisting with serologic diagnosis of some of these people. All patients have recovered. In the course of enhanced surveillance, one Florida resident infected with *V. cholerae* non-01, one Florida child infected with *Vibrio cholerae* 075, and one visitor infected with both *Vibrio cholerae* non-01 and *Vibrio parahaemolyticus* (exposed to raw oysters in the DR) have been identified.

High-concern situations include cholera while working as a food-handler or providing health care, or in a person living in severely substandard sanitary circumstances. One confirmed, probable or suspected case is in a hospital phlebotomist. No transmission of *V. cholerae* from imported cases to household residents or other Florida contacts has been identified. Travelers may bring contaminated food items, such as seafood, back to the United States, resulting in cases; one such situation is currently under investigation.

In response to the threat and reality of imported cholera, DOH developed guidance for clinicians on recognition, reporting and management of returned travelers with cholera; developed guidance for County Health Departments on the recommended public health response to cases; modified our surveillance case definition to include probable and suspect cases in people with appropriate travel histories; developed an outbreak-specific case report from within our Merlin surveillance application; requested emergency departments participating in our ESSENCE syndromic surveillance system to mention Haiti and cholera in the chief complaints of persons presenting for care with appropriate histories; collaborated with County Health Departments on public advisories; contributed to an MMWR article about Haiti-related cholera outside Haiti; collaborated with CDC on the development and distribution of Travel Health Alert Notices to travelers arriving in the US from Haiti; and assisted the CDC in evaluating the impact of those notices.

Acknowledgements: Tom Torok, Janet Hamilton, Aaron Kite-Powell, Madsen Beau de Rochars, Florida Bureau of Laboratories

61. Genetic variation and GacA regulation of iron response in *Vibrio vulnificus*

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Vibrio vulnificus is an opportunistic human pathogen and is the most common cause of fatal seafood related illnesses in the U.S. This ferrophilic bacterium typically infects individuals with compromising conditions such as hemochromatosis (iron overload), hepatic disease, or diabetes. The GacS/GacA two-component signal transduction system is found in all *Vibrio* species and is associated with regulation of virulence and biofilm formation. As this system also regulates iron acquisition in *V. fischerii*, the present study investigated the role of GacA in the catechol siderophore system of *V. vulnificus*, which includes genes encoding siderophore synthesis (*venB*), siderophore surface receptor *vuuA*, and a cytoplasmic hydrolase *viuB* respectively. Growth studies showed the *gacA* mutant and the vector plasmid control for complementation were significantly decreased in growth yields as compared to either the wild-type or the complemented mutant strains ($p<0.01$). In regards to gene regulation, qRT-PCR demonstrated that transcript levels of all three genes were not significantly changed under iron replete conditions. Under iron limiting conditions the mutant exhibited large fold decreases for all three genes ($p<0.001$) compared to wild-type. Of these genes, *viuB* may provide a marker for virulence, as DNA sequences segregated the clinical and environmental isolates into two phylogroups. Type 1 consisted of strains that were primarily of clinical origin (94%) with increased virulence, while Type 2 strains were predominately environmental isolates (78%) with relatively reduced virulence in a mouse model. Therefore, mutations of this gene should correlate to decreased virulence, with allelic exchange having similar results if the gene type corresponds to virulence. Further studies are ongoing to investigate mutational analysis of genes involved in the *V. vulnficus* iron response and their specific role in virulence.

62. Individual characteristics and travel patterns associated with distinct HIV-1 subtype infections in Agadir, Morocco

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Introduction: In Morocco, HIV infection rates have doubled in the previous 6 years and continue to increase by approximately 15% per year. The epidemic in Morocco is notable in its shift from HIV subtype B, originating in Europe, to subtype AG, originating in sub-Saharan Africa. This shift of HIV subtypes may offer clues as to the reasons that HIV is continuing to increase in Morocco. However, the specific behavioral or travel patterns associated with the shift from subtype B to subtype AG are not known. **Methods:** Participants included 76 persons receiving care for HIV infection in the city of Agadir, which has the highest number of known HIV cases in Morocco. Each participant provided a blood sample for HIV subtyping, and completed a face-to-face interview regarding their demographic characteristics, travel history and risk behaviors. We compared behavioral and travel characteristics of persons with subtype B vs. subtype AG using bivariate analysis and GIS mapping. **Results:** The sample included 38 men and 38 women with HIV. Compared to the women, men were statistically significantly more likely to be older, married, more educated, more likely to drink alcohol, and more likely to have traveled outside of Morocco. The 76 HIV subtypes included 17 with AG (22%), 40 with B (53%), and 19 with other subtypes (25%). When comparing persons with subtype AG to subtype B, subtype AG was significantly more common in men than in women (44% vs. 17%, $P<0.05$), and especially common in men who have sex with men (4/5 MSM had subtype AG). Alcohol consumption was more common in those with subtype AG than subtype B (70% vs. 55%, $p<0.05$); however, HIV subtype was not significantly associated with age, marital status, education, occupation, or other drug use. GIS mapping indicated much broader travel patterns in men than in women, but the travel patterns did not appear to vary dramatically by HIV infection subtype in this sample. **Conclusion:** The emergence of HIV-1 subtype AG into Morocco appears to be associated with specific risk groups and behaviors. Phylogenetic analysis of HIV viral sequences and a larger study sample are proposed to better identify persons with related HIV virus, to provide further clues as to how and why the HIV epidemic continues to grow in Morocco, and to suggest specific prevention strategies.

63. Efficient Transmission and Evolutionary Dynamics of SIVmac251 Quasispecies Infecting CD8-depleted Rhesus Macaques

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The progression of SIV infection in experimentally infected rhesus macaques follows a similar course to that of human immunodeficiency virus (HIV). CD-8 depleted rhesus macaques infected with the genetic heterogeneous SIVmac251 viral swarm rapidly develop Simian-AIDS (SAIDS) and SIV-encephalitis (SIVE). We analyzed the phylogenetic relationship of 1,135 envelope viral sequences derived from lymphoid and non-lymphoid tissues taken 21-118 days post-infection (dpi) from six CD8+ T lymphocyte depleted SIV-infected macaques and 193 sequences derived from the SIVmac251 inoculum. Nucleotide diversity was calculated and phylogenies were inferred for each macaque. No evidence of a transmission bottleneck reducing overall diversity was observed in the peripheral virus at 21 dpi. On the other hand, two haplotypes accounting for less than 3% of the inoculum sequences were present in the plasma in at least 4 monkeys at 21 dpi. The haplotypes persisted in the later plasma time and were found in the

brain tissues at moderate to high frequencies (4-100%). Additionally, a macrophage-tropic variant, not detected in the viral swarm (<0.3%), was observed at high frequencies (29-30%) in brain derived sequences from two macaques with meningitis or SIVE. This study demonstrates the utility of the rapid AIDS macaque model for understanding the intra-host evolutionary dynamics and the efficient transmission of low frequency SIVmac251 founder variants, characterized by gp120 motifs that may be linked to neuropathogenesis.

64. Impact of Virus Dose, Extrinsic Incubation Temperature, and Incubation Period on Vector Competence of *Culex nigripalpus* (Diptera: Culicidae) for West Nile Virus and St. Louis Encephalitis Virus

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Culex nigripalpus, a vector of West Nile and St. Louis encephalitis virus (family *Flaviviridae*, genus *Flavivirus*; WNV, SLEV) in the southeastern US, was characterized for vector competence. For WNV, we assessed the impact of virus dose and incubation period (IP), while for SLEV we assessed the impact of dose and extrinsic incubation temperature (EIT). Vector competence was evaluated with chi-square analyses ($P<0.05$) using rates of infection (% virus-positive bodies out of total tested), dissemination (% virus-positive legs out of those infected), and transmission (% virus-positive saliva out of those infected). Virus titer in bodies, legs, and saliva was also tested. *Culex nigripalpus* were fed blood containing a low dose (LD: 6.3 ± 0.01) or high dose (HD: 7.3 ± 0.1) of WNV (logs plaque-forming units (pfu)/mL ± SE) and held at 28°C for IPs of 6 or 12 d. WNV infection rates were high (100%) and not affected by dose or IP. At 6 d, WNV dissemination rates were highest at the HD, but not different between doses at 12 d. Transmission of WNV was only observed under permissive conditions (HD, 12 d) and was low (11%). *Culex nigripalpus* were fed blood containing a LD (4.0 ± 0.1) or HD (4.6 ± 0.1) of SLEV (logs pfu/mL± SE) and held at 25°C or 28°C for 12 d. SLEV infection rates (≥85%) were not affected by dose or EIT. SLEV dissemination rates were lowest at 25°C for each dose group but rates did not differ between doses, showing a greater impact of EIT than dose. SLEV dissemination rates for the LD (91%) and HD (100%) at 28°C were higher than observed for WNV. Transmission of SLEV occurred at only 28°C. The SLEV doses tested were significantly lower than the WNV doses, yet *Cx. nigripalpus* showed higher SLEV dissemination rates under these conditions. *Culex nigripalpus* vector competence for SLEV and WNV is discussed and compared to previous similar studies of *Cx. pipiens quinquefasciatus* SLEV and WNV vector competence. Studies of environmental and biological factors and their influence on vector competence are essential to understand the role of vectors in virus transmission cycles in nature.

65. Non-tuberculous Mycobacteria in Florida Surface and Municipal Waters

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Non-tuberculous mycobacteria (NTM) are ubiquitous in the environment, including within municipal water distribution systems. Incidence of NTM infection in humans has dramatically increased over the past 20 years, with the number NTM cases exceeding that of tuberculosis cases within the United States. NTMs such as *M. avium, M. kansasii, M. xenopi, M. scrofulaceum, and M. marinum,* have been associated with pulmonary disease, bone and soft tissue disease, as well as disseminated diseases. Disseminated infection is primarily due to *Mycobacterium avium complex* (MAC), an opportunistic bacterial infection. Disseminated MAC infection is most commonly found in patients with HIV-1, organ transplant recipients, persons with kidney or liver failure, or the elderly and is associated with significant mortality in these persons. Incidence of pulmonary NTM infections is also increasing in otherwise healthy subjects with the majority of clinical isolates associated with environmental sources. Primary route of NTM infection is believed to be through exposure to water through ingestion, or inhalation of aerosolization of water colonized with mycobacteria. We hypothesize that the chemistry of natural waterways may regionally alter the number and diversity of mycobacteria, and that these effects may also change the relative risk to the general population for NTM infections. In this study NTMs were detected in environmental samples using novel qPCR probes. Genus- and MAC-specific probes detected mycobacteria and MAC in all samples tested from surface waters and waters sampled from municipal water distribution lines pre- and post- treatment. Phosphorus and pH appeared to play a significant role in the presence of mycobacteria among the different samples ($p<0.01$). Results indicate that mycobacteria are growing within water distribution systems and various water quality parameters may play a role in the density of these organisms within both surface waters and municipal water distribution systems. The presence and growth of these bacteria within municipal sources may provide a means for the distribution of these organisms to households throughout the study area, and may serve as a source of infection for high-risk patients.

66. The HPV Oral Prevalence Investigation (HOP-IN) Study: Rationale & Design

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Background: Oral human papillomavirus (HPV) infection has recently been identified as an important cause of cancers of the head and neck, particularly those of the oropharynx. In the United States, approximately two-thirds of oropharyngeal cancers are attributable to oral HPV infection, resulting in up to 4,600 HPV-related cases. Importantly, the incidence of potential HPV-related head and neck cancer between 1973 and 2004 in the United States has increased by 0.8% annually, whereas the incidence of non-HPV-related head and neck cancer has decreased by 1.85% annually over the same period. Despite this new appreciation for HPV involvement, data on the incidence, prevalence and persistence of oral HPV infections are scarce, particularly in college-aged women. Furthermore, little is known about risk factors for contracting oral HPV infections or whether HPV vaccination prevents oral HPV infection. **Design:** Using a combined cross-sectional and cohort study design, the HOP-IN study plans to enroll 1000 women at the University of Florida starting in February 2011. Eligible participants are females, aged $\geq$ 18 years, currently enrolled in college. After providing informed consent, fully eligible subjects will be asked to provide an oral rinse sample with 15 mL of mouthwash. Samples will then be processed in the Emerging Pathogens Core Laboratory and sent for determination of HPV-status, including specific HPV subtype using the Roche linear array assay. Participants will also use a small portable computer to complete an electronic questionnaire covering demographic characteristics, sexual behaviors (lifetime and recent), contraceptive use, substance use, smoking behavior, concomitant HPV-related diseases, lesions or sores in the mouth, and HPV vaccination status. Any participant with a positive baseline oral test will be informed and asked to participate in a follow-up cohort study to determine the persistence of oral HPV infections and risk factors for persistence at 3-, 6- and 12-months after the baseline visit. **Study Outcomes:** The primary outcomes will be the prevalence of oral HPV infection, overall and by specific HPV subtype (e.g. HPV16). We will identify factors associated with prevalent oral HPV infection, the proportion of HPV-infected individuals with persistent infections at 3-, 6-, and 12-months, and risk factors for persistent infections. **Conclusions:** Given the current burden and recent increase in HPV-associated

head and neck cancers in the U.S. and other developed countries, quantifying the prevalence and risk factors associated with oral HPV infections is important, especially among those with greater risk of contracting oral HPV. The HOP-IN study will include the largest sample of college women to date, and will provide opportunities for other research collaborators interested in the epidemiology of HPV infections.

67. Survival of *Salmonella enterica* in water under different environmental conditions

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Salmonella enterica can be considered a re-emerging pathogen. Survival of *S. enterica* in the natural environment has been studied before, but survival in surface water is not well understood. Therefore, the survival of a gfp-marked strain of *S. enterica* serovar Typhimurium in water was studied under different eutrophication and temperature conditions. A similar experiment was carried out twice: once with five eutrophication levels (30, 50, 100, 500 and 1000 mg C / L water) over a four-month period and again with three eutrophication levels (30, 100 and 200 mg C / L water) over a three-week period. The carbon levels were obtained by mixing cattle manure with spring water. Each flask was inoculated with the gfp-strain of *S. enterica* at a concentration of 10^{10} CFU/ml. The flasks were placed in two sets of three incubators at 7, 17 and 27 °C in the first experiment and at 27 °C in the second. In the four-month experiment, *S. enterica* colonies were counted after dilution plating on kanamycin-amended LB agar daily for a one-week period and weekly or bi-weekly thereafter. In the 3-week study, CFU's were determined daily. The plates were incubated at 22 °C for 48 hr. *S. enterica* concentrations declined quickly within one week and then slowly during one month. After 50 days, *S. enterica* concentrations dropped quickly at the lowest three carbon concentrations and more slowly at the highest two concentrations. However, *S. enterica* survived for almost 4 months, even at the lowest carbon concentration. The decline in *S. enterica* concentration was significantly faster at the lowest three eutrophication levels than at the highest two levels in the first experiment. Effects of temperature were not significant. In the second experiment, effects of eutrophication level were not significant in the first two weeks; additional analyses are needed for the third week. When daily observations were plotted over time, a wave-like pattern was discerned in CFUs of *S. enterica* per ml of water; the occurrence of oscillations will be verified by harmonics analysis. Based on similar research on *E. coli* O157:H7, it is expected that the amplitudes of the oscillations are greater at higher eutrophication levels, so that the predictability of the survival period would be less precise at those eutrophication levels.

68. Altered T cell differentiation mediated by heterozygous SOCS1 expression: implications for autoimmunity

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Suppressor of cytokine signaling-1 (SOCS1) is an intracellular protein that negatively regulates cytokine signaling. Heterozygous expression of SOCS1 leads to T lymphocyte mediated autoimmunity, suggesting that reduced expression of SOCS1 can disrupt immune system tolerance through aberrant cytokine signaling in lymphocytes. Immune system tolerance to self-tissues is dependent upon proper differentiation of naïve T lymphocytes (Tresp) subsequent to activation. Although it is known that cytokines are essential for Tresp differentiation, it is poorly understood how heterozygous expression of SOCS1 affects this process. We demonstrate that heterozygous expression of SOCS1 does not affect Treg induction from naïve Tresp. However, it does disrupt Th17 differentiation and results in a Th1 bias, as SOCS1 heterozygous Tresp that have been placed in Th17 inducing conditions have decreased IL-17 and increased IFN-gamma message expression. These results suggest that the systemic autoimmunity experienced by SOCS1 heterozygous mice may not be due to decreased Treg induction, but rather a predominant Th1 bias caused by dysregulated IFN-gamma signaling as a result of reduced expression of SOCS1.

69. Gut Microbiota Composition Correlates with Colorectal Polyp Prevalence

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Distortions in human gut microbiota have been associated with various diseases. However, little is known about gut microbiota contributions to colorectal carcinogenesis in humans although animal models are suggestive. We performed a nested case control study in human volunteers undergoing a screening colonoscopy. Data on dietary habits and medical history, a fecal sample, and multiple colon biopsy samples were collected from 91 subjects. We analyzed microbiota composition in 30 individuals presenting with at least one polyp and 30 age and gender matched controls. Denaturing Gradient Gel Electrophoresis (DGGE) profiling did not show any differences between cases and controls in overall diversity index. In addition, pyrosequencing was performed on stools samples and colon biopsy samples. There was no difference between cases and controls in diversity index and overall microbiota pattern. However, we did detect differences in the prevalence of specific sequences. Specifically, *Eubacterium ramulus, Acidovaraz sp. ZO22,* and three OTUs matching closest to the groups *Parabacetroides, Collinsella,* and *Clostridiales* were detected in higher numbers in subjects with polyps. These sequences were found when subjects were classified based only on polyp presence and were also found when these subjects were reclassified by relative risk. Sequences found in controls at significantly higher numbers included those corresponding to *Ruminococcus sp. CO41* and several OTUs matching closest to the groups *Lachnospiraceae, Clostridiales,* and a human intestinal firmicute. These sequences were likewise found using both classification methods described above. Our current data are consistent with the hypothesis that differences in gut microbiota composition correlate with the prevalence of colorectal polyps.

70. Molecular Analysis of the Gut Microbiota associated with *Clostridium difficile* Infection

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Clostridium difficile accounts for 3 million cases of diarrhea and colitis in the US annually. Increasing evidence suggests that human gut microbiota plays an important protective role against *C. difficile* infection (CDI), and yet basic features of gut microbial communities associated with CDI remain poorly understood. We used barcoded deep sequencing to examine 60,798 partial prokaryotic 16S rDNA gene sequences (V1-V3) from feces of 42 individuals with CDI and 40 healthy adults. Using a modified GAST (Global Alignment Sequence Taxonomy) algorithm, we identified a total of 2,896 bacterial phylotypes. Phylogenetics-based analysis revealed a significant alteration of organismal lineages in CDI. Compared to healthy controls, the microbial communities associated with CDI were significantly less diverse and had fewer phylotypes. These changes were driven primarily by the loss of phylotypes within the *Firmicutes* phylum. The abundance of several members of the *Ruminococcaceae* family, including *Faecalibacterium* spp., were significantly decreased in CDI (3.9%) compared to healthy controls (15.0%), suggesting a role for protection against CDI. These findings provide the most detailed sequence-based analysis of gut microbiota associated with CDI to date. Longitudinal analysis of these diverse communities focusing on the putative probiotic bacteria identified here is a critical next step toward understanding the role of gut bacteria in CDI, and more broadly, the clinical implications of gut microbiota in infectious diseases.

71. A SEROEPIDEMIOLOGICAL STUDY OF CANINE ZOONOSES AMONG PERSONS OCCUPATIONALLY EXPOSED TO DOGS

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Background – Man often lives or works in close contact with dogs, yet epidemiological studies examining human infections with emerging canine pathogens are few. We sought to examine evidence that three relatively new canine pathogens might be infecting man: *Brucella canis*, H3N8 canine influenza virus (CIV), and canine respiratory coronovirus (CRCoV). Human infections with CIV or CRCoV have not been reported or serologically studied. Clinical human *Brucella canis* infections have been documented, but are rare. **Methods** – Using an informed consent process, healthy adults whose work or hobbies involved exposure to dogs completed an enrollment questionnaire and permitted a serum sample collection. Targeted populations included breeders, veterinarians, and shelter, kennel, and racetrack workers. Sera were studied for evidence of *B. canis*, CIV, and CRCoV infections through various serological assays. Persons not exposed to dogs were enrolled as a control group. **Results** – 306 dog-exposed workers and 101 non-exposed controls have been enrolled and provided a sera sample. Preliminary data suggests a small percentage of participants have evidence for previous infections with either *B. canis* or CIV. A serological assay to detect CRCoV antibodies in humans is under development. **Conclusions** – An emerging infectious disease first seen in persons with intense dog exposures could indicate an animal pathogen has gained the ability to spread across species. Identifying specific disease risks in this occupational group would bring more awareness of zoonotic diseases to persons who are occupationally exposed to dogs. Enrollments and laboratory analyses are ongoing.

72. GacA regulates the survival of *Vibrio vulnificus* in the Eastern oyster (*Crassostrea virginica*)

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Background: *Vibrio vulnificus* is an opportunistic human pathogen and the most common cause of reported seafood-related deaths in USA. Consumption of raw oysters that contain *V. vulnificus* can lead to life-threatening systemic disease. The GacS/GacA two-component signal transduction system contributes to the virulence of this species in a mouse model of disease (Gauthier et al., 2010) and also causes a cascade of gene regulation in other *Vibrio spp*. Our research investigated the role of GacS/GacA in the attachment and survival of *V. vulnificus* in oysters. **Methods:** Growth and survival was analyzed *in vitro* at room temperature and at 37°C in Luria Burtani with 1% NaCl (LBN) broth and in oysters incubated at room temperature and 16 ppt salinity over a 6 day period. The contribution of GacA was evaluated in competitive studies using kanamycin resistance (200μg/ml) and loss of mannitol fermentation to compare recovery of a *gacA* deletion/substitution mutant (*ΔgacA*::*aph*) to a wild-type surrogate strain marked only by kanamycin resistance and loss of mannitol fermentation (*ΔmtlA*::*aph*). Following dual inoculation of the two strains in the freshly harvested oysters, samples (n=6) were assessed for bacterial content at 24, 48, 72h and 6 days post-inoculation. **Results:** The *V. vulnificus ΔmtlA*::*aph* was used as a surrogate for wild-type strains as it showed similar *in vitro* growth patterns but could be differentiated from wild-type on LBN agar containing mannitol with a pH indicator. Kanamycin selection was used to differentiate inoculated strains from background *Vibrios* in oysters. Results showed that the number of *V. vulnificus* Δ*gacA* mutant did not differ significantly from wild-type surrogate at 24, 48, 72h but was significantly ($p<0.001$) decreased at 6 days. **Conclusions:** In summary, the GacS/GacA two-component signal transduction system did not appear to contribute to initial attachment to oyster but showed significant contribution to the long-term survival of *V. vulnificus* in oysters. Further studies are ongoing to investigate the function of GacA inhibitors that interrupt GacS/GacA pathway influence on bacterial survival in oysters.

73. Role of the *E. coli* Common Pilus in Biofilm Formation and Adherence in Clinical Isolates of *Klebsiella pneumonia*

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Klebsiella pneumoniae is an opportunistic nosocomial pathogen that primarily affects immunodeficient people. Clinical diseases caused by this bacterium include pneumonia, bacteremia, urinary tract infection, diarrhea, upper respiratory tract infection, and wound infection. Biofilm formation (BF) is a property associated with virulence of pathogens colonizing of the upper respiratory tract. Pili or fimbrial adhesins are virulence factors that adhere to different surfaces and promote interbacterial interactions for biofilm formation. *K. pneumoniae* produces two major fimbrial types, type 1 and type 3 fimbriae. The *E. coli common pilus* (ECP) is a recently described adhesive structure produced by most *E. coli* pathogroups. A search for the major pilus subunit gene *ecpA* homologs among the *Enterobacteriaceae* indicated that *K. pneumoniae* contains the *ecp* gene cluster, *ecpRABCDE*. The goal of this study was to investigate the frequency of *ecpA* among *K. pneumoniae* clinical isolates and to explore the role of ECP in cell adherence and biofilm formation. Ultrastructural analysis using electron microscopy on two different strains of *K. pneumoniae* showed long peritrichous pili that reacted with anti-ECP antibodies by immunogold labeling. In agreement, pili purified from one of the strains showed a 21-kDa band in SDS-PAGE corresponding to EcpA. No antigenic cross reactivity was found between the type 3 pilus MR/K and ECP. We then tested a collection of 69 laboratory and clinical isolates among which, *ecpA* was found in 66 (96%) strains and 62 (94%) of these *ecpA* positive strains produced ECP after 6 h of incubation with HeLa cells as shown by immunofluorescence. Forty-eight (70%) of the 55 BF+ strains displayed ECP in biofilms produced after 24 h of incubation. In contrast, the *mrkA* gene was found in 100% of the strains examined, but only 55% of them produced MR/K when adhering to HeLa cells. ECP and MR/K were co-produced by 51% of the strains. The high incidence of *ecpA* and its presence on *Klebsiella* adhering to host cells and in biofilms suggest a role of ECP as an important adhesive structure in this species. Future studies will aim to construct *ecpA* mutants to determine the role in adherence to epithelial cells and biofilm formation.

74. Estimating the reproductive numbers for the 2008-2009 cholera outbreaks in Zimbabwe

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Cholera remains an important global cause of morbidity and mortality, capable of causing periodic epidemic disease. Beginning in August, 2008, a major cholera epidemic occurred in Zimbabwe, with 98,585 reported cases and 4,287 deaths. The dynamics of such outbreaks, particularly in non-estuarine regions, are not well understood. We explored the utility of mathematical models in understanding transmission dynamics of cholera, and in assessing the magnitude of interventions necessary to control epidemic disease. Weekly data on reported cholera cases were obtained from the Zimbabwe Ministry of Health and Child Welfare (MoHCW) for the period from 13 November 2008 to 31 July 2009. A mathematical model was formulated and fitted to cumulative cholera cases to estimate the basic reproductive numbers $\mathcal{R}_0$, and the partial reproductive numbers from all 10 provinces for the 2008-2009 Zimbabwe cholera epidemic. Estimated basic reproductive numbers were highly heterogeneous; ranging from a low value of just above unity to 2.72. Partial reproductive numbers were also highly heterogeneous, suggesting that the transmission routes varied by province; human-to-human transmission accounted for 41% to 95% of all transmission. Our models suggest that the underlying patterns of cholera transmission varied widely from province to province, with a corresponding variation in the amenability of outbreaks in different provinces to control measures such as immunization. These data underscore the heterogeneity of cholera transmission dynamics, potentially linked to differences in environment, socio-economic conditions, and cultural practices. The lack of traditional estuarine reservoirs combined with these estimates of $\mathcal{R}_0$ suggest that mass vaccination against cholera deployed strategically in Zimbabwe and surrounding regions could prevent future cholera epidemics and eventually eliminate cholera from the region.

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