EPI RESEARCH DAY 2010:

BOOK OF ABSTRACTS

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LETTER FROM THE DIRECTOR

January 26, 2010

Welcome to the third annual EPI Research Day! This is a particularly special day for us, as it is also marks the dedication of our new building. My hope is that today's sessions will give you a feel for the wide range of emerging pathogens-related research conducted on the University of Florida campus, and an appreciation of the ways in which our new facility will further enhance collaborations among investigators. As part of these activities I especially welcome investigators from the Florida Department of Health; the University of North Florida; the USDA/ARS Center for Medical, Agricultural, and Veterinary Entomology; and UF investigators from outside of Gainesville, including the Florida Medical Entomology Laboratory at Vero Beach, the Whitney Laboratory at St. Augustine, the North Florida Research and Education Center at Quincy, and the Citrus Research and Education Center at Lake Alfred, all of whom are presenting posters today.

As part of our dedication we are particularly honored to have in attendance Florida Surgeon General Dr. Ana M. Viamonte Ros; Florida Department of Agriculture Deputy Commissioner Dr. Joanne Brown; Infectious Disease Society of America President-elect and former Director of the Center for Infectious Diseases, CDC, Dr. James Hughes; and U.S. Department of Agriculture Deputy Undersecretary for Food Safety Jerold Mande. The research being done by EPI investigators and their partners has impact at a state, national, and international level, and we are pleased to have these individuals present to see the type of work being done and to join in our celebration.

While the completion of our building is an important landmark, EPI itself continues to grow: new faculty are being recruited, and new projects are underway. I appreciate your being here today to be part of our Research Day activities. 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.
EPI Director, and Professor of Medicine

RESEARCH DAY 2010:

SCHEDULE OF EVENTS

8 AM - 10 AM	Poster set up
10 AM - NOON	Dedicated Poster Session *(presenters, please stand by your posters)*
NOON -1 PM	Lunch
2 PM - 3 PM	Dedication Ceremony on the EPI lawn

01. *Rickettsia parkeri*: emerging spotted fever in Florida

Danielle Stanek1, DVM; Beth Radke1, MPH; Valerie Mock2; and Carina Blackmore1, DVM, PhD

[1]Florida Department of Health, Zoonotic and Vector-Borne Disease Program, [2]Florida Department of Health Bureau of Laboratories

Background: Approximately 20 cases of Rocky Mountain spotted fever (RMSF) are reported in Florida annually, with testing primarily performed using serologic assays. Cross-reaction of serologic testing between *Rickettsia* species is known to be common. The Gulf Coast tick, *Amblyomma maculatum*, is relatively resistant to drying conditions, making it suitable for arid coastline climates, and is distributed across Florida. The emerging rickettsia *Rickettsia parkeri* is associated with this tick and with symptoms of "mild" RMSF in the 12 previously reported cases identified in the United States. In addition, an eschar lesion has also been associated with this infection and but not with *R rickettsii*. In 2007 the first probable *R. parkeri* case was identified in the Florida Panhandle. In 2009, *R. parkeri* was confirmed in a central Florida resident and the tick that bite him using polymerase chain reaction (PCR) performed at the Centers for Disease Control (CDC). A second patient from the same region was also found to have compatible symptoms including a skin lesion at the site bite site, and a 4 fold change in serology for *R. parkeri* (also performed at CDC). A third suspect patient from southwestern Florida is undergoing confirmatory testing at this time including serologic testing and IHC and PCR testing of a skin biopsy.
Methods: Describe clinical presentation of suspect, probable or confirmed *R. parkeri* patients in Florida. Describe new sampling methodology, outreach and syndromic surveillance tools that may be applied to improve case finding in Florida.
Results: One suspect, two probable and 1 confirmed *R. parkeri* cases have been identified in Florida in the past 2 years. Distinguishing clinical presentation (eschar lesion), and further developing outreach tools (Florida Department of Health public webpage) may assist with case finding. Assessing syndromic surveillance of hospital admittance data suggest *R. parkeri* activity may be more broad spread than initially realized and may also assist with case finding.
Conclusions: Cases previously attributed to RMSF are likely at least in part caused by other spotted fever rickettsia including *R. parkeri*. Information about clinical presentation, improved sampling methodology, and geographical and temporal activity should be provided to health care providers. On-line information and syndromic surveillance data may be useful adjuncts to case finding.

02. *Aedes aegypti* Brush Border Membrane Vesicles Complement *Xenopus laevis* Oocytes for Characterizing Novel Mosquito Membrane Transporters

William R. Harvey[1, 2]; Kenneth M. Sterling Jr.[1]; Paul J. Linser[1] and Bernard A.Okech[2, 3]

[1]Whitney Laboratory, UF; [2]Emerging Pathogens Institute, UF; [3]College of Public Health & Heallth Professions

Understanding the physiology of disease vector mosquitoes and developing new mosquitocides are hindered by three roadblocks. (1) In the pre-genomic era many transporters were characterized physiologically in membrane vesicles, especially those from apical brush borders of *Manduca sexta* (MsBBMV) but few transporters have ever been cloned from this source. Conversely, in the post-genomic era many membrane transporters have been cloned from the genomic mosquitoes, *Anopheles gambiae* and *Ae. aegypti*, but few have been characterized physiologically. (2) A highly promising Na^+/H^+ Antiporter has been cloned from *An. gambiae* (AgNHA1) and expressed in *Xenopus laevis* oocytes but characterization has been frustrated by activation of an endogenous oocyte Na^+ conductance. (3) Two aromatic nutrient amino acid transporters (NATs) have been cloned from *An. gambiae* and characterized in *Xenopus* but have never been characterized in BBMV from mosquitoes since it would require 1,000 larvae to yield the same quantity of membranes vesicles as 1 *M. sexta* larva. MacIntosh has patented a method to isolate BBMV from whole larvae (BBMVW) that yields BBMVW in 2 hours. Abdul-Rauf and Eller used such BBMVW in studies on the *Bacillus thuringiensis* subsp. *israelensis* (Bti) receptor and others have shown that amino-peptidase 1 (APN1) is the receptor for the Bti crystal protein, Cry11Aa, and that amino-peptidase 2 (APN2) is the receptor for Cry11Ba. Our immuno-histochemical studies show that the AeH^+ V-ATPase, which generates a voltage (outside positive) that AeNHA1 and AeNATs 6 and 8 use for cation exchange and amino acid uptake respectively (the trio is called VAN for short), are all highly expressed in BBMV from *Ae. aegypti* whole larvae. Dr. Okech is presenting a poster that describes these studies.

Until recently, studies that identify new targets for the development of novel mosquitocides have been justified as agents for the control of diseases in sub-Saharan Africa. The moral outrage over the ~3,000 children who die each day of malaria there has led philanthropists and governments to provide billions for aid but has never led to the fear and outrage that 09/11/2001 or the 12/25/2009 have generated. Yet a US Senate-commissioned committee headed by Senators Bob Graham and Jim Talent concluded that the US homeland is at greater risk from Bioterrorism than from the Atom Bomb. Jeffrey A. Lockwood's 2009 book, "Six Legged Soldiers Using Insects as Weapons of War" concludes with a graphic example of how a single terrorist could paralyze an American city by releasing millions of adult *Ae. aegypti* females infected with Yellow Fever, for which we have no cure. The Emerging Pathogens Institute is sponsoring a workshop in May of 2010 to investigate the seriousness of the threat and to recommend actions to counter it. Novel mosquitocides may be part of a solution.

03. Characterization of five Yersinia pestis phages for developing a phage cocktail for surface decontamination

Chythanya Rajanna[1], Mohammed H Rashid[1], Tamara Revazishvili[1], Timothy Dean[2], Alexander Sulakvelidze[1]

[1]University of Florida, Department of Molecular Genetics and Microbiology; [2]Environmental Protection Agency

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

Five Y. pestis-lytic phages (YpsP-G, YpP-Y-ATCC, YpP-R-ATCC, YpsP-PST-ATCC, and YpP-G) obtained from various sources were characterized by (i) electron microscopy, (ii) pulsed field gel electrophoresis, (iii) restriction fragment length polymorphism, (iv) protein profiling, and (v) determining their host ranges, burst sizes, and lysis kinetics. All of the phages belonged to the Podoviridae or Myoviridae families of DNA-containing, tailed phages. The approximate genome size of four phages was 40 kb, and it was ca. 180 kb for the fifth phage (YpsP-PST). The host range of each bacteriophage was determined against (i) fifty-nine Y. pestis strains (46 strains were from the Republic of Georgia), and (ii) eight Y. pseudotuberculosis strains obtained from various countries. At a concentration of ca. 109 plaque-forming units (PFU)/ml, each phage lysed 100% of the Y. pestis strains. YpP-G was highly specific for Y. pestis, even more so than the PhiA1122 phage commonly used for diagnostic purposes. The lysis time for all phages (examined against Y. pestis strain NR642) was ca. 90 min at 37°C, and the burst sizes for YpsP-G, YpP-Y-ATCC, YpP-R-ATCC, YpsP-PST-ATCC, and YpP-G were ca. 45 ± 10, 5 ± 1, 52 ± 12, 71 ± 13, and 179 ± 49 PFU/cell, respectively. Our data support the idea that the five Y. pestis-specific phages we characterized may be useful in designing a preparation that effectively decontaminates surfaces naturally or intentionally exposed to many strains of that bacterium.

04. Draft Genome of Two Georgian *pestis* Isolates

Chythanya Rajanna[1], Tamara Revazishvili[1], Mohammed H. Rashid[1], Steve Ehrman[2], Alvin Liem[2], Paata Imnadze[3], Lela Bakanidze[3], Kevin O'Connell[2], Colin Stine[4], Henry S. Gibbons[2], Alexander Sulakvelidze[1]

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pestis, the causative agent of plague, exists today in discrete, geographically defined foci each of which presents different microevolutionary pressures. These isolates vary in virulence towards animals and in their biochemical phenotypes. To gain insight into a clad of atypical *Y. pestis* isolates from the FSU, we have obtained draft genome sequence of two *Y. pestis* isolates (G3768 and G1670) isolated from mountain voles (*Microtus arvalis*) from the Republic of Georgia. *Insilico* analysis of VNTR and CRISPR regions indicate that these strains are highly similar to previously determined sequences from another Georgian strain G8786 (1, 2), previously grouped with other *Y. pestis* subsp. Caucasus strains. The pMT1plasmid resembles that of G8786 and Pestoides F (also known as *Y. pestis* subsp. *microtus*), being approximately 30 kb larger than the pMT1 plasmids of most *Y. pestis* strains. The chromosomal organization closely resemble that of Pestoides F- when mapped using Projector 2 (3). 172 0f 192 gaps yielded PCR products, most of which produced the expected sequence. Three gaps have been repeatedly refractory to PCR-directed gap closure attempts, suggesting possible genome rearrangement at those loci. We are currently attempting high-throughput IS-element mediated PCR strategies and Paired end sequencing to scaffold these loci. Analysis of genome content revealed that, like most Antiqua and Medeivalis biovar strains, G3768 and G1670 lack the phage locus YPO2271-2278. G3768 further resembles Antiqua and Medeivalis strains in that its genome encodes the entire flagellar locus at YPO0739-0754, and a repeat within this region resembles most closely that found in another *microtus* strain 91001. YPO0757-0780 are missing in these strains. In general, our draft genome sequences confirm the microarray data of Hinchliffe *et al*. for G8786 (4). As in other Pestoides strains, leucine replaces serine in the *aspA* gene encoding aspertate ammonia lyase. This variant of AspA produces an active enzyme, unlike that found in canonical YP strains. G3768 also contains an *lcrV* gene that closely resembles *Y. pestis* subsp. *pestis* strains. Finally, in both strains, non-synonymous SNPs result in the formation of previously unreported pseudogenes and frame shift errors. Notably strain G1670 contains mutations in *manX* and *clpA*, which may correlate with the inability to ferment arabinose and slow *in vitro* growth, respectively. Particularly intriguing is the discovery of a nonsense mutation in the *phoR* gene of G3768. The *phoR* gene encodes a phosphate-responsive regulatory protein that is required for virulence in diverse enterobacterial species. Deletion of this activity in G3768 may have dramatic consequences for virulence of this strain. The effect of these substitutions on gene expression, protein profiles and virulence of G3768 and G1670 is the subject of ongoing investigation.

05. SOLiD Pyrosequencing of Four *Vibrio vulnificus* Genomes Enables Comparative Genomic Analysis and Identification of Candidate Clade-Specific Virulence Genes

Paul A. Gulig[1]*, Valérie de Crécy-Lagard[2], Anita C. Wright[3], Brandon Walts[1], Marina Telonis-Scott[1], and Lauren M. McIntyre[1]

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Vibrio vulnificus is the leading cause of reported death from consumption of seafood in the United States. Despite several decades of research on molecular pathogenesis, much remains to be learned about the mechanisms of virulence of this opportunistic bacterial pathogen. The two complete and annotated genomic DNA sequences of *V. vulnificus* belong to strains of the same clade, clade 2, which tends to be associated with clinical samples and generally possesses higher virulence potential in animal models of disease. We therefore used SOLiD pyrosequencing of four *V. vulnificus* strains representing different clades (clades 1 and 2) and biotypes (biotypes 1 and 2) to be able to perform comparative genomic analysis to gain insight into molecular pathogenesis and evolutionary genetics. Greater than 4,100,000 bases were sequenced of each strain yielding approximately 100-fold coverage for each of the four genomes. Although the read lengths of SOLiD genomic sequencing were only 35 nt, we were able to make significant conclusions about the unique and shared sequences among the genomes, including identification of single nucleotide polymorphisms. Comparative analysis of the newly sequenced genomes to the existing reference genomes enabled the identification of the 3,459 core *V. vulnificus* genes shared among all six strains and 80 clade 2-specific genes. We identified 523,161 SNPS among the six genomes. In summary, although SOLiD pyrosequencing of these bacterial genomes did not yield assembled genomes, we were able to perform the desired comparative genomic analyses. These results will enable the design and execution of follow-up genetic and biological (e.g., virulence) studies to elucidate the virulence of *V. vulnificus*.

06. Validation and Evaluation of the ¡Vivir a Todo Pulmón! fotonovela series: The patient perspective

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Southeastern National Tuberculosis Center (SNTC). College of Medicine, University of Florida

OVERVIEW: The goal of ¡Vivir a Todo Pulmón! Fotonovela Series is to provide tuberculosis (TB) information to foreign-born Spanish speaking persons in the general community and to assist state/local TB program staff in their interactions with clients from this population. This series seeks to address the most important topics/concerns in a format that is attractive and familiar to the target population. The materials were developed by the researchers in collaboration with the Project Advisory Committee (PAC), a multidisciplinary team with different expertise (health, medical anthropology, intercultural communication, cultural competency, among others). To determine the quality of the materials a four-phase evaluation plan was designed. This poster presents the results of Phase 1, namely Patient Perspective. For this stage, quality of the materials includes: ease of understanding, cultural sensitivity, persuasiveness, relevancy of the content, and credibility.

METHODS: The objective of this phase is to asses patients' perceptions on 1) appropriateness of language/literacy level. 2) Attractiveness of design/stories. 3) Appropriateness of primary "teaching points". 4) The patient's perspective about the potential positive impact of the messages prior to/at the time of their diagnosis. 5) The need for additional information based on patients' experiences since their diagnosis. Data was obtained through structured observation and focus group interview with five Hispanic hospital patients with an existing diagnosis of tuberculosis (TB). The focus group was conducted and recorded in Spanish. Verbatim transcription was obtained from the audio files and open coded for content analysis.

PRELIMINARY RESULTS: It was observed that although the materials have low literacy level, participants had had a hard time reading through in the period of time planed. Extra time was given to allow participants finishing the booklet. Four main themes were found in participants' responses: Attractiveness, identification, impact, and changes and additions. Participants perceived that the materials are responsive to their needs, they could identify themselves in the stories, the topics were relevant to their experiences and the stories could help others.

CONCLUSION AND RECOMMENDATIONS: Based on observations, we conclude that it is necessary to adjust the length of the text to better serve the intended population. We need to consider alternatives for those persons that may be illiterate.

It is recommended that the fotonovelas clearly indicate that they are free of cost, and to include health department information within the packet to ensure that the material is utilized appropriately and to direct people to the right place. Based on attitudes and key phrases said by focus group participants we can note that they perceive a need for TB awareness within the medical community. Based on results, there is room for improvement of the fotonovela series to better the quality and accessibility in order to properly reach target population.

07. Non-Tuberculous Mycobacteria in Florida

Stephanie Yarnell[1], James Wellehan[2], Youliang Qiu[3,4], Jason Blackburn[3,4], Glenn Morris[4,5], Michael Lauzardo[1,4,6], David Ashkin[6,7], Judith Johnson[1,4], Andrew Kane[4,8]

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The incidence of disease caused by non-tuberculous mycobacteria (NTM) in humans has dramatically increased over the past 20 years, with the number of 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*, can cause pulmonary disease, bone and soft tissue lesions, and disseminated infections. Disseminated infection with *Mycobacterium avium complex* (MAC) is the most common opportunistic bacterial infection in adults infected with HIV-1 in the developed world, and is second only to AIDS Wasting Syndrome as the most common cause of death in these patients. The incidence of NTM disease is also increasing in otherwise healthy subjects, with the majority associated with environmental sources. The primary route of NTM infection of humans is via ingestion or aerosolization of water colonized with mycobacteria. We hypothesize that the chemistry of different surface waterways alters the number and diversity of mycobacteria, and therefore alters the relative risk for NTM infections. This presentation will discuss NTM disease, methods to relate NTM disease prevalence and surface water chemistry in Florida using a Geographic Information System (GIS) database, and the application of novel sequence-based methods to discern different clades of mycobacteria in environmental samples. The GIS database will be used to predict waterways that may foster NTM growth and increase risk of NTM disease. Use of our non-culture based molecular probes is critical, since existing methods for NTM identification rely on the ability to culture isolates in the laboratory, and many NTMs are not readily cultured or exhibit very slow growth. These efforts are expected to lead to species-specific methods for the rapid and accurate detection of NTMs directly from clinical or environmental samples, and to discern risk factors for NTM transmission, infection and disease.

08. Paleobacteriology and phylogeography of cholera; seventh pandemic origin and spread.

Marco Salemi[1,2], Andrew Tatem[1,3], Rebecca Gray[1,2], Yuansha Chen[1,2], and Judith A. Johnson[1,2]

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The factors responsible for modern disease epidemics are likely multi-factorial and involve ecological, political, social, and evolutionary influences. The purpose this project is to develop a highly interdisciplinary framework that integrates state-of-the-art phylogenetic, population genetic and geospatial analysis techniques to investigate the origin and spread of the 7th cholera pandemic. Cholera, a life-threatening diarrheal disease, is endemic in Asia where it is characterized by large seasonal epidemics that intermittently become pandemic. Seven cholera pandemics have occurred since 1817. The ongoing 7th pandemic, due to El Tor biotype, erupted in Indonesia in 1961, spread to Africa in the 70s, and reached South America in the 90s.

We sequenced housekeeping (*recA*) and variable number tandem repeat (VNTR) regions of 48 strains of *Vibrio cholerae* to identify phylotypes. The collection contained both El Tor and classical biotypes from as early as 1905. Genetic relatedness of the isolates was determined by a neighbor joining tree using *recA* sequences and by e-burst analysis incorporating all loci. Yearly cholera incidence data from the World Health Organization as well as the occurrence specific phylotypes was used to construct GIS maps.

Phylogenetic analysis of *recA* sequences separated El Tor and classical (6th pandemic) biotypes of toxigenic *V. cholerae*. El Tor- like pre-7th pandemic strains(isolated in 1905 and 1933) fell between these clusters, but strains other pre-7th pandemic isolates (1958-1960) clustered with 7th pandemic strains. VNTR analysis provided further discrimination within these clusters. E-burst identified 1 clonal complex within the El Tor strains that included isolates from seven countries and 2 clonal complexes within classical strains. The combination of sequencing methods provides excellent discrimination among toxigenic O1 *V. cholerae* strains. Although only a few strains were examined in this study, our data suggests that earliest El Tor isolates may represent a transitional phase between 6th and 7th pandemics. Further analysis of our unique and unrivalled collection of approximately 2500 *V. cholerae* strains will provide input to novel phylogeographic techniques that will integrate spatial datasets describing those factors known to be relevant to cholera dispersal and epidemics, including climatic variations, human travel patterns and land use changes across the time period under study. Determining which of these factors or combination of factors best describes the evolution, dispersal and outbreak patterns seen through the proposed phylogeographic framework will provide a unique understanding of the main causes of the past pandemics, and valuable information to guide future control.

09. Non-Target Toxicity Evaluation of Novel Carbamate Insecticides for Control of the Malaria Mosquito, *Anopheles gambiae*

Ying Jiang[1], Paul Carlier[2], Josh Hartsell[2], Ming Ma[2], Fredrik Ekstrom[3], and Jeffrey R Bloomquist[1]

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Malaria is a major cause of morbidity and mortality in the world, and each year takes the life of more than 1million children. Anopheles mosquitoes are the only natural vectors of human malaria and Anopheles gambiae is a principal vector of malarial parasites. Historical data has proved that vector control is an effective way to tackle malaria. Currently, pyrethroids are mostly used to control malaria mosquitoes, especially when formulated on insecticide-treated nets (ITNs). However, insecticide resistance to common insecticides is threatening to unravel the progress of malaria control, so alternatives are urgently needed.

Carbamate insecticides, because of their relatively low cost and good killing capacity, have potential for use in malaria vector control. However, carbamate insecticide neurotoxicity is a concern. The target of carbamate insecticides is acetylcholinesterase (AChE, EC 3.1.1.7), which hydrolyzes the neurotransmitter acetylcholine (ACh) at cholinergic synapses. By inhibiting AChE, carbamate insecticides cause ACh accumulation at synapses and induce neurotoxicity. Carbamates tend to react indiscriminately with the ubiquitous catalytic serine residue at the active site of AChE and cause neurotoxicity in livestock and aquatic animals (Taylor 1994; Keifer 2007).

This study is part of a broader effort in our laboratory to offer an effective and selective option in malaria vector control. Along these lines, we have discovered a series of carbamates with unprecedented selectivity for Anopheles gambiae AChE, compare to human AChE. The purpose of the present work is to confirm the safety of these new carbamates for other non-target organisms.

Accordingly, we have tested novel carbamates on 6 non-target species. By comparing in vitro AChE inhibition capacity (IC50 non-target/IC50 mosquito), the novel carbamates show good selectivity over An. gambiae, with values ranging from 0.47- to 19587-fold compared to the vertebrates we screened. In contrast, less selective commercial carbamates ranged from 0.3- to 5.6-fold. Next, neurotoxic esterase (NTE) assay evaluated. Inhibition of this enzyme is involved in nerve demyelination, but the new carbamates we have prepared had no blocking activity, in vitro, while some of the commercial carbamates did had some activity. The overall results show that the novel carbamates we identified have improved toxicological properties for use in malaria vector control.

10. Prevalence of Phage in Surface Waters

Judith A Johnson[1,2], James E Maruniak[3], Jianli Dai[1], Yuansha Chen[1,2], Jason Mclean[4], Kim Dennis[4], Chris Maggio[4], Pablo Plasencia[4], Stacy Ranson[4], Victoriya Struko[4], Yevgeniy Popov[4], Brittney Cobb[4], Adam Rosen[4]

[1]Department of Pathology, Immunology and Laboratory Medicine; [2]Emerging Pathogens Institute; [3]University of Florida Entomology and Nematology Department, [4]Department of Microbiology and Cell Science

In an effort to enrich and expand the research skills of undergraduate students at the University of Florida, the Emerging Pathogens Institute and the student body have collaborated to conduct an ongoing research project involving human pathogens. The current goals of this venture are as follows:
1) Determine the presence of human pathogens in water sources within Gainesville, Florida via phage plaque counting. Create a geospatial projection of pathogen occurrences with ArcGIS software
2) Based on the derived projection, correlate against other geospatial data projections (i.e. hydrology data, zoning maps, municipal waste lines, etc…) to form dependence statements 3) Tentatively collect a library of wild-type phages that successfully lyse human pathogenic bacteria This project was conducted by the Society for Viral Studies, an undergraduate organization funded by Student Government and composed primarily of first and second year undergraduate students. Due to the undergraduate participants, this study attempts to focus on rudimentary microbiology techniques and utilizes bacteriophage plaque assays for detection and enumeration of pathogens. Between September and December 2009, 38 samples were collected from natural and man-made water sources within the city of Gainesville in 50cc aliquots; water temperature and GPS data were recorded during these collections. Samples were filtered with 0.45 μm Millex HV luer-lok filters and pH was determined prior to conducting soft agar overlay plaque assays with E. coli C3000 in LB enriched agar. Phage plaques were counted and results were stored as a geospatial projection with ArcGIS ArcView 9.3 with a digital ortho map of Alachua county from the Alachua County GIS Service Center. Currently, no plaques have been identified in any of the samples. During the spring semester, participating students who have developed familiarity and skill with soft agar overlay assays will repeat those procedures on additional samples.
Future research will include testing the effectiveness of Plaque Assays Modified with Antibiotic (PAMA) and spatial analysis of the pathogen projection; this will include spatial autocorrelation via Moran's I and/or Geary's C and correlations against other geospatial variables.

11. Nutrient (Amino Acids and D-glucose) Transport Activity in Brush Border Membrane Vesicles (BBMV) Isolated from larvae of *Aedes aegypti* (Diptera: Culicidae) mosquito

Bernard A Okech[1,2], Kenneth Sterling[3], William R. Harvey[3]

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Abstract: Characterization of nutrient transport by the use of brush border membrane vesicles (BBMV) in insects has previously been done using *Manduca sexta* (Tobacco hornworm) and *Cecropia* (Silk moth) but not using mosquito larvae. The reason is that the isolation of BBMV from larval mosquitoes has seemed impossible because of their small size – it would take 1,000 excised mosquito midguts to provide the same amount of vesicles as 1 excised caterpillar midgut. But improved techniques have enabled the use of intact mosquito larvae for the isolation of BBMV, which yield virtually identical results to BBMV from isolated midguts with respect to binding capacity of *Bacillus thuringiensis* Cry1C toxin, marker enzymes enrichment, and morphology under electron microscopy examination. We have used similar techniques to isolate BBMV and for the first time have evidence that these vesicles are suitable for nutrient amino acid and sugar transport studies. The purity of the BBMV was confirmed in western blots by antibody labeling to mosquito specific BBMV markers including *Anopheles gambiae* amino peptidase 2 (AgAPN2) and alkaline phosphatase (ALP), and to a membrane protein (AgNHA1) that expresses exclusively in the apical brush border of the posterior midgut in mosquito larva. The well known basal membrane marker Na^+K^+ ATPase did not label in the western blots. To determine the transport activity in BBMV, we used [^{3}H] labeled amino acids (L-Phe and L-Leu) and [^{3}H] labeled D-Glucose. Preliminary experiments using [^{3}H] L-Phenylalanine showed a Na+ dependent transport activity whereas leucine transport was Na+ independent. In separate experiments done at pH 6.8, [^{3}H] labeled D-glucose uptake was demonstrated in BBMV from *Aedes aegypti* in the presence of 50 mM NaCl or with the NaCl replaced by choline chloride. Uptake increased sharply and linearly for the first 30 seconds. When Na+ was added there was an overshoot which peaked within the first 30 seconds; glucose uptake was twice as large with Na^+ as without it. In the presence of 1 mM phloridzin or 10 mM phloretin (glucose transporter inhibitors), with and without Na^+, a Na^+-dependent and Na^+-independent (facilitative) glucose uptake were observed. Uptake of glucose without Na+ was decreased by 10 mM phloretin indicating a facilitative glucose uptake. The uptake of glucose, with or without Na+ was abolished by 1 mM phloridzin. Our results demonstrate the utility of using BBMV from whole mosquito larvae in nutrient transport studies.

12. Environmental conditions affecting eastern equine encephalitis virus transmission in North Florida.

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The spatio-temporal transmission patterns of eastern equine encephalitis virus (EEEV) in Florida have always been difficult to forecast, yet EEEV has the capability to cause widespread outbreaks, especially in horses. From January 1982 through December 2009 there have been 2,051 EEE-positive horses reported in Florida. The vast majority (>70%) of these have resulted in the death of the horse. During this 27 year period, four years emerge as major EEEV transmission events: 1982 (with 202 reported horse cases), 1991 (160 cases), 2003 (207 cases), and 2005 (150 cases). Transmission of EEEV to horses has been reported for every month of the year with the highest transmission levels in June, July, and August. The major focus of EEEV transmission in Florida is from Orlando north to Jacksonville and West to Pensacola. Since 1955, there have been 73 human EEE cases reported from 35 Florida counties. Thirty-one percent of the Florida human EEE cases have been fatal. Thirteen (18%) of the human EEE cases reported in Florida have been recorded during the past 10 years. As is the case with horses in Florida, human cases of EEE are most frequently reported during June, July, and August. As land development expands from the coastal regions of Florida to the interior of the state where freshwater swamps known to cycle EEEV are more abundant, the risk of human encounters with EEEV-infected mosquitoes will increase. Therefore, it is highly desirable to be able to predict high EEE transmission years well in advance of human transmission to allow mosquito control and public health agencies time to mitigate EEEV transmission events (Day 2001). Environmental factors, especially the cycling of drought and rainfall, drive the amplification and transmission of EEEV in Florida. These factors can be monitored in real-time and used to establish an EEE-transmission risk profile that will predict the likelihood of EEE transmission to humans and horses in Florida. Here we present current and historical real-time Keetch-Byram Drought Index datasets to establish environmental profiles that forecast EEEV transmission throughout Florida. The blending of EEEV biology with environmental surveillance techniques will greatly enhance the predictability of EEEV amplification and transmission in Florida. Real-time monitoring and forecasting of EEEV transmission events will facilitate and focus insecticide treatments by Florida Mosquito Control agencies that are directed toward the threat of EEEV transmission and will allow a timelier issuance of Medical Alerts and Medical Advisories by local and state public health agencies.

13. Florida's front line of protection from mosquito-borne diseases: Florida Mosquito Control and the University of Florida

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Organized mosquito control programs began to appear in Florida in 1925. By 2009, there 57 of Florida's counties with mosquito control programs operating to manage disease-causing and pestiferous mosquitoes. The University of Florida's Florida Medical Entomology Laboratory (FMEL), established in 1956, has a long rich history of working with Florida's mosquito control programs. The FMEL has provided guidance during mosquito-borne disease outbreaks, in developing surveillance programs for monitoring mosquito vectors, and to ensure the public health of Florida citizens and tourists. FMEL provided information in support of mosquito control and public health efforts during in outbreaks of St. Louis encephalitis, eastern equine encephalitis, West Nile virus encephalitis, and recently, during an epidemic Dengue fever in the Florida Keys. FMEL developed and provides an on-line surveillance and prediction system to assist mosquito control personnel in making informed decisions concerning potential mosquito-borne diseases in their regions. The FMEL also provides training programs for mosquito control technicians, biologists, managers, and staff. Though the FMEL is not an action agency, providing operational anti-disease measures, the FMEL is recognized as Florida's think tank for protection against mosquito-borne diseases.

14. MChip, a low density microarray, differentiates among seasonal human H1N1, classical swine H1N1, and the 2009 pandemic influenza A H1N1

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Background: Current influenza molecular characterization methods often take at least 2-3 days. The MChip system is a low density RNA binding microarray coupled with an artificial neural network (ANN) designed to determine the subtype of influenza A isolates based on hybridization to 15 unique sites in the Matrix gene. It has shown promise in the ability to perform this task in less than 8 hours. Previous success reported that this system has the ability to distinguish between human influenza A isolates and highly pathogenic avian influenza A (HPAI) isolates. In this report we sought to use the microarray to distinguish H1N1 strains.
Methods: Fluorescence intensity and pattern from the scanned images of characterized isolates is used to train the ANN to recognize images representative of specific subtypes. The ANN then compares image intensity from uncharacterized specimens to this database, weighing the relative intensities to classify the influenza A virus by subtype.
Results: Additional training of the ANN database with 71 well-characterized influenza A isolates, yielded relatively high MChip system accuracy in distinguishing unique H1N1 strains (little misclassification): 10 huH1N1 strains (90% correct), 22 huH3N2 (95.5% correct), 12 North American swH1N1 (80.6% correct), 14 2009 H1N1 (85.7% correct) and 23 negative samples (91.3% correct) A number of experimental chip images (~25.3%) could not be classified by the ANN, largely attributable to poor signal intensity or to chip processing errors.
Conclusions: The MChip system has considerable potential to rapidly and accurately distinguish previously recognized and unique influenza subtypes.

15. Utilizing Mechanism-Based Pharmacokinetic/Pharmacodynamic Models to Understand and Prevent Antimicrobial Resistance

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Background: Despite decades of antimicrobial usage, the relationship between antimicrobial drugs and the development of drug resistant has not been fully delineated. Several mechanisms of resistance published in recent years warrant exploration of new optimized models. We have developed semi-mechanistic pharmacokinetic/pharmacodynamic (PK/PD) models mimicking various resistance hypotheses to describe drug-bacteria kill curve relationships. Methods: *In vitro* kill-curves of *E. coli 204* up to 48 hours following initial ciprofloxacin (CIP) treatment at 0.0, 0.1, 0.2, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0 and 125 times the minimum inhibitory concentration (MIC) totaling 193 data points were obtained from a previously published data. The *in vitro* system simulated the clinical half-life of CIP (4 hours). The resistant mechanisms evaluated in the mathematical models include (1) common susceptible to resistant conversion (2) drug enhanced distribution of dividing cells to nondividing cells (dormant) (3) sequential compensatory mutation to restored fitness, and (4) dual mechanisms of dormant distribution and mutation. Model parameters were estimated from simultaneous fitting of 11 dose groups using NONMEM VI and Adapt II software. Bootstrap was performed to obtain confidence intervals of NONMEM estimates. Model selection criteria were based on AIC values, experimental data, residual plots, visual

predictive checks, and observed versus predicted plots. Results/Conclusions: The *E. coli* kill curves after CIP treatment were best described by the compensatory mutation hypothesis (Model 3). The model estimated the first order synthesis and degradation rates to be 0.42 h^{-1} and 0.20 h^{-1}. The overall drug effect in the resistant population decreased by more than 2-folds as compared to that of the susceptible population. The PK/PD models infer that the sequential mutation to restore fitness may account for a second growth phase that occurs while the CIP concentration is well above the initial MIC.

16. Evaluation of Tissue Distribution of TR-701 in Healthy Volunteers, Using Microdialysis

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Background: Plasma concentrations of antimicrobial drugs have long been used to correlate exposure to effect, yet one cannot always assume that unbound plasma and tissue concentrations are similar. Knowledge about unbound tissue concentrations is important in the development of antimicrobial drugs, since most infections occur localized in tissues. Therefore, a clinical microdialysis study was conducted to evaluate the distribution of TR-700, the active moiety of the antimicrobial prodrug TR-701, into interstitial fluid (ISF) of subcutaneous adipose and skeletal muscle tissues following a single, oral 600mg dose of TR-701 in fasting conditions.
Methods: 12 healthy, adult subjects were enrolled. Two microdialysis probes were implanted into the thigh of each subject, one into the vastus medialis muscle and one into subcutaneous adipose tissue. Probes were calibrated using retrodialysis. Dialysate samples were collected every 20 min for 12 hours after a single, oral dose of 600 mg TR-701 and blood samples were drawn at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, 24 h post dose.
Results: Unbound TR-700 levels in plasma were similar to those in muscle and adipose tissue. The ratios of $fAUC_{0\text{-}12,\,tissue}/fAUC_{0\text{-}12,\,plasma}$ were 1.1 ± 0.2 and 1.2 ± 0.2 for adipose and muscle tissues, respectively. The median half-life was 8.1, 9.2 and 9.6 h for plasma, adipose and muscle tissues, respectively. Mean protein binding was 87.2 ± 1.8 %. The study drug was very well tolerated.
Conclusions: The results of the study show that TR-700 distributes well into ISF of adipose and muscle tissues. Unbound levels of TR-700 in plasma, adipose and muscle tissues were well correlated. Free plasma levels are indicative of unbound levels in the ISF of muscle and adipose tissues.

17. Active Surveillance in Key West, Florida Following a Locally-Acquired Case of Dengue Fever

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Florida Department of Health

BACKGROUND: A case of dengue fever in a traveler to Key West, Florida was reported to the Florida Department of Health in September, 2009. The primary mosquito vector of dengue virus, *Aedes aegypti*, is widespread in the Florida Keys, making autochthonous transmission possible. Enhanced mosquito control efforts were implemented immediately. Active surveillance was initiated to identify additional infections and determine whether transmission was ongoing. This entailed a serosurvey to identify asymptomatic cases, and the methodology described here to identify symptomatic patients.
METHODS: A medical record search was performed at three clinics in Key West. Records from 7/15/09 through 9/15/09 were pulled based on ICD discharge diagnoses consistent with dengue. Specified ICD codes were dengue and dengue hemorrhagic fever, persistent fever of unknown origin, infectious disease NOS, myalgia), bleeding, hemorrhage, blood in urine, thrombocytopenia, rash, arthralgia, petechiae, leukopenia, generalized pain, and eye pain. Influenza and respiratory symptoms were excluded. Records were examined for likelihood of dengue infection. Suspect patients were contacted and blood samples were drawn from those willing to be tested. Testing for anti-dengue IgM antibody levels using enzyme-linked immunoassay (ELISA) was performed at the CDC laboratory in Puerto Rico.
Local physicians were asked to report suspect cases to Monroe County Health Department (CHD). Serum samples were requested from patients with fever and at least one other symptom characteristic of dengue fever, who also reported mosquito exposure and time spent on Key West in the 2 weeks prior to symptom onset.
RESULTS: The medical record search yielded 211 records based on discharge diagnoses consistent with dengue fever. Six were identified as possible cases; all were willing to supply a blood sample and four were confirmed as recent dengue virus infections. The four positives reported fever (4/4), myalgia (2/4), headache (1/4), arthralgia (1/4), and hematuria (1/4). Physicians submitted 21 samples for testing. Eleven patients were confirmed as recent dengue infections with onset dates ranging from 7/26/09 to 10/19/09.
CONCLUSIONS: These results suggest that dengue virus transmission was occurring prior to when the traveler was infected, and continued after mosquito control efforts were accelerated. The medical record search was useful in identifying dengue fever cases. Searching using fever *and* any of the other ICD codes would have improved efficiency of the search. Physician submission identified the highest number of cases, but this would likely not have occurred without a broad notification from Monroe CHD of dengue risk. Increasing physician awareness during this type of event is critical for sensitive surveillance.

18. IL17A Production Mediated by Orally-fed *Lactobacillus johnsonii N6.2* is correlated with Diabetes resistance in the Bio-Breeding Diabetes Prone Rat Model

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Although it is known that resident gut flora contribute to immune system function and homeostasis, the role of gut flora in the progression of autoimmune disease is poorly understood. It has recently been shown that distinct bacterial populations are present within rodent models prone (DP) and resistant (DR) to Type 1 Diabetes (T1D). The oral transfer of *Lactobacillus johnsonii N6.2 N6.2* from DR rodents to DP rodents conferred T1D resistance to DP rodents. Diabetes resistance in *L. johnsonii* N6.2 fed DP rodents was correlated to a TH17 bias within the mesenteric lymph nodes which was associated with high levels of IL6 and IL23. Moreover, in vitro assays showed that *L. johnsonii* N6.2 mediated high IL6 levels in antigen presenting cells which can mediate TH17 differentiation in the presence of sufficient TCR stimulation. Together, these data suggest an interesting paradigm whereby autoimmunity can be circumvented by gut flora-mediated T cell TH17 differentiation.

19. Ultrasensitive and Rapid Monitoring of Bacteria and Cells Using Bioconjugated Nanomaterials

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The rapid, sensitive and selective determination of pathogenic bacteria is extremely important in biotechnology, medical diagnosis, and the current fight against bioterrorism. Under the appropriate conditions, bacterial pathogens can survive and spread easily in environment. The estimated foodborne illnesses in 1999 were 76 million cases annually in United States, resulting in 5,000 deaths. In considering the trace concentrations (~10-100 cells) of bacterial pathogens in food, water, or the environment can pose a serious threat to human health, fast, sensitive and multianalyte detection systems are needed which identify multiple contaminants preferably within minutes. However, most current bacterial detection methods are time-consuming and laborious because of the complicated assay procedures, and can detect only one bacterial pathogen at a time.

Over the past decade, rapid development in nanoscience and nanotechnology has resulted in the successful synthesis and characterization of various inorganic nanomaterials, including nanoparticles, nanocrystals (quantum dots), nanorods, nanowires, and carbon nanotubes (CNTs). The unique physical properties of these materials induced by their extremely small size, make them highly suitable for a wide range of biological, electronic, optical, environmental, and medical applications. Bionanotechnology is the convergence of biology, genomics and nanotechnology. When combined with molecular biological tools, nanomaterials offer more diverse capabilities in bioanalysis and biomedical applications.

Here we would like to present the research work developed in our lab in the past few years using biocongugated nanomaterials for bacteria and cells detection. Our bioassay could achieve the accurate determination of a single bacterial cell within 20 min by using bioconjugated nanoparticles in a fluorescence-based immunoassay. Applied with multicolored FRET (fluorescence resonance energy transfer) silica nanoparticles, we extended the method to simultaneous and multiplexed pathogen monitoring. Meanwhile, we also built up several platforms for cell detection and therapy. For example, a colorimetric assay for the direct detection of diseased cells was developed using aptamer-conjugated gold nanoparticles. The distinct color change of samples with target cells could be simply observed by naked eye. We also used Au-Ag nanorods as a nanoplatform for multivalent binding by multiple aptamers on the rod to increase both the signal and binding strengths of these aptamers in disease cell recognition. By shining with NIR light, 90% of cells could be efficiently and selectively killed. All these sensitive and selective methods for disease cell detection can be taken advantageous for the recognition of those related to pathogens by choosing suitable biomolecules. Therefore, the incorporation of these functionalized nanomaterials into current pathogen detection methods could lead to the development of new generation methods for the rapid and sensitive detection of pathogens in mixed microbial populations with emphasis on device portability and simplicity in sample preparation.

20. Multiple genome comparison of Vibrio parahaemolyticus

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In the effort to understand the evolution of Vibrio parahaemolyticus and the genetic changes that may contribute to the sudden increase of virulence in the pandemic strains, four additional strains of V. parahaemolyticus were sequenced in the J. Craig Venter Institute. Comparative genomic analysis of these strains plus the other two strains of V. parahaemolyticus sequenced previously was performed. Strains analyzed were isolated from either Asia or Peru. Five of these are serotype O3:K6 with varying degree of pathogenicity and include three pandemic isolates and two pre-pandemic strains representing the tdh group or trh group. A never before sequenced O4:K68 pandemic isolate was also included to understand the nature of serotype conversion. The genomic data suggests

that the pandemic strains of V. parahaemolyticus are closely related and form a distinct lineage and that the epidemics in South America are tightly linked to the outbreaks in India. The presence of pandemic strain specific islands was confirmed in this study. In addition, genomic analysis revealed that the trh+ strain and the tdh+ strains have different types of pathogenicity islands. Major structural difference exists in the tdh pathogenicity island between pre-pandemic and pandemic strains. Single nucleotide polymorphism (SNP) analysis indicated that a recombination event involved a region much larger than the O and K antigen genes may have resulted in the serotype conversion of pandemic strains from O3:K6 to O4:K68. The "core" genes of V. parahaemolyticus were also compared to the ones in Vibrio cholerae and Vibrio vulnificus to understand the genetic conservation and difference in these important human pathogens. About half (49-59%) of the "core" genes from each species are conserved across species. On the other side, 14-24% of the "core" genes are species specific and they are in different functional categories. This is the first multiple genome comparisons of V. parahaemolyticus with various serotypes and different pathogenic mechanisms. It will improve our understanding of how V. parahaemolyticus evolves to become a pandemic pathogen. In addition, our across species genomic analysis have categorized those genes that likely define the organisms at the species level.

21. Aptamers bind glycosylated hemagglutinin expressed on poxvirus infected cells

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Traditional methods for detection and identification of pathogenic viruses or bacteria tend to be slow and cumbersome. We have developed aptamer probes with the capacity to rapidly detect the presence of viral infection with specificity and sensitivity. For our model, we chose vaccinia virus (VV), which is closely related to variola virus that causes smallpox. Using a method known as cell-SELEX (Systematic Evolution of Ligands by Exponential Enrichment), we describe the generation of very selective and highly specific aptamers designed to recognize proteins expressed on the surface of VV-infected cells. Characterization of the aptamers showed that the virus-encoded hemagglutinin, a protein expressed on the surface of infected cells, is the preferential binding target. These studies show the feasibility of generating aptamers against a given specific infectious agent and will enable us to further develop aptamers as diagnostic and/or therapeutic tools against a broad range of infectious agents.

22. Biochemical and Toxicological Assessment of Novel Insect Anticholinesterases for Control of Mosquito Vectors and Agricultural Pests

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Insecticides for mosquito control remain the most important and effective aspect of the vector management programs for the control of vector borne diseases. Malaria is vectored by the mosquito *Anopheles gambiae* in Sub-Saharan African and infects 500 million people annually. To minimize transmission, residents utilize insecticide-treated nets (ITNs) that are impregnated with a variety of pyrethroids; however the increasing prevalence of pyrethroid resistant mosquitoes, through a sodium channel mutation (*kdr*), has amplified the need for development of new, selective mosquitocides for the use on ITNs.

Accounting for mosquito resistance toward insecticides is vital when developing mosquitocides for disease control. Insecticide resistance of mosquitoes due to agricultural uses has been documented and specifically effects insecticide design for disease control. Widespread agricultural use of pyrethroids has been implicated in exacerbating development of resistance to insecticides with the same mode of action when used in ITNs. Development of highly selective insecticides with poor toxicity to agricultural pests, and therefore less ancillary uses, can mitigate resistance due to limited selection pressure within breeding sites.

Through collaboration with the chemistry department at Virginia Tech, we have begun development of new and highly selective anticholinesterase mosquitocides (e.g., carbamates). Our experimental insecticides inhibit acetylcholinesterase (AChE) and were developed based on structural knowledge of the AChE gorge of *An. gambiae*, allowing for a high degree of selectivity through utilization of unique differences between human and mosquito AChE active sites. Toxicokinetic analysis of the novel AChE inhibitors yielded IC_{50}'s on the mosquito vectors/agricultural pests. Bioassays (LD_{50}) were then performed to test *in vivo* toxicity of the highly selective carbamates.

The experimental carbamates have shown 100 – 1000 fold selectivity of *An. gambiae* enzyme over human AChE and an LD_{50} of 4 ng/insect. A similar degree of selectivity and toxicity was observed for other mosquito species studied. For example, the compound, 3-tert-butylphenyl-N-methylcarbamate yielded: *A. aegypti* $IC_{50} \leq 30$ nM and $LD_{50} \sim 4$ ng; *A. albopictus* $IC_{50} \leq 40$ nM and $LD_{50} \sim 5$ ng; *C. quinquefasciatus* portrayed an IC_{50} of 120 nM and an LD_{50} of 11 ng. Surprisingly, the experimental carbamates displayed poor enzyme inhibition toward the honey bee (*Apis mellifera*) ($IC_{50} > 10^{-6}$) and toward lepidopteran agricultural pests ($IC_{50} > 10^{-6}$), indicating unusual insect selectivity. Accordingly, there should be fewer agricultural applications of these new compounds, and subsequently enhanced properties in resistance management and malaria vector control programs.

23. Non-Target Toxicity Evaluation of Novel Carbamate Insecticides for Control of the Malaria Mosquito, *Anopheles gambiae*

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Malaria is a major cause of morbidity and mortality in the world, and each year takes the life of more than 1million children. *Anopheles* mosquitoes are the only natural vectors of human malaria and *Anopheles gambiae* is a principal vector of malarial parasites. Historical data has proved that vector control is an effective way to tackle malaria. Currently, pyrethroids are mostly used to control malaria mosquitoes, especially when formulated on insecticide-treated nets (ITNs). However, insecticide resistance to common insecticides is threatening to unravel the progress of malaria control, so alternatives are urgently needed.

Carbamate insecticides, because of their relatively low cost and good killing capacity, have potential for use in malaria vector control. However, carbamate insecticide neurotoxicity is a concern. The target of carbamate insecticides is acetylcholinesterase (AChE, EC 3.1.1.7), which hydrolyzes the neurotransmitter acetylcholine (ACh) at cholinergic synapses (Massoulié 1993). By inhibiting AChE, carbamate insecticides cause ACh accumulation at synapses and induce neurotoxicity. Carbamates tend to react indiscriminately with the ubiquitous catalytic serine residue at the active site of AChE and cause neurotoxicity in livestock and aquatic animals (Taylor 1994; Keifer 2007).

This study is part of a broader effort in our laboratory to offer an effective and selective option in malaria vector control. Along these lines, we have discovered a series of carbamates with unprecedented selectivity for *Anopheles gambiae* AChE, compare to human AChE. The purpose of the present work is to confirm the safety of these new carbamates for other non-target organisms.

Accordingly, we have tested novel carbamates on 6 non-target species. By comparing *in vitro* AChE inhibition capacity (IC_{50} non-target/IC_{50} mosquito), the novel carbamates show good selectivity over *An. gambiae*, with values ranging from 0.47- to 19587-fold compared to the vertebrates we screened. In contrast, established less selective commercial carbamates ranged from 0.3- to 5.6-fold. Next, neurotoxic esterase (NTE) assay evaluated. Inhibition of this enzyme is involved in nerve demyelination, but the new carbamates we have prepared had no blocking activity, *in vitro*, while come of the commercial carbamates did had some activity. The overall results show that the novel carbamates we identified have improved *toxicological properties for use in malaria vector control*.

24. The Ecological Effects of PaV1 Infection on the Caribbean Spiny Lobster

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Pathogens can profoundly impact the ecology of the organisms they infect through changes in host behavior that influence demographic processes. For example, juvenile Caribbean spiny lobsters infected with the PaV1 virus (*Panulirus argus* Virus 1) are avoided by their normally social conspecifics, which alters local spatial distributions and rates of disease transmission. PaV1 infections are nearly always lethal, but prior to succumbing to the disease, infection may impact other host dynamics that effect transmission of the virus. We used a suite of field and laboratory studies to determine the impact of PaV1 infection on lobster ecology, including: movement, physiological condition, shelter use, and survival. Lobsters with early-stage infections moved at an equivalent rate to healthy lobsters, but as infection progressed infected lobsters moved less and ultimately remained sedentary. Although lobsters infected with PaV1 eventually become sedentary, during the early stages of infection they remain active and are thus capable of dispersing the virus throughout the population. However, heavily infected lobsters in field mark-recapture studies were recaptured less frequently than healthy lobsters, indicating either greater emigration from the area (i.e., greater movement) or greater mortality. Infected individuals were lethargic and had significantly lower blood protein levels, indicating poor physiological condition, which supported the probability of increased mortality from predation. Field tethering experiments revealed that predation was indeed higher on infected individuals and on all tethered lobsters deprived of shelter. Moreover, in shelter competition experiments, neither healthy nor diseased lobsters dominated access to shelters, but lobsters shared shelter less often when diseased lobsters were present relative to controls with only healthy lobsters. These results offer striking evidence of how pathogenic diseases shape not only the behavior of social animals, but also their use of shelters and risk of predation.

25. Structural and functional compartmentalization in larval *Anophles gambiae* alimentary canal

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The cell and tissue biology of mosquitoes is only poorly understood. A detailed understanding of an organism's biology and biochemistry can provide the bases for new approaches to population control. In this vein, we have been working toward a detailed picture of larval cell biology and tissue architecture. Among many biochemical features that can distinguish and highlight cell function and specialization is the nature of macromolecules synthesized by or associated with defined tissues and cells. The distribution

of specific proteins and carbohydrate chains added to cellular proteins and lipids frequently provide distinguishable characteristics to the cells. Specific antibody probes as well as fluorescently labeled lectins (Vector Lab) were applied to cross sections of the early 4th instar. Lectins have very specific binding characteristics and recognize sugars in the context of the carbohydrate chains that are frequently added as post translational modifications to many proteins and are also enzymatically added to some lipids. Our confocal microscopy analyses of the lectin labeling showed a remarkable diversity in tissues, cells and extracellular matrices with regard to glycoconjugate character. Among the most interesting observations was that the peritrophic matrix of the midgut and the physically similar matrix of the caecal membrane were differentially labeled. This shows that the biochemical nature of the peritrophic matrix is distinct from that of the caecal membrane. Additionally, the larval salivary glands showed remarkably intense and specific labeling for certain glycoconjugates both within the gland cells and in the lumen (saliva). This observation is consistent with the role of the salivary glands in producing a mucin-rich saliva. Furthermore, antibodies to cell surface digestive peptidases highlight distinct compartmentalization of specific protein degradation pathways. Other labeling specificities will be discussed in reference to specific glycoconjugate structure.

26. Spatial and Temporal Characterization of Gene Expression Changes in Culex nigripalpus Theobald (Diptera: Culicidae) Following a Blood Meal

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Gene expression in midgut tissue of blood fed and non-blood fed *Culex nigripalpus* has been previously characterized by differential display analysis, showing an increase in expression of 11 cDNA fragments related to blood ingestion. Three of the 11 fragments have been characterized both over time and in different mosquito tissues. Of the remaining fragments, the putative translation product of the cDNA fragment CN-G3D is 88% similar to a salivary gland protein of unknown function in *Culex pipiens* quinquefasciatus and fragment CN-G8A is 45% similar to a B3 esterase protein in *Culex tarsalis*. Spatial and temporal expression analysis of CN-G3D was performed on RNA from midgut, ovary, head, thorax, and abdomen tissues of blood fed and non-blood fed *Cx. nigripalpus* females. CN-G3D is uniformly expressed over time (0-72 h post-blood meal) in all mosquito tissues tested, regardless of blood ingestion, indicating a possible function as a housekeeping gene. CN-G8A expression was also analyzed over time (0-72 h) in RNA from midgut and ovary tissues from blood fed and non-blood fed females. CN-G8A was not expressed in ovary tissues or non-blood fed midgut tissues. However, from 0-12 h post-blood meal, expression of CN-G8A is increased in midgut tissue. After 12 h post-blood meal, expression completely ceases, suggesting a role in blood ingestion or early digestion.

27. Characterization of a Gene Cluster Regulating Stress Tolerance in *Streptococcus mutans*

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The oral cavity is a very dynamic environment with fluctuations in oxygen tension and nutrient availability. *Streptococcus mutans*, one of the primary etiological agents of dental caries, has evolved many pathways to overcome these environmental stresses and establish itself as a member of pathogenic biofilms. One conserved strategy of *S .mutans* to cope with nutrient limitation is through the accumulation of (p)ppGpp to adjust global transcription to enhance the survival of the organism. Most gram-positive bacteria, including *S. mutans*, possess a bifunctional RelA enzyme that governs production and degradation of (p)ppGpp. There are two additional (p)ppGpp synthetase enzymes RelP and RelQ found in *S. mutans*, which also participate in production of (p)ppGpp. RelP appears to be the major enzyme for (p)ppGpp production under non-stresses conditions and the *relP* gene is co-transcribed with a two-component system encoded by *relRS*. Our hypothesis is that the RelRS system senses environmental signals to modulate (p)ppGpp by RelP so as to optimize persistence in the oral cavity. A gene cluster (*SMu0835-0839*) directly upstream of the *relPRS* operon appears to have some regulatory overlap with the RelPRS system and encodes genes that play a role in stress tolerance. Importantly, *Smu0836* and *0837* encode ABC exporters and are co-transcribed with *SMu0835*, which encodes a MarR transcriptional regulator. The SMu0836 and SMu0837 exporters appear to be exporting a growth inhibitory compound from *S. mutans*. When both of the transporters are deleted, the cells have a much slower growth phenotype and are sensitive to low pH and oxidative stress. There is also a significantly smaller zone of inhibition compared to the wild-type strain, when the cells are overlaid with a mutacin-sensitive strain of *Streptococcus sanguinis*. Thus, the SMu0836 and SMu0837 ABC exporters are integrated into a complex sensing network that may enhance the resistance of *S. mutans* to environmental stress while contributing to antagonism of growth of competing species in the oral biofilm. This study was supported by DE13239 to RAB and the T32 Training Program in Oral Biology (DE07200)

28. GacA regulates virulence, biofilm, and iron acquisition of *Vibrio vulnificus*

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GacS/GacA is a two-component signal transduction system (also referred to as VarS/VarA) that regulates transcription of small

RNAs to globally influence behavior of _ –proteobacteria, including control of biofilm, virulence, and symbiosis of *Vibrio* species. Phase variation from opaque to rugose (wrinkled) colony types increases biofilm formation of *V. vulnificus* due to extracellular polysaccharide (EPS) in rugose types. Conversely, virulence in mice is related to Group 1 capsular polysaccharide (CPS) expressed by opaque strains and correlates with increased survival in oysters but reduced biofilm *in vitro*. CPS and EPS differ in genetics, carbohydrate composition, hydrophobicity, and charge, but both have been related to GacS/GacA in other bacteria. We hypothesized that GacS/GacA system regulates virulence and biofilm of *V. vulnificus* and investigated relevant phenotypes using mutational analysis. Deletion mutants (Δ*gacA::aph*) in opaque clinical isolates of *V. vulnificus* (CMCP6/O and MO6-24/O) were confirmed by DNA sequencing and were shown to be deficient in expression of GacA-related sRNAs by quantitative RT-PCR. Mutants were compared to the wild type (WT) for virulence, biofilm, motility, phase variation, iron acquisition, and uptake by oysters. Virulence was determined by subcutaneous inoculation of mice to evaluate localized (CFU/g of skin) and systemic (CFU/g of liver and rectal temperature) disease with or without pretreatment with iron dextran.
Analysis of sRNAs confirmed the GacS/GacA pathway in *V. vulnificus* but indicated strain differences in response. Virulence of *V. vulnificus* Δ*gacA::aph* was significantly reduced compared to WT for both localized (p=0.0003) and systemic (p=0.017) infections in non-iron-treated mice. Conversely, the mutant was not affected for any measure of virulence in iron-treated mice. Mutants showed decreased growth relative to WT under *in vitro* iron limitation conditions. Mutants retained opaque morphology indicative of Group 1 CPS and showed decreased phase variation (p=0.001). Biofilm was reduced in the *V. vulnificus* MO6-24/O mutant (p=0.01), but was unchanged in the CMCP6/O mutant. Motility is necessary for both biofilm and virulence and was unchanged for all mutants. No significant differences were observed for initial uptake by oysters, and long-term survival of bacteria in oysters is under investigation.

These studies delineated the relationship of GacS/GacA to virulence and survival of *V. vulnificus*. Strain differences in sRNA and biofilm response are consistent with the diversity of the GacS/GacA pathway. These results strongly link the GacS/GacA pathway to virulence and iron acquisition and utilization, which is consistent with prior data showing susceptibility to *V. vulnficus* disease correlates with host iron availability.

29. Evaluating Peanut (Arachis hypogaea L.) cv. Florida-07 for Tolerance to Late Leaf Spot (Cercosporidium personatum (Berk and M. A. Curtis) Deighton)

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Florida-07, a peanut cultivar recently released by the University of Florida, displays classic symptoms of leaf spot susceptibility, having numerous lesions and heavy defoliation. However, previous observations showed that it maintained good yields. Therefore, our hypothesis is that Florida-07 possesses tolerance to leaf spot. To test this hypothesis, Florida-07 was compared to a known leaf spot susceptible cultivar, AP-3. Experiments were conducted in Gainesville, FL in 2008 and in Marianna, FL in 2008 and 2009. During both years, late leaf spot (Cercosporidium personatum (Berk and M. A. Curtis) Deighton) was the predominant pathogen. The experimental design was a randomized complete block with a split-plot treatment arrangement and three replications. The cultivars were assigned to the sub-plots and fungicide treatment (full-season vs. no spray) was assigned to the main plots. Data collection began at the onset of disease symptoms and continued weekly until harvest, for a total of three collection dates. Data collected included a visual leaf spot rating (Florida 1-10 scale), lesion area percentage, lesion-count ratio, and final yield. In the non-sprayed treatment, Florida-07 performed similarly to AP-3 in lesion area percentage at the first (13.1% and 9.9%,respectively) and third (23.6% and 21.5%, respectively) collections, in lesion-count ratio at the first collection (3.4 and 4.0, respectively), and in the final leaf spot rating (7.4 and 7.1, respectively). Based on these data, we conclude that Florida-07 and AP-3 possess the same degree of susceptibility to late leaf spot disease. The impact of leaf spot on pod yield of Florida-07 was similar to its impact on pod yield of AP-3 in two out of three tests, but in the third test, leaf spot impacted pod yield of Florida-07 (968 lbs/A loss) less than it did AP-3 (1778 lbs/A loss) (p>t=0.0524). On average, however, yield loss (sprayed minus non-sprayed) of AP-3 (1440 lbs/A) was not different than that of Florida-07 (1026 lbs/A). Therefore, we can also conclude that in some environments, pod yield of Florida-07 in the presence of leaf spot may prove more resilient than AP-3, but on average, Florida-07 does not possess significant tolerance to leaf spot.

30. UF-HHMI ICORE: Emerging Pathogens Partnership Program

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The University of Florida Center for Precollegiate Education and Training (UF CPET) coordinates science enrichment programs for precollege students and teachers, forging partnerships between students, educators, and researchers. One of UF CPET's most recent programs is the Interdisciplinary Center for Ongoing Research/Education (ICORE) Partnerships: Emerging Pathogens which proposes innovative and outcome-oriented professional development for high school teachers through extended collaborations with University of Florida Emerging Pathogen researchers across the state. 136 teachers from limited-resource rural and urban schools will attend the partnership program during the five years of the HHMI funded precollege grant.

The learning activities and outcomes of the ICORE Partnerships are organized around Emerging Pathogens, a new initiative at UF and a topic of major importance to the health and economy of Florida and the nation. The program addresses HHMI and

UF goals to strengthen science education across the K-20 continuum; builds on measurably successful precollege laboratory and career exploration programs; establishes a new paradigm for collaboration among bench, translational, and social scientists through interdisciplinary courses and integrated opportunities for teachers, graduate students, and faculty; and importantly, magnifies and measures the broader impacts on Florida's high schools of Emerging Pathogen research.

The ICORE program begins with a two week Institute integrating multidisciplinary concepts, skills and activities into experiments illustrating the molecular basis of host-pathogen interactions and the development and production of controls/treatments. Example modules include: investigations of Tomato Spotted Wilt Virus and genetic engineering, HIV, arthropod vectors, and human and animal pathogens. At the conclusion of the Institute, teachers produce Action Proposals to incorporate knowledge, skills, applications and career information into classroom and inservice instruction. During the school year, teachers receive funds, equipment and personnel resources to facilitate implementation of their action research. Online message boards, reunions at professional meetings, and visits to and from scientists nourish the partnerships. Teachers return to a UF Symposium to present classroom results to ICORE faculty, students, and evaluators. Graduate credits awarded for successful completion can be applied towards a degree program or the Certificate in Biotechnology Education generated by this proposal.

As part of the mission of UF CPET, ICORE is an important program designed to facilitate collaborations across the K-20 continuum. UF CPET assists University faculty in communicating research to a broader audience by providing the infrastructure for their research outreach efforts. In return, science and engineering faculty and graduate students have the opportunity to enrich the science curriculum in the classroom and serve as mentors and role models for precollege students. More information about the numerous UF CPET programs can be found at www.cpet.ufl.edu.

31. Multiple Genome Comparison Reveals dnaK Typing Locus for Mycobacterium

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Besides the notorious Mycobacterium tuberculosis and M. leprae, a large number of ubiquitous environmental nontuberculous mycobacteria (NTM) also post great threat to the public health. The steadily increasing NTM infection rate in the U.S., especially in Florida, imposes us to put more research effort onto this area. To enhance our understanding of the population structure and genetic variability of Mycobacterium, and to provide more advanced diagnostic tools for mycobacterial infection, we have conducted bioinformatic studies of sequenced genomes from Mycobacterium and related genera. The results revealed numerous informative loci for species identification as well as evolutionary relations among these species. Among these loci, the dnaK locus is analyzed with several ATCC reference strains and clinical isolates. Result is compared to the most commonly used hsp65 locus and the newly identified rpoBC locus. Besides the general similarity of the phylogenic trees, the dnaK locus provides more details in certain clusters and is excellent tools for species identification. A multilocus sequence typing system including these loci will be ideal for accurate identification of Mycobacterium species in diagnosis of mycobacterial infections and environmental surveys and will greatly improve the typing method for mycobacteria.

32. Fate of Microbial Contaminants in Florida Tomato Packinghouses

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The spread of infectious diseases due to consumption of tomatoes contaminated with microbes is a significant health risk to consumers and an economic concern for the tomato industry. Our objective in this study was to determine the presence and accumulation of microbes in tomato packinghouses in Florida. We collected pre-processed tomatoes, post-processed tomatoes, and washwater (water used to clean tomatoes) and assayed for total heterotrophic bacteria (THB) and *E. coli* by traditional membrane filtration methods, and *Salmonella* spp., *E. coli* O157:H7, and *Listeria* spp. by molecular-based methods. The concentrations of THB in the pre-processed tomatoes were 6.1×10^4 to 3.4×10^5 colony forming units (CFU) per 100 mL of the phosphate-buffered saline solution applied over ~500 g of tomatoes. The post-processed samples had an 81% reduction (range: 73–93%) in THB concentration, suggesting that washwater disinfection at packinghouses was effective at killing a large proportion of the bacteria. Concentrations of THB were 25 to 30 CFU per 100 mL of washwater at the beginning of packinghouse operation, which decreased to <5 CFU per 100 mL after 30 min of operation. No *E. coli* were detected by membrane filtration assays in any of the washwater samples. Even with the use of highly sensitive DNA-based assays, no *Salmonella* spp., *E. coli* O157:H7 or *Listeria* spp. were detected on the surfaces of pre- and post- processed tomato samples or in the washwater samples. The high level of microbial inactivation in the packinghouses' washwater suggests that the possible pathogens introduced into the packinghouse washwater by field tomatoes are effectively inactivated by chlorinated treatments. Moreover, periodic tests of microbial inactivation at tomato packinghouses is a useful protocol in food safety programs.

33. Plasmepsin 4-Deficient Plasmodium berghei Are Virulence Attenuated and Induce Protective Immunity against Experimental Malaria

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Plasmodium parasites lacking plasmepsin 4 (PM4), an aspartic protease that functions in the lysosomal compartment and contributes to hemoglobin digestion, have only a modest decrease in the asexual blood-stage growth rate; however, PM4 deficiency in the rodent malaria parasite *Plasmodium berghei* results in significantly less virulence than that for the parental parasite. *P. berghei pm4* parasites failed to induce experimental cerebral malaria (ECM) in ECM-susceptible mice, and ECM-resistant mice were able to clear infections. Furthermore, after a single infection, all convalescent mice were protected against subsequent parasite challenge for at least 1 year. Real-time *in vivo* parasite imaging and splenectomy experiments demonstrated that protective immunity acted through antibody-mediated parasite clearance in the spleen. This work demonstrates, for the first time, that a single *Plasmodium* gene disruption can generate virulence-attenuated parasites that do not induce cerebral complications and, moreover, are able to stimulate strong protective immunity against subsequent challenge with wild-type parasites. Parasite blood-stage attenuation should help identify protective immune responses against malaria, unravel parasite-derived factors involved in malarial pathologies, such as cerebral malaria, and potentially pave the way for blood-stage whole organism vaccines.

34. Inoculation with a Non-replicating Herpes Simplex Virus Vector Confers Protection from Wild-type HSV

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Herpes keratitis, caused by Herpes Simplex Virus (HSV), is the leading cause of infectious blindness in the U.S. It is characterized by recurrent outbreaks, including corneal inflammation and shedding of virus from the corneal epithelium, which lead to an accumulation of corneal scarring and eventual blindness. Previous studies in rabbits demonstrated that corneal inoculation with KD6, a non-replicating HSV recombinant that lacks the ICP4 gene, conferred localized protection against subsequent infection with wild-type HSV. We hypothesized that inoculation of murine footpads may be able to confer systemic protection from challenge, since the footpad is not an immunoprivileged site like the cornea.

Methods: Following saline pre-treatment and abrasion of the keratinized epithelium, $1x10^5$ PFU of KD6 was applied to the dermis of the right feet of female Swiss-Webster mice. The right feet of control animals were mock-infected. The left feet of all animals were untreated. At one month post-treatment, mice were challenged bilaterally with $1x10^5$ PFU of HSVlacZgC, a recombinant virus containing an *E. coli lac*Z gene cassette inserted at the gC locus. At 4 days post-challenge, mice were sacrificed and perfused with a 4% formaldehyde solution. Feet were taken for X-gal staining.

Results: Following challenge with HSVlacZgc, heavy blue X-gal staining of feet from control animals revealed the replication and spread of virus on the epithelium. In contrast, staining was sharply reduced in KD6-inoculated mice, suggesting a restriction of viral replication. Contrary to previous experiments in the rabbit cornea, this result was observed bilaterally, suggesting a systemic response to viral infection.

Conclusion: We have demonstrated that unilateral murine footpad inoculation with KD6 confers bilateral protection against subsequent infection with wild-type HSV. Further studies will reveal if seroconversion has occurred in these animals. The observed protective response contrasts sharply with the localized protection observed in rabbit corneas and suggests that response to HSV infection is dependent upon the route of infection. Specifically, immune response in the cornea may be limited by the immunoprivileged nature of the cornea itself. Studies of inoculated tissues in rabbit cornea and mouse footpad model systems may yield insights into aspects of local innate and adaptive immune responses to viral infection.

35. Kinetic Characterization of and Newly Discovered Inhibitors for Various Constructs of Human T-cell Leukemia Virus-I Protease and Inhibition Effect of Discovered Small Molecules on HTLV-1 Infected Cells

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Human T-cell leukemia virus type 1 (HTLV-1) is the first retrovirus that has been shown to be causative for a human cancer, adult T-cell leukemia (ATL) as well as other diseases. It is estimated that up to 30 million people worldwide are infected with HTLV-1, which is endemic in Melanesia, Japan, the Caribbean, sub-Saharan Africa, and the United States. Only an estimated 3–5% of people infected with the virus develop ATL in their lifetime, but those that do have a poor prognosis. The HTLV-I protease (PR) is an es-

sential component of the virus. It is a 28 kDa homodimeric aspartic acid protease composed of two identical subunits of 125 amino acids each. The viral protease is necessary for the virus' lifecycle, as it cleaves the polyproteins Gag, Pro, and Pol into their constitutive components that enable the virus to infect a host cell. Thus, the protease is an ideal target for drug design. The recombinant HTLV-1 PR was expressed in E. coli and purified by ionic exchange chromatography. Various truncated forms of HTLV-1 PR have been used to compare kinetic activity and folding properties. Current clinically used inhibitors of HIV-1 PR and plasmepsin inhibitors were tested for inhibition of the HTLV-1 PR. In addition; the Dock program was used to identify new potential inhibitors using binding energy values and this has led to the identification of 40 small molecules. We tested these for inhibition of the HTLV-1 PR, and found that 24 of them gave K_i's ranging from 0.8 to 40 μM. The best five inhibitors were then selected based on K_i values (K_i < 5 μM) to be tested in HTLV-1 infected (MT-2) cells and the best inhibitor (compound 667746 K_i=1.09 μM) has been shown to lower gag processing of HTLV-1. These best inhibitors are now being utilized in crystal trials. This approach has the potential to identify a novel drug that can be used to treat HTLV-1 infections.

36. Microarray Analyses of the Transcriptional Responses of Human Peripheral Monocytes to *Bacillus anthracis'* Lethal Toxin

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Background: Anthrax lethal toxin (LT), produced by the Gram-positive bacteria *Bacillus anthracis*, is a potent zinc dependent metalloprotease that cleaves the N-terminus of MAPKKs (MEKs) and is known to play a major role in impairing the host immune system during an inhalation anthrax infection. Here, we present the transcriptional responses of LT treated human monocytes in order to further elucidate the mechanisms of LT induced inhibition of the host immune system. Results: Using flow cytometry, we found that 4 hour treatment of 500ng/mL LT with human monocytes did not induce cell apoptosis. Furthermore, using Western Blot analysis we confirmed human monocyte susceptibility to 500ng/mL LT by demonstrating cleavage of MEK1 and MEK3 after a 4 hour incubation. Using Affymetrix Human Genome U133 Plus 2.0 Arrays, we identified over 820 probe sets differentially regulated after LT treatment at a 0.001 significance, interrupting the normal transduction of over 60 known pathways. As expected, the MAPK signaling pathway was most drastically affected by LT, while the p38 pathway was also highly impacted. Numerous genes outside the well-recognized pathways were also influenced by LT including the IL-18 signaling pathway, the Toll-like receptor pathway and the IFN alpha signaling pathway. It was also found that multiple genes involved in actin regulation, signal transduction, transcriptional regulation and cytokine signaling were impacted by LT treatment. Conclusion: We conclude human peripheral monocytes are a direct target of anthrax LT. We show that LT caused multiple defects in human monocytes normal signaling transduction pathways, along with inducing multiple aberrant gene responses associated with monocyte functioning. Using these results, we can further understand how anthrax LT impairs normal human monocyte function by focusing on the unique transcriptional responses and their contribution to host immune system dysfunction.

37. Detection of Enteric Pathogens in Young Children from Developing Countries by Molecular Approaches

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Worldwide, enteric diseases cause up to one fifth of deaths in young children. Enteric diseases contribute to growth shunting, immunosuppression, and cognitive impairment. A major obstacle in designing strategies to combat enteric disease is the large number of etiologic agents and a lack of efficient diagnostic technologies to detect them. New technologies offer promise in providing rapid, sensitive and specific detection. In an ongoing study at four international sites (Mali, The Gambia, Kenya and Bangladesh) we have to date collected fecal samples from more than 750 children suffering from severe diarrhea and 750 matched controls to evaluate three technologies: PCR/ESI-MS (Ibis Biosciences, Carlsbad, CA), GoldenGate (Illumina, San Diego, CA), and metagenomics (UF, UMD and Sanger Centre, UK). Here we report initial findings of differences in microbiota profiles as determined by Denaturing Gradient Gel Electrophoresis and 454 based 16S rRNA sequencing. Of 1293 samples successful PCR amplification was achieved in 1198 samples. There was no PCR bias against samples from cases. 1153 samples passed our QC. QC failed in 30% of samples from Mali but in less than 10% of samples from the other study sites. The proportion of distorted microbiota profiles was higher in cases and similar at all sites. To date, we have generated 454 16S rRNA sequencing data for more than 600 samples. Initial analysis of conventional microbiological and 16S rRNA sequences suggests that we can identify many molecular signatures of pathogens at similar frequency in cases and controls. This finding, if confirmed in the final analyses, suggests that in developing countries pathogens are present more frequently in healthy controls than previously thought. Such finding would have important implications for future public health interventions aimed at decreasing mortality and morbidity from enteric diseases.

38. Canine Influenza Virus among Persons Occupationally Exposed to Dogs

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Background: In 2004, a novel H3N8 equine-like influenza virus was detected as the cause of a canine epizootic in racing greyhounds in Florida. Since its isolation, canine influenza virus (CIV) widely spread among canine racing and pet populations in North America. Thus far human infections with CIV have not been reported.

Methods: Beginning in 2007, we enrolled dog breeders, dog show enthusiasts, veterinarians, shelter workers, kennel workers, and racetrack workers in a cross-sectional study to assess their exposure to CIV. Participants completed an enrollment questionnaire and permitted a serum sample collection. Sera were studied for evidence of previous CIV and human H3 influenza virus infections by microneutralization and hemagglutinin inhibition assays. Persons not exposed to dogs were enrolled as a control group.
Results: As of December 2009, 196 dog-exposed workers and 36 non-exposed controls have been enrolled and provided a serum sample. Thus far, 18 of 196 (9.2%) exposed subjects had titers ≥1:10 (run in duplicate) against H3N8 CIV, and 189 of 194 (97.4%) had H3 titers ≥1:40 against human H3N2 influenza virus. Occupations with elevated titers against CIV included breeders, dog show enthusiasts, veterinarians, pet store owners, and racetrack workers.
Conclusions: While cross-reactivity of antibodies cannot be ruled out, the lack of strong correlation between these two assays supports the hypothesis that some dog-exposed participants have been infected with CIV. Enrollments and laboratory analyses are ongoing.

39. Canine and Equine Influenza Virus Replication in Mice and Isolated Canine Alveolar Macrophages

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Canine influenza virus (CIV) is a highly contagious virus that induces severe tracheitis and bronchitis as well as pneumonia of variable severity in dogs. We have demonstrated that CIV replicates in alveolar macrophages and induces TNF-alpha which may be important in pathogenesis. Although CIV originated from direct transmission of equine influenza virus (EIV) to dogs, mutations in EIV genes that facilitated dog-to-dog transmission and disease induction are not well characterized. Our objective was to identify cost-effective animal and cell models to elucidate genetic evolution important in adaptation of EIV to dogs. BALB/c mice inoculated with CIV (A/canine/Florida/43/2004) supported moderate levels of pulmonary viral replication and developed tracheitis, bronchitis, bronchiolitis and pneumonia of moderate to mild severity. EIV (A/equine/Kentucky/1/1991) replicated to a much lower extent and only induced very mild inflammatory lesions in mice. Isolated primary canine alveolar macrophages had similar kinetics of virus matrix gene expression following CIV and EIV inoculation. CIV induced significantly greater TNF-alpha protein and mRNA than did EIV. Experimentally-inoculated mice and primary alveolar macrophages may be suitable models for molecular pathogenesis studies focused on adaptation of equine influenza virus to dogs.

40. Lessons Learned from a School Based Influenza Immunization Program in Alachua County, Florida

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Background: Children are "super spreaders" of influenza. Models suggest an entire community will be protected from influenza by immunizing 70% of its school children, demonstrating herd immunity.
Method: In the 2005/2006 flu season, the Alachua County Department of Health immunized 25% of K-8 public and private students through a school-based influenza immunization program with Live Attenuated Inactive Vaccine, (LAIV). Due to technical issues, there was inadequate time for necessary community involvement. Telephone surveys targeting parents with children enrolled in schools were conducted six months afterwards to evaluate the program.
Results: Of 400 households contacted, 83.8% were aware of the program. 34.9% of "aware" households had at least one child immunized. 8.1 % reported flu-like symptoms following immunization. Most (93.3%) parents surveyed would have their child participate in the program again. Factors encouraging parent participation in the program were convenience, use of a needleless spray-based vaccine, and a lack of cost or fees for participants. Roughly 23% of children could not participate due to health problems. Of these, 53% received a flu shot from their physician. Approximately 96% households believed children should receive vaccines in general, but only 74% thought it was important to receive flu vaccine. Conclusions: School-based influenza immunization programs could theoretically immunize enough children to protect the community from an epidemic. The local school system's and health department's full support was not sufficient in reaching 70% of students. Participation of local pediatric physicians, community groups, and news media are necessary for maximum success.

41. Neutralization of *Francisella tularensis* virulence regulators by FDA-approved small molecules

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Francisella tularensis ssp. *tularensis* is considered a potential bioweapon by the CDC because of its extreme virulence, low infectious dose, ease of aerosol dissemination, and capacity to cause severe illness and death. Indeed, only ten cells are enough to develop the disease tularemia. *F. tularensis* is found in animal hosts (i.e. voles, mice, squirrels, rabbits) where they serve as natural reservoirs of infection. In addition, *F. tularensis* can be recovered from contaminated water, soil, and vegetation, serving as a threat in agriculture. The current treatment of choice is antibiotics, however the ease of genetic modification of these microorganisms could intentionally render these treatments ineffective. With this in mind, it is necessary to develop novel treatments for the effective control of this pathogen.

Experimental data have shown that the transcriptional regulators MglA and SspA interact with RNA polymerase to regulate the expression of genes located in the Francisella Pathogenicity Island (FPI), which are required for virulence and intramacrophage growth. In this study, we screened a FDA-approved chemical library to identify small molecules that have the ability to interact with purified MglA and SspA. Compound # 527 was able to increase the thermal stability of MglA, which could be translated into a change in the functionality of the protein *in vivo*. To test the effect of this compound *in vivo*, an *E. coli* two-hybrid system was used to determine if the interaction between MglA and SspA is disrupted. We found that compound # 527 has the ability to decrease the interaction between MglA and SspA by 40 %. In conclusion, compound # 527 could be used as a novel anti-*Francisella* therapeutic. Interference with the expression of virulence genes would affect their intracellular viability, and ultimately help neutralize infection.

42. Adaptive Photoperiodic Egg Diapause is Retained in Florida Populations of Invasive *Aedes albopictus*

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More than 20 years have passed since *A. albopictus*, descendents of invasive colonizers from temperate Japan, became established in northern Florida and spread rapidly southward to become the State's most common day-biting mosquito. Adults of this species may be active in south Florida in every month, but many eggs laid in December and January of 2006-07 in West Palm Beach County did not respond to a standard hatching stimulus. Females from geographic populations, collected in 2008 from north and south Florida, exposed to short (10L:14D) daylengths at 21 degrees C laid eggs of regionally variable diapause incidence. Short days induced strong diapause responses in north Florida populations (Pensacola and Jacksonville). Some subtropical populations (e.g., Vero Beach) showed decreased diapause compared to ten years previously (vide: Lounibos et al. [2003] Ann. Entomol. Soc. Am. 96:512), while others had unexpectedly high current incidence (e.g., Card Sound), suggesting possible recolonization from more northern localities. Eggs derived from short-day Vero Beach females survived January exposures in the field significantly better than eggs laid by long day controls, substantiating the adaptive nature of the polymorphic diapause response.

43. Genetic Analyses of *Vibrio vulnificus* Strains Isolated from Tilapia Fish in Bangladesh

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V. vulnificus, a gram-negative bacterium, distributed worldwide in estuarine and marine environments, can cause serious human illnesses, including wound infections, septicemia and gastroenteritis. People with underlying chronic diseases acquire the bacterium upon consumption of raw and undercooked seafood, especially oysters and fish. While oysters are considered to be a major reservoir of *V. vulnificus* in the U.S., necrotizing wound infections associated with injuries caused by aquacultured fish such as tilapia are increasingly being recognized as a serious consequence of *V. vulnificus* infection, as evidenced by the recent report of 62 cases of *V. vulnificus* infection in Israel attributed to the handling and eating of tilapia.

To meet the growing demand for protein, policymakers in a number of countries, including Bangladesh, are encouraging their citizens to increase aquaculture production of tilapia. To assess the potential health risk from *V. vulnificus* linked to the handling and consumption of tilapia in aquaculture environments in areas such as Bangladesh, we screened coastal aquaculture ponds, and tilapia, for the presence of *V. vulnificus* for a three month period beginning in May, 2008.

During our study period, we collected a total of 42 tilapia and water samples from coastal aquaculture ponds. As controls, we also collected 54 tilapia and water samples from fresh water aquaculture ponds. While none of the tilapia fish and water samples obtained from fresh water aquaculture ponds yielded *V. vulnificus*, 24 (57%) of 42 samples collected from coastal aquaculture ponds

yielded a total of 33 *V. vulnificus* isolates. A preliminary characterization using a battery of biochemical tests revealed that all 33 *V. vulnificus* isolates belonged to biotype 1. PCR and ribotyping analyses confirm that all of the strains possess the virulence gene, *vvhA*, with strains coming from 5 different ribotypes. On rep-PCR and multilocus sequencing typing (MLST) analysis, the Bangladeshi strains tended to cluster with clinical biotype 1 strains, or with newly described biotype 3 strains linked with tilapia-associated infections in Israel.
Our data suggest that tilapia grown in coastal aquaculture ponds in Bangladesh serve as reservoir for *V. vulnificus*. While the clinical significance of these findings remains to be determined, it is concerning that these Bangladeshi strains appear to be closely related genetically to strains associated with human illness in other parts of the world. Currently there is minimal surveillance for human *V. vulnificus* infections in Bangladesh; our data suggest the need for such surveillance, particularly among persons working in the coastal aquaculture industry.

44. Vertilmicin, a novel aminoglycoside

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As a novel semisynthetic amnoglycoside antibiotic derived from verdamicin, vertilmicin is currently in the stage of clinical development in China. Its pharmacological role in clinical practice is not yet established, compared with gentamicin, amikacin, netilmicin and verdamicin. Nevertheless, preclinical study with rodents has suggested that in vivo therapeutic efficacy of vertilmicin was approximately equal to that of netilmicin, much better than that of gentamicin and verdamicin in the majority of bacteria including gram-positive and gram-negative strains.

Furthermore, in vitro experiment has suggested vertilmicin has a broad antimicrobial spectrum with high activity against both gentamicin-susceptible and gentamicin-resistant clinical isolates.
According to minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and time-kill curve tests compared with other aminoglycosides appearing in the market, it was proved that the antibacterial activity of vertilmicin is similar to that of netilmicin and pretty higher than that of gentamicin and verdamicin, but lower than that of amickacin.

However, the toxicity of vertilmicin is still unclear. Further pharmacodynamic assay was badly required to ensure the efficacy and safety of the drug before clinical study.
On the other hand, high-performance liquid chromatographic using derivatization was originally developed as a sensitive and reliable method of qualitative and quantitative analysis, and is successfully applied to the pharmacokinetic investigation of vertilmicin in rats. The remarkable advantages of the validated method include: 1) the lower limit of quantification was as low as 10 ng/ml; 2) the volume of the plasma sample needed to be collected from rat is only 50 ??g/ml. This analytical method may provide powerful technique to get valuable data for the clinical study of vertilmicin in the future.
In our lab, static time-kill curve and dynamic time-kill curve experiment of vertilmicin is in progress. Determination the free concentration of vertilmicin in tissue and muscle by microdialysis in animals is under preparation. Finally, the in vitro assay will be combined with the data from pharmacokinetic profile to simulate the antibacterial activity of vertilmicin in vivo by Pharmacokinetic/Pharmacodynamic model, and will offer a useful guidance for dose or regimen selection of vertilmicin for clinical studies.

45. Pandemic Influenza Virus Among Healthy US Swine Show Pigs

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Background: In recent years, a number of swine influenza virus (SIV) infections in man have been associated with swine show exposures. During the summers of 2008 and 2009 we studied swine show participants and their pigs at US state fairs for evidence of SIV.
Methods: Using an informed consent process, swine show participants ≥7yrs of age completed an enrollment questionnaire and permitted a serum sample collection. Nasal swabs were collected from at least one of each participant's show pigs. Sera and swab specimens were studied for evidence of SIV through hemagglutinin inhibition and real-time RT-PCR assays. Influenza viruses were cultured in MDCK cells and compared to other influenza viruses by their sequence data.
Results: In the late summers of 2008 and 2009 we enrolled 123 swine show participants and collected nasal swabs from 149 show pigs at the Minnesota and South Dakota state fairs. In 2008 no influenza viruses were detected in the pigs. In 2009, influenza A virus was detected in 12 (11.8%) of 102 healthy pigs by RT-PCR. Culture and sequence studies yielded 7 influenza isolates - 6 isolates were very similar to A/California/07/2009(H1N1) and 1 isolate was a triple reassortant H1N2 virus. Human serological results against common SIV strains are pending.
Conclusions: During August and September 2009, a significant number of Minnesota and South Dakota state fair show pigs had evidence of asymptomatic carriage of influenza A. Available data suggest that most viruses were from the human pandemic.

46. Induction and Inhibition of a Neuronal Phenotype in Spodoptera frugiperda (Sf21) Insect Cells.

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Virginia Polytechnic Institute and State University

Due to the increasing resistance demonstrated by insects to conventional insecticides, the need for compounds with novel modes of action is becoming more urgent. Also, the discovery and production of new insecticides is vital as regulations and restrictions on conventional insecticides become increasingly stringent (Casida and Quistad 1998). Research in this area requires screening of many candidate compounds which is costly and time-consuming. The goal of this research was to produce *in vitro* insect neurons from Sf21 insect ovarian 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 a culture of Sf21 cells and a mixture of differentiation agents to produce viable neuron-like cells. In the presence of the molting hormone 20-hydroxyecdysone (20-HE), or insulin, in the growth medium, Sf21 cells began to express neuronal morphology, or the production of elongated, axon-like processes within 2-3 days. Maximal differentiation occurred when in the presence of 42 µM 20-HE or 10 µM insulin. Effects were maximal on day 2 for 20-E and day 3 for insulin. Insulin was more potent at day 2 for inducing differentiation (EC_{50} = 247 nM) than 20-HE (EC_{50} = 13 μM). In combination, 20-HE and insulin produced apparent synergistic effects on differentiation. Caffeine, a central nervous system (CNS) stimulant, inhibited induction of elongated processes by 20-HE and/or insulin. Caffeine was a potent inhibitor of 42 μM 20-HE, with an IC_{50} of 9 nM, and the inhibition was incomplete, resulting in about one quarter of the differentiated cells remaining, even at high concentrations (up to 1 mM). The ability to induce a neural phenotype simplifies studies with of insect cells, compared to either the use of primary nervous tissue or genetic engineering techniques. The presence of ion channels or receptors in the differentiated cells remains to be determined. If they are present, high throughput screening for new insecticides will be accelerated and made more economical by the utility of this method.

47. VASP Phosphorylation Impairs Neutrophil and *Listeria* Actin-Based Motility

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Enabled/Vasodilator-stimulated phosphoprotein (Ena/VASP) localizes to regions of dynamic actin remodeling and promotes filament formation. Phosphorylation affects the interaction of VASP with actin; however, the crucial role of phosphorylation in the regulation of actin filaments remains to be clarified. We find that anthrax edema toxin (ET) an exotoxin that impairs neutrophil and *Listeria monocytogenes* actin-based motility induces VASP phosphorylation at serine 157 (S157). Introduction of pseudo-phosphorylated VASP into MVd7 cells lacking VASP, Ena, or Mena slows *Listeria*-induced actin assembly as compared to MVd7 cells transfected with wild-type VASP, and transfection with pseudo-unphosphorylated VASP accelerates *Listeria*-induced actin motility. Introduction of these proteins into human neutrophils has identical effects on chemokinesis. We conclude the phosphorylation of VASP at S157 slows and dephosphorylates actin-based motility in living cells.

48. Reduction of *Salmonella* in almond hulling and shelling dusts following application of different sanitizers

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Introduction: Almond dust is generated during removal of hulls and shells from almond kernels. This fine particulate matter is extremely difficult to eliminate from the huller-sheller facility, given current equipment and facility design, and was identified as a potential source of *Salmonella* in almonds.
Aim: The objective of this study is to evaluate the efficacy of aqueous and alcohol based sanitizers for reducing *Salmonella* in dusts generated in almond hulling and shelling facilities.
Material and methods: Five different nalidixic acid resistant *Salmonella* strains were inoculated into almond dust at 5 log CFU/g. One g dust, 1 ml inoculum, and 2 ml of sterile water, aqueous based sanitizer (200 ppm) or alcohol based sanitizer were mixed in 50 ml Falcon tubes. At 0 and 48 h, *Salmonella* was enumerated on bismuth sulfate agar supplemented with Nalidixic acid (50μg/ml) following incubation for 48 h 37°C.
Results: Sterile water and aqueous based sanitizers led to a ca. 3 log CFU/ml increase in *Salmonella* populations after 48 hours. Alcohol based sanitizers lead to instant reduction (at 0 h) of ca. 1 log CFU/ml and a subsequent reduction to below the level of detection (≤1.3 log CFU/ml) after 48 hours.
Significance: Alcohol based sanitizer is effective at reducing *Salmonella* populations, while the addition of aqueous based sanitizers and water allow for the growth of Salmonella in almond dust. The use of alcohol based sanitizer is recommended for sanitizing in Almond hulling and shelling facility.

49. Enhancement of Antiviral Immunity by a Peptide Antagonist of SOCS

Prof. Howard M. Johnson, Ph.D.

University of Florida

Enhancement of Antiviral Immunity by a Peptide Antagonist of SOCS Chulbul M. I. Ahmed, Rea Dabelic, and Howard M. Johnson Department of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611 Suppressors of cytokine signaling (SOCS) are negative regulators of both innate and adaptive immunity via inhibition of signaling by cytokines such as interferons (IFNs). We have developed a small peptide antagonist of SOCS-1 that corresponds to the activation loop of the Janus kinase JAK2. SOCS-1 inhibits both type I and type II IFN activities by binding to the kinase activation loop via the kinase inhibitory region (KIR) of the SOCS. The antagonist, pJAK2(1001-1013), inhibited the replication of vaccinia virus and EMC virus in cell culture, suggesting that it possesses broad antiviral activity. In addition, pJAK2(1001-1013) protected mice against lethal vaccinia and EMC virus infection.

pJAK2(1001-1013) increased the intracellular level of the constitutive IFN??, which may play a role in the antagonist antiviral effect at the cellular level. pJAK2(1001-1013) also synergizes with IFNs as per IFN?? mimetic to exert a multiplicative antiviral effect at the level of transcription, the cell, and protection of mice against lethal viral infection.

pJAK2(1001-1013) binds to the KIR of both SOCS-1 and SOCS-3 and blocks their inhibitory effects on the GAS promoter. In addition to a direct antiviral effect and synergism with IFN, the SOCS antagonist also exhibits adjuvant effects on the humoral and cellular arms as well as an enhancement of polyI:C activation of TLR3. The SOCS antagonist thus presents a novel and effective approach to enhancement of host defense against viruses.

50. Role of the Porphyromonas gingivalis SerB Phosphoserine Phosphatase in Periodontal Disease

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One of the predominant polymicrobial infections of humans is expressed clinically as periodontal disease. P. gingivalis, an invasive pathogen, has been strongly implicated in the initiation and progression of periodontal disease and secretes haloacid dehydrogenase family phosphoserine phosphatase enzyme (SerB) when in contact with gingival epithelial cells in vitro. A P. gingivalis SerB mutant was shown to be deficient in internalization and persistence in gingival epithelial cells. Objective: This study examined the in vivo role of P. gingivalis SerB in colonization, immune response, and induction of periodontal disease in rats. Methods: Adult rats were infected with P. gingivalis or P. gingivalis SerB mutant at 1 x 1010 cells mixed with 2% carboxymethylcellulose. Oral bacterial samples were collected and colonization/infection assessed by PCR. Rats were euthanized at 12 weeks following infection. Serum IgG antibody responses to the infections were evaluated by ELISA. Defleshed rat jaws were also evaluated for morphometric (horizontal) and radiographic (interproximal) analysis of alveolar bone resorption (ABR). Results: The PCR results demonstrate an appropriately sized amplicon for P. gingivalis (600 bp) present in DNA isolated from rat oral microbial samples. Both the wild-type and SerB mutant induced significant ($P < 0.05$) levels of IgG, and IgG isotype (IgG1, IgG2a, IgG2b) antibody responses to infection compared to control rats. Both strains induced significantly ($P < 0.05$) higher levels of horizontal and interproximal ABR than control rats. However, ABR induced by the SerB mutant was significantly ($P < 0.001$) lower than that induced by the parent strain. Conclusions: This is the first in vivo study demonstrating a role for the P. gingivalis SerB phosphatase in the induction of periodontal disease in rats. This research was supported by a UFCD Student Summer Research Fellowship, NIH/NIDCR Grant 5T32 DE007200, U24DE016509, and NIH/NIDCR 1R01 DE11111.

51. Norovirus Outbreaks Among Wedding Attendees in Northeast Florida: A Comparison of Transmission Methods with Recommendations for Future Investigations

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Introduction: In November 2008 and February 2009, two separate weddings in Northeast Florida experienced large outbreaks of gastrointestinal illness among guests. The guest lists and catering staff for both events combined totaled more than 300 people. Interviews were conducted on guests and food service workers for each wedding. Following epidemiological investigations, both outbreaks were confirmed positive for norovirus GII strain. The November outbreak, or Outbreak A, was linked to the ingestion of contaminated food. However, the February outbreak, or Outbreak B, was likely the result of person-to-person spread. Follow-up

involved contacting additional health departments throughout the country. The investigation team worked with the Maryland Health Department and with the Centers for Disease Control's internal alert system, Epi-X.
Summary of Outbreaks: Outbreak A's epidemic curve was typical of a common source outbreak. For Outbreak A, the odds of illness among those attending the rehearsal dinner was 41.03 (99% Confidence Interval [CI] =2.38, 707.55) times the odds of illness among those not attending. For those attending the rehearsal dinner, the odds of illness among those who ate meatloaf were 5.67 (95% Confidence Interval [CI] =1.61, 19.98) times the odds of illness among those who did not eat meatloaf. For Outbreak B, an epidemic curve at 24-hour intervals indicated a person-to-person exposure rather than a point source infection. The median incubation time between illness onset and the rehearsal dinner was 50.2 hours. The documented incubation period for norovirus is 24 to 48 hours, indicating that the exposure for the majority of cases did not occur at the rehearsal dinner.
Case Comparison: Each wedding had a rehearsal dinner and a catered wedding reception. Outbreak A featured a Jewish wedding with a "kosher-like" menu and traditional Jewish ceremonial activities. Outbreak B was comprised primarily of Palestinian or Lebanese families. Outbreak B wedding events included a groomsmen's party at the groom's sister's house and a gathering for the bridal party at the parents of the bride's house, both prior to the wedding. The two weddings represented different cultural groups with diverse social norms, customs, and food.
Findings and Recommendations: Due to the pathogenicity of norovirus, there is significant potential for outbreaks associated with weddings. Transmission between guests can occur through foodborne exposure, person-to-person spread, touching contaminated surfaces, or breathing in the aerosolized vomitus of an infected person. Because weddings tend to be events in which a large number of people gather, virus transmission between guests can occur rapidly. The development of investigation tools, such as food questionnaires, must pay close attention to the cultural aspects of the event and look at every activity separately and as a potential source of infection.

52. Antimicrobial effect of Sodium Metasilicate on *Salmonella* Typhimurium and Psychrotrophs in Refrigerated Chicken Breast Meat

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The objectives of this study were to determine the antimicrobial effects of sodium metasilicate (SMS, U.S. Food and Drug Administration approved food additive) against *Salmonella* and psychrotrophic organisms in fresh ready to cook chicken breast meat, and to ascertain the effects of the treatment on pH. Boneless chicken breasts (with skin-on) were inoculated with *Salmonella* Typhimurium, treated with 0% SMS and no inoculum (negative control), 0% SMS and inoculum (positive control), 1% SMS (w/w) and 2% SMS (w/w) and stored at 4 ± 1°C. All samples were analyzed after 0, 1, 3, 5 and 7 days for recovery of *Salmonella*, psychrotrophic organisms and pH. Treating the breast meat with 1 and 2% SMS resulted in 1 and 2 log cfu/gram reductions ($P < 0.05$) in *Salmonella*, respectively, when compared to the positive control. Psychrotrophic counts for breast meat treated with 1 and 2% SMS were lower ($P < 0.05$) than the control samples on all sampling days. The results of the present study indicate that SMS has significant antimicrobial potential against gram negative foodborne pathogens, such as *Salmonella* Typhimurium, in chicken meat and can play a role in safeguarding the health of consumers in terms of food safety. The data also suggested that SMS could function in extending the shelf life of poultry by retarding the growth of psychrotrophic bacteria.

53. Attempts to isolate the causal agent of citrus Huanglongbing on solid media

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Candidatus Liberibacter sp. is the suspected causal agent of citrus Huanglongbing (HLB), transmitted by psyllids. Based on its 16S rRNA gene sequence it was grouped in the alpha subclass of proteobacteria. It is hitherto uncultured. Other suspected causal agents of HLB are phytoplasma species, which are also uncultured. Therefore, there are no confirmed causal agents of HLB. There have been two recent reports on cultivation of Liberibacter species (in one case a dual culture with an Actinomycete) on various media, but no pure cultures are currently available. Here an attempt to isolate the causal agent of citrus HLB on S medium is presented. S medium was previously used to isolate the fastidious bacterium *Rhizomonas suberifaciens* (belonging to the alpha-proteobacteria) from lettuce roots with corky root symptoms. Leaves, stems and fruits of citrus trees that were PCR positive for Liberibacter were collected from Fort Pierce, Southwest Florida REC and the Orlando area. Eight attempts were made to isolate Liberibacter between May and November 2009. Two-cm pieces of stems, midribs, petioles and peduncles were cut from the samples and surface sterilized in ethanol. The cortex, including phloem, was peeled off the plant parts with blades. The tissue was ground in sterile water and filtered through 0.65μm nitrocellulose membrane. The 10^{-1} to 10^{-4} dilutions of the filtrate were plated on S medium, incubated at 28°C and checked after one and two months. Translucent small colonies were isolated from Valencia orange material from Fort Pierce after two months. No other colonies were observed on these plates. Similar colonies were observed on other plates, but not in pure culture. These colonies consisted of slow growing, very small bacteria (about 0.2x0.6 micron), with variable shapes under the light microscope; they appeared gram variable, but mainly gram negative. Genomic DNA was extracted and subjected to bead beating. PCR with phytoplasma and Liberibacter specific 16S rRNA primers showed that the unknown bacterium was neither phytoplasma nor Liberibacter. Sequencing of PCR product obtained with eubacterial 16S rRNA primers hinted at the presence of an uncultured *Pseudomonas* species. The PCR product was cloned and sequenced again. Two of three clones, when blasted against the NCBI nucleotide database, showed 99% identity with an uncultured *Pseudomonas sp*. The isolate has been grown in liquid S medium, and

will be subjected to fatty acid analysis and additional sequencing to make sure that it is still the same as the original isolate. Since it has not been confirmed that Liberibacter or phytoplasma sp. are the pathogens causing HLB, this isolate will be used to carry out Koch's postulates on citrus and periwinkle plants by direct injection into the phloem as well as by the use of psyllids as vectors.

54. Detection of Virus Using Photo-Electric Sensors

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Abstract: Photo-electric microbes sensors (PEMS) are being investigated for aqueous detection of viruses (patent pending). Recognition between molecules coating sensor surfaces with biological analytes delivered in test solutions change the photovoltages observed. This technology has been successfully used to detect Sindbis virus (SIN), Porcine parvovirus (PPV), and several bacterial species. Currently the sensors are being used to target mouse-adapted influenza virus A/WSN/33. Influenza virus, a member of the Orthomyxoviridae, is the causative agent of the flu in birds and mammals. Hemagglutinin, an integral membrane protein of influenza is known to bind sialic acid (SA) attached to sugars on host cells prior to virus entry. Tethering SA to the sensor surface resulted in detection of influenza. Exposure of SA coated electrodes to solutions spiked with influenza virus produced photovoltages of increased magnitude. Monoclonal antibodies against influenza A nucleocapsid are currently under investigation in an effort to refine sensor specificity to this target. We have now detected membrane bounded and naked viruses using PEMS.

55. Detecting E. coli with photo-electric sensors

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Photo-electric microbe sensors (PEMS) are currently being used in electrodes for aqueous sensor technology, which relies on recognition between molecules on the sensor surface with biological analytes in aqueous solution. The time constant of light-induced molecular events that displace charge on the sensors are taken from the measurement of voltage versus time readings. PEMS are being used for detection of bacteria; photo-voltage changes may indicate detection of microbes. Successful detection of *Escherichia coli* bacterium has been established : increased titers of *E. coli* have yielded increased photo-voltages.

56. Detecting of HLB(Citrus Greening) using Hyperspectral imaging

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Huanglongbing (HLB) or citrus greening is the world's most destructive and devastating of all citrus diseases. The disease obstructs the flow of nutrients in citrus trees and HLB infected trees die within 3-5
years. There is no cure for the infected trees, which will have to be removed and destroyed. HLB has now emerged as the major threat to the Florida's $9 billion citrus industry. As of February 2009, citrus trees
in 1891 different sections (square mile) in 33 counties were infected in Florida. The disease in Florida is caused by a bacterium, Candidatus Liberibacter asiaticus, that is transmitted by a tiny insect, the Asian
citrus psyllid (Diaphorina citri), which thrives on young citrus leaves.

Growers urgently need diagnostic tools for early detection, because infected trees may not show symptoms for months or years, during which they are contagious. Current molecular diagnostic tests do not detect the disease soon enough to stop its spread. The objective of this study was to develop a method to detect HLB infected areas in citrus groves using airborne hyperspectral imaging such that it would allow rapid detection of potentially infected areas. This would prevent further spread if followed by development of efficient management plans of these areas. A ground-based inspection, an existing method which is much prevalent and conducted by trained personnel, is subjective, labor intensive and time consuming. On the other hand, airborne hyperspectral imaging would provide much faster results over a wide range of area. An aerial hyperspectral image with a spectral range of 400-1000 nm spanning
across 128 spectral bands was acquired from an HLB infected citrus grove in Florida in 2007. The imagery had a 5 nm spectral resolution and 0.7 m spatial resolution. Ground truthing of this area had been carried out and infected tree coordinates were recorded. The images were divided into smaller blocks and sections in order to create training and validation sets. A hyperspectral imaging software (ENVI, ITT VIS) was used for the analysis of these images. Disease infected areas were identified using image-derived spectral library, the mixture tuned match filtering (MTMF), the spectral angle mapping (SAM), spectral feature fitting (SFF), and spectral analyst tool in the hyperspectral imaging software.

57. Could tomato seeds be a source of Salmonella?

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The goal of this study is to test the hypothesis that external and internal contamination of tomato seeds with *S. enterica sv* Typhimurium is possible from infection of leaves or flowers, and that the probability of contamination depends on the complexity of the competing microbial community. Specific objectives are to determine whether: (i) *Salmonella* can enter tomato plants through leaves and flowers; (ii) there is a negative correlation between the diversity level of microbial communities inside the plant and the rate of ingress; (iii) *Salmonella* can be transmitted through seed and if so, further test if seed-borne Salmonella can invade plants from these seeds and contaminate fruits; (iv) there are differences in the rate of ingress between two strains. A two-step experiment is being conducted in a BSL II greenhouse where tomato plants were inoculated with *Salmonella* in the first step and contaminated seeds will be grown and tested for the presence of *Salmonella* inside plants in the second step. A split-plot design was used with plants inoculated with two strains of *S.* Typhimurium-gfp (110 and 119) and control plants in main plots and soil types (sand, conventional soil and organic soil) in subplots in five randomized complete blocks. Water was applied at a 2-day interval and fertilization was applied weekly with 8 g Black Kow (Oxford, Fl) for organic soil and 150 ml half-strength Hoagland solution for sand and conventional soil. Stationary phase inoculum was prepared in LB broth with kanamycin after 48 h incubation at 37 °C, and adjusted to 10^9 with sterile distilled water. Inoculation was carried out by wounding the upper surface of leaflets with Carborundum and then placing two 10-μl droplets on each leaflet. A total of 3 leaflets were inoculated on each plant. At each sampling time, one inoculated and one non-inoculated leaflet was removed at random from one plant in each block. A 12-mm disc was cut from the inoculation site and the base part of an inoculated and non-inoculated leaflet. Leaf discs were either surface sterilized with pure alcohol for 15 S or not, ground in 1 ml sterile distilled water and the suspensions were dilution plated on LB agar with kanamycin. Preliminary results indicated that *Salmonella* entered the leaf through wounds at the inoculation site, but did not move to uninoculated leaflets during a 2-week period after inoculation. Strain 119 had a larger population size on/in the leaf than 110. There was a significant interaction between *Salmonella* strain and soil type. In the second week after inoculation, the population densities dropped significantly, especially inside the leaflets. The plants will be reinoculated on new leaves and flowers, and fruits and seeds will be tested for the presence of *Salmonella*.

58. Callose accumulation in the phloem plasmodesmata and inhibition of phloem loading in the Liberibacter-infected citrus leaf

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Huanglongbing (HLB) is a serious disease for the citrus industry around the world. A citrus tree infected with the disease can die in 2 years and there is no effective treatment yet. The causal agent of HLB is the Gram-negative bacterium *Liberibacter* and it was observed that massive amount of starch granules accumulate in the parenchyma cells of HLB symptomatic leaves. To better understand disease development process, we examined Liberibacter infected (Las+) leaves by fluorescence, electron, and immunoelectron microscopy.

In Las+ leaves, plasmodesmata pore units (PPUs) that connect companion cells to sieve elements were swollen and abnormally large amount of callose was seen in at PPUs, plasmodesmata between the sieve elements and sieve pores when immunogold labeled by an anti-callose antibody. In contrast, PPUs were not swollen and far less amount of callose was detected in the PPUs, plasmodesmata and sieve pore in uninfected (Las-) samples. Then, we imaged callose in the citrus leaf phloem tissues after staining with aniline blue. In Las-, no callose was detected in the phloem while callose deposition in sieve pores and PPUs was observed in Las+ samples. It was shown that callose accumulates in the phloem cells of senescing citrus leaves, raising a question as to whether the callose deposition is a secondary phenomenon following leaf deterioration by Liberibacter infection. To address this question, we examined callose deposition in Las+ citrus leaves but display no HLB signs yet. Callose was detected in the sieve pores and PPUs of the asymptomatic leaves as much as those in the symptomatic leaves. This demonstrates that callose synthesis in the Liberibacter-infected phloem precedes development of HLP symptoms.

Callose deposition in the plasmodesmata is a natural plant defense reaction but it reduces solute transport efficiency through the plasmodesmata. This led us to hypothesize that the callose synthesis in the PPU accompanying Liberibacter infection may affect phloem loading in the citrus leaves. To test the possibility, we injected carboxyfluorescein into intercellular spaces of Las-, Liberibacter-infected asymptomatic Las+, and symptomatic Las+ leaves. After 24 hours after injection, the dye was seen to accumulate in the vein and spread through the vein in Las- leaves. However, dye remained at the injection site in the Las+ leaves, indicating that phloem loading is inhibited in the infected leaves. The massive starch accumulation in the parenchyma cells suggests a blockage in photosynthetate export and inhibition in phloem loading of the infected leaves provides an explanation for the blockage. Our results also suggest that cell death in the Las+ trees is due to a plant defense response rather than damages actively done by the bacterial cells, which is consistent with the fact that this phloem-limited bacterium devastates the entire tree at a low titer.

59. Advanced computational algorithms for deep interrogation of microbial communities using massive 16S rRNA pyrosequencing data

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Aided by advances in next-generation DNA sequencing technology, researchers may obtain millions of DNA sequences rapidly and economically. Consequently, large-scale DNA sequencing is increasingly applied as a primary research tool for applications as diverse as human epidemiological studies involving hundreds of individuals, as in the Human Microbiome Project, to global ocean survey efforts such as the International Census of Marine Microbes. Developing advanced computational strategies to maximally extract pertinent information from massive nucleotide data collections has become a major focus of the bioinformatics community. Here, we describe an innovative analytical strategy enabling researchers to deeply explore the hidden world of microbial communities, far beyond basic microbial diversity estimates. We demonstrate the utility of our strategy by performing a computational study using a human gut microbiome dataset consisting of more than one million 16S rRNA sequences obtained from the fecal material of 154 individuals of known body weight status. Application of our computational strategy enabled us to describe the diversity of gut microbiota, specific microbial signatures associated with obesity, microbial community structure, and microbe-microbe interactions with far more detail than previously achievable. Our strategy is broadly applicable to other sequence-based microbial studies, and provides a rigorous statistical analysis for the identification of organisms, known or unknown, that correlate with physiological or environmental conditions. Ultra-deep DNA sequencing techniques combined with our computational approach will significantly advance discovery and understanding of microbial community structures and their relationship with the environment.

60. Molecular Analysis of Surface Polysaccharide Genes in Pandemic *Vibrio parahaemolyticus* O3:K6

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In order to understand the rapid serotype changes occurring in pandemic *Vibrio parahaemolyticus*, we set out to identify and characterize genes that encode the O3-antigen and capsule (K6) antigen of pandemic *V. parahaemolyticus* O3:K6 isolate VP53. We first identified regions related to surface polysaccharide in the published genome and then investigated the functions of these regions by deleting genes in each region. Mutants were examined for colony morphology, by immuno-blots of SDS-PAGE gels and by immuno-gold EM.

The region previously reported to encode capsule genes is located on chromosome 2. Examination of the published sequence of O3:K6 pandemic strain RIMD2210633 found that the first 4 genes in that region are similar to exopolysaccharide (EPS) genes in *Vibrio cholerae* that cause a rugose phenotype when expressed, sharing the same gene orders and 31-54% amino acid identity. In frame deletion of the first four genes in this region in VP53 abolished the ability of the strain to undergo rugose-like phase variation but did not change the colony opacity. Immuno-blots of SDS-PAGE gels indicated that neither the K-antigen nor the O-antigen was affected in the mutant.

In *V. cholerae* and *V. vulnificus*, the O antigen genes lie between *gmhD* and *rjg* on chromosome I and this region is also responsible for capsule production in *V. cholerae* serogroup O31 strain and in *V. vulnificus*. Deletion of the homolog region in *V. parahaemolyticus* VP53 changed the colony morphology to translucent. It eliminated the K6-antigen but not affected the O3-antigen in *V. parahaemolyticus* as shown by immuno-blot. This finding was confirmed with Immuno-gold EM with gold particles labeled with K6 antiserum which revealed a capsule in wild type but not deletion mutants. These data indicate that this region encodes the K antigen and is the real capsule region. In addition, deletion mutations demonstrated that the exportation of the capsule (K-antigen) depends on *wzm* and *wzz* genes but not the Wza system.

In summary, we have clarified the function of the EPS gene regions in *V. parahaemolyticus* O3:K6. The region on chromosome 2 reported previously as the capsule region appears to be an EPS region similar to that seen in *V. cholerae* and *V. vulnificus*. The K-antigen does appear to be a capsule antigen and is encoded in the region between *gmhD* and *rjg* on chromosome 1. The O-antigen genes remain to be identified, and unlike other vibrios do not seem to share genes with capsule and lie between *gmhD* and *rjg*.

61. Survival of E. coli O157:H7 in manure-amended soil and rhizosphere: research data and initial model

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The human pathogen *E. coli* O157:H7 has increasingly been associated with enteric disease outbreaks traced back to contaminated vegetables. Its main reservoir is the colon of cattle. Survival of *E. coli* O157-H7 in manure and soil depends mainly on substrate availability as affected by microbial competition. Survival curves show an oscillatory decline pattern. When applied with cattle

slurry (as opposed to manure), *E. coli* O157:H7 can reach high densities in the rhizosphere of lettuce plants. To combine effects of environmental and substrate variables on the risk of *E. coli* O157:H7 in manure-amended soil, a simulation model was developed in the Matlab programming environment. State variables are the pathogen, autochthonous bacteria and substrate concentration. Forcing variables are temperature and moisture condition. The overall decline in E. coli O157:H7 was primarily determined by competition with autochthonous copiotrophic bacteria simulated by an inter-specific competition term according to Lotka-Volterra. Oscillations of bacterial populations were attained by the relationships between relative growth and death rates with readily available substrate content. The model contains a logistic and exponential relation of relative growth and death rates, respectively, of E. coli O157:H7 and copiotrophic bacteria with temperature, resulting in optimum curves for net growth rates similar to the curves reported in the literature. Oscillatory decline curves are simulated accurately for survival in manure and manure-amended soil in relation to oxygen availability and temperature. This is the first time that oscillations are described for decline curves, and that temperature optimum curves are modeled by separating the responses of the relative growth and death rates to temperature. The model was used for sensitivity analyses and to determine effects of different management strategies on the survival period of *E. coli* O157:H7 in manure and manure-amended soil. The relative effects of changes in temperature on simulated survival time of E. coli O157:H7 were more pronounced than changes in oxygen condition. Alternating temperature and oxygen availability and high microbial competition, as in a regularly turned manure heap, lead to the shortest survival periods. The simulation model provides a new approach to investigating dynamic changes of invasive microorganisms in natural substrates such as manure or manure-amended soil.

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

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Since the discovery of soybean rust (SBR) in 2004 in the Southeastern United States, its severity has been variable from year to year. Still, it is important to understand the epidemiology of the pathogen in Florida as it may serve as an inoculum source for other areas of the country. This study examined the incidence and severity of SBR in relation to prevailing weather data, growth stage, and maturity group (MGIII, MGV, MGVII) in soybean plots (15 m square) across the Panhandle of Florida that were part of the national sentinel plot network from 2005 through 2008. Of the three maturity groups, the MGIII soybean became infected first the least often. Plots became infected first at growth stage R4 (full pod) or later. On average, plots became infected 40 days earlier and 30 days closer to planting in 2008 than 2005. Precipitation was the principle factor affecting disease progress, where disease increased rapidly after rain events and was suppressed during dry periods. The area under the disease progress curves (AUDPC) for incidence was the lowest in 2007, most likely due to dry conditions. In 2008, there was a significant increase in disease incidence and severity as reflected in the AUDPC. This was associated with the occurrence of Tropical Storm Fay, which deposited up to 290 mm of water in the plot locations during the third week of August. Results from this study may lead to a better understanding of the impact of weather on the epidemiology of this pathogen.

63. The Stochastic Properties of High Daily Maximum Temperatures

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The statistical properties of the excursions of maximum daily temperatures above various critical thresholds of interest are analyzed with a view to developing models of heat wave events using the 115 years of record from the meteorological station in Lake City, Florida. These stochastic variables include; event density (numbers of such events per unit time), duration, timing, and peak values over the threshold. The theoretical basis for the modeling is found in Crossing Theory which states that as the threshold of interest becomes particularly large with respect to the mean of a Gaussian process, the number of crossings (up or down) becomes Poisson distributed. The changing seasonal intensity of such events can be incorporated by utilizing a temporally non-homogeneous Poisson model, with time varying rates.

Environmental health studies indicate that both the magnitude and duration of the excursion above the critical threshold are important. As both the number of upcrossings and downcrossings follow a Poisson distribution it is reasonable to approximate the length of time between the two (the duration of an event) by an exponential, or exponential-like distribution. Similarly, the peak magnitudes of event over the threshold (POT) represent the extreme tail of the distribution of daily maximum temperatures and might be assumed to follow the same sort of distribution.

Although this study only considers a single site, the threshold that constitutes a critical value may well vary spatially, and be dependent upon the ultimate application of the results. It is therefore necessary to be able to extrapolate findings derived at one level of interest to others, particularly in seeking to determine the risks of extremely rare events like those of Chicago(1995), France (2003), London (2006) and Melbourne (2009), which had seldom if ever been observed in historic series. The methodology has the flexibility to extrapolate to such levels while also having the advantage of being applied to data from across the state to determine risks associated with high temperature events during any time period or at any location of interest.

64. Rift Valley fever Prediction and Assessment in East and Southern Africa 2006 - 2008 and Possible Vector Control Strategies

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Historical episodic outbreaks of Rift Valley fever (RVF) since the early 1950s have been associated with cyclical patterns (El Niño and La Niña) of El Niño Southern Oscillation (ENSO) phenomenon which results in elevated and widespread rainfall over the RVF endemic areas of Africa. Using satellite measurements of global and regional elevated sea surface temperatures, and subsequent elevated rainfall and satellite derived-normalized difference vegetation index data, we predicted with lead times of 2- 4 months specific areas where outbreaks of RVF in humans and animals were expected and occurred in the Horn of Africa, Sudan and Southern Africa at different time periods from September 2006 to March 2008. Predictions were confirmed by entomological field investigations of virus activity in the areas we identified and by reported cases of RVF in human and livestock populations. This represents the first series of prospective predictions of RVF outbreaks and provides a baseline for improved early warning, control, response planning and mitigation into the future.

65. West Nile Virus Infection Alters Midgut Gene Expression in *Culex pipiens quinquefasciatus* Say (Diptera: Culicidae)

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Alterations in gene expression in the midgut of female *Culex pipiens quinquefasciatus* exposed to blood meals containing 6.7 logs plaque-forming units/mL West Nile virus (WNV) were investigated by Fluorescent Differential Display. Results from the display revealed 26 cDNA bands exhibiting reproducible differences after feeding on infected blood. Of these, 21 bands showed an increase in expression and 5 showed a decrease in expression as a result of WNV presence. Eleven cDNA bands exhibiting increased expression following infection with WNV were cloned and four of these were sequenced. VectorBase and BLAST database searches revealed that one clone, CQ G12A2, shares identity with a leucine-rich repeat-containing protein (LRR) from *Cx. p. quinquefasciatus* and Toll-like receptors from *Aedes aegypti* (95 and 42 %, respectively); one clone, CQ G1A1, shares high identity (98%) to the gram-negative bacteria binding protein family (GNBBP) from *Cx. p. quinquefasciatus*; the third clone, CQ G43A2, shares identity (95%) to members of the defensin-A protein family from *Cx. p. quinquefasciatus, Armigeres subalbatus, and Ae. aegypti*; and the last clone, CG G35A, shares high identity (98%) to chorion peroxidase proteins from *Cx. p. quinquefasciatus*. Here, we present the expression analysis of three cDNA clones isolated from female *Cx. p. quinquefasciatus* midgut tissue whose protein products may interact with WNV and play a role in the immune response to viral infection.

66. Esterase Isolation, Expression, and Population Analyses of *Culex nigripalpus* Theobald (Diptera: Culicidae) in Manatee County, FL

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Mosquitoes play an important role as vectors for a wide variety of pathogens. In an attempt to control mosquito-borne disease outbreaks around the world, the use of insecticides has risen; however, this has also resulted in selection for mosquitoes that possess high tolerance/resistance to many insecticides. It is important to understand the mechanisms involved with the development of mosquito resistance to insecticides to be able to predict where targeted control measures may be needed and how mosquitoes will react to new insecticides. Because esterase is an enzymatic protein known to play a role in insecticide resistance formation, we amplified an esterase gene segment (Temsha est-1, TE-1) from *Culex nigripalpus* using esterase primers. Through expression studies, we found that TE-1 consistently showed high expression within thoraces and abdomens in unfed mosquitoes and high expression in heads, thoraces, abdomens, and midguts after blood feeding. This suggests TE-1 has a role in feeding/digestion. We also found that the level of expression of TE-1 differed depending on where field mosquitoes were collected. If this difference in expression can be correlated to differences in susceptibility towards insecticides, we may be able to use TE-1 as an indicator of the formation of tolerance/resistance. This would greatly enhance mosquito control efforts.

67. Effects of Forced Egg Retention on the Temporal Progression of West Nile Virus Infection in *Culex pipiens quinquefasciatus* Say (Diptera: Culicidae)

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Environmental factors that impact the biology of mosquito vectors can have epidemiological implications. Lack of oviposition sites facilitated by environmental factors such as temperature and drought can often force *Culex* spp. mosquitoes to retain their eggs. *Culex pipiens quinquefasciatus* were fed blood meals containing West Nile virus (WNV, family *Flaviviridae*, genus *Flavivirus*) and either allowed to oviposit or forced to retain their eggs through different time points post-infection (9, 13, 20, 27 d) at 28°C. Oviposition status did not significantly affect rates of WNV infection (% with virus-positive bodies), dissemination (% with virus-positive legs), or transmission (% with virus-positive saliva) for any of the tested time points. As expected, WNV titers in bodies and legs were significantly ($p < 0.05$) higher at late time points compared to early time points. No significant differences were observed in WNV titers in saliva between time points. There were no significant effects of oviposition status on virus titers of bodies, legs, or saliva. However, we found that egg retention may increase vector competence at early and late time points post-infection and that a single oviposition event may decrease vector competence, possibly by activating an anti-viral immune response. Environmental changes that influence mosquito biology are important determinants of virus transmission and further studies are needed to assess the effects of drought on virus transmission risk and how these interactions affect our interpretation of field data.

68. ATP-dependent activation of an inflammasome in primary gingival epithelial cells infected by *Porphyromonas gingivalis*

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Production of IL-1b typically requires two-separate signals. The first signal, from a pathogen associated molecular pattern, promotes intracellular production of immature cytokine. The second signal, derived from a danger signal such as extracellular ATP, results in assembly of an inflammasome, activation of caspase-1 and secretion of mature cytokine. The inflammasome component, Nalp3, plays a non-redundant role in caspase-1 activation in response to ATP binding to P2X7 in macrophages. Gingival epithelial cells (GECs) are an important component of the innate-immune response to periodontal bacteria. We had shown that GECs express a functional P2X7 receptor, but the ability of GECs to secrete IL-1b during infection remained unknown. We find that GECs express a functional Nalp3 inflammasome. Treatment of GECs with LPS or infection with the periodontal pathogen, *Porphyromonas gingivalis*, induced expression of the *il-1*b gene and intracellular accumulation of IL-1b protein. However, IL-1b was not secreted unless LPS-treated or infected cells were subsequently stimulated with ATP. Conversely, caspase-1 is activated in GECs following ATP treatment but not *P. gingivalis* infection. Furthermore, depletion of Nalp3 by siRNA abrogated the ability of ATP to induce IL-1b secretion in infected cells. The Nalp3 inflammasome is therefore likely to be an important mediator of the inflammatory response in gingival epithelium.

69. Fish Deformities and Stress in the St. Lucie Estuary System

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The St. Lucie Estuary, located in Southeast Florida, is threatened by increasing residential and commercial development, industry and agriculture. Construction of extensive agricultural and urban drainage projects has substantially altered the St. Lucie watershed, and the effects of these anthropogenic changes are associated with the distribution, quality, and volume of freshwater entering the estuary. Chemical contamination emanating from regional industry, agriculture and golf courses also contributes to stress on this estuary that serves as a vital component of the environmental and economic well-being of Martin, St. Lucie and surrounding counties. The health of the fish that live in potentially impacted waters can serve as a biotic index to integrate the effects of multiple stressors. This study reports observations of physical deformities from fish collected in the St. Lucie River and adjacent waterways from 2006 September to 2008 February. Thirty-four fish with deformities were archived frozen and digitally photographed and radiographed to generate observational data. Eleven species of fish, representing 7 taxonomic families, were observed; black margate and pinfish were the two most common species. Deformities primarily included missing or deformed dorsal fin spines or pyterygiophores, with or without concave defects along the dorsal surface. The prevalence of these anomalies in the field-sampled population in the St. Lucie system was approximately 0.18%. These deformities may be genetic, developmental, and/or associated with a variety of other

etiologies including trauma, parasites, infection or environmental chemical exposure. There is no direct evidence, however, making such a link at this time. A website has been developed to provide details of the study for other investigators and to allow input from complimentary disciplines [http://aquaticpath.epi.ufl.edu/deformities]. Ongoing studies are focusing on histopathology, as well as examination of water and sediment data to explore relationships between deformities within the different fish species and various environmental stress agents.

70. Vector Competence of Florida *Culex* and *Aedes* Mosquitoes for Chikungunya Virus

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Chikungunya virus (CHIKV) is a re-emerging arbovirus of worldwide public health importance that has been imported into the United States via infected travelers in recent years. The primary vectors of CHIKV are *Aedes albopictus* and *Ae. aegypti* due to vector competence and propensity to blood feed on humans. *Culex pipiens quinquefasciatus* is a principal enzootic and epidemic vector of other arboviruses in Florida, also feeding on humans; however, vector competence has not been evaluated for this species. In this report, we evaluate vector competence for an emergent strain of CHIKV in Florida populations of *Ae. albopictus*, *Ae. aegypti*, and *Cx. p. quinquefasciatus* and also examine the effects of extrinsic incubation temperature. This information informs risk prediction models as mosquito control and public health agencies prepare for the possibility of a CHIKV outbreak in the United States, thereby helping target control measures to the most relevant mosquito populations in advance of epidemics.

71. Effect of Incubation Period on Vector Competence Relationships for *Culex pipiens quinquefasciatus* (Diptera: Culicidae) and West Nile Virus

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Culex pipiens quinquefasciatus fed a blood meal containing 6.8 ± 0.3 logs plaque-forming units of West Nile virus (WNV) / mL were maintained at 28°C for incubation periods (IP) of 7 d, 14 d, or 21 d. Aspects of vector competence were determined at each IP using rates of infection (% with WNV-positive abdomens), dissemination (% infected with WNV-positive legs or thoraces), and transmission (% infected with WNV-positive saliva), as well as WNV titer in abdomens, legs, thoraces, and saliva. Rates of infection, dissemination, and transmission increased or were equivalent with increasing IP. The WNV titers in abdomens and thoraces were higher than titers in legs and saliva. Mosquitoes that transmitted WNV showed significantly higher titers in abdomens, legs, and thoraces and this was dependent on IP. The titers of abdomens and thoraces were correlated with the presence of WNV in saliva and the degree of these associations differed between IPs. However, although correlated, preliminary regression analyses showed that WNV titers in abdomens or thoraces did not predict the presence of WNV in saliva ($P > 0.05$) suggesting that these proxy measures of WNV in other tissues were not good predictors of transmission under the conditions of our test. These results are consistent with the hypothesis that the presence of WNV in the saliva of infected *Cx. p. quinquefasciatus* is due to processes involved in salivary infection and escape that are, in part, independent from the infection processes in other tissues. The causes of limits to virus replication in different mosquito tissues are unknown. However, an understanding of the relationships between various measures of vector competence will improve our ability to characterize mosquito populations for vector competence to ultimately improve risk assessment for disease and develop novel control strategies.

72. West Nile Virus Affects the Rate of Blood Digestion in *Culex pipiens quinquefasciatus* (Diptera: Culicidae)

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Culex pipiens quinquefasciatus were fed blood meals containing a high virus dose (6.2 logs plaque-forming units (PFU) West Nile virus (WNV)/mL), low virus dose (5.3 logs PFU WNV/mL), or no virus and incubated at 28°C. Twenty mosquitoes per group were collected daily from one to six days post-infection (dpi) and the rate of blood digestion was scored using the Sella scale. Bodies and legs of mosquitoes fed blood meals containing WNV were separated and tested for virus to determine rates of midgut infection and viral dissemination out of the midgut. There were no significant differences in rates of infection, dissemination, or digestion between mosquitoes given blood meals containing a low or high virus dose ($p \geq 0.05$). However, at two dpi, mosquitoes given either virus dose showed significantly faster digestion rates compared to mosquitoes given an uninfected blood meal ($\chi^2 = 10.85$, df = 1, $p = 0.004$). This finding suggests that WNV increases the rate of blood digestion in *Cx. p. quinquefasciatus*. Increased digestion rates in virus-infected mosquitoes may shorten the gonotrophic cycle and increase the chance that an infectious mosquito will take a subsequent blood meal. This phenomenon may serve to facilitate and increase the transmission rate of WNV in nature.

73. Effects of West Nile Virus Dose and Extrinsic Incubation Temperature on Temporal Progression of Vector Competence in *Culex pipiens quinquefasciatus* (Diptera: Culicidae)

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Culex pipiens quinquefasciatus were fed blood containing either 7.0 ± 0.1 logs plaque-forming units (PFU)/mL (high dose) or 5.9 ± 0.1 logs PFU/mL (low dose) of West Nile virus (WNV) and held at extrinsic incubation temperatures (EIT) of 28˚C or 25˚C. Approximately 20 mosquitoes per dose were collected after incubation periods (IP) of 4, 6, 8, and 12 days post-infection (dpi). Infection rates were influenced by EIT and virus dose but not by IP. Body titer was significantly higher for mosquitoes fed the high dose and held at 28˚C at the later IPs (6, 8, and 12 dpi). However, leg titer was significantly higher for mosquitoes at the later IPs, but did not differ between EITs or doses. Because infection rates varied with EIT and dose, there is likely a midgut infection barrier influenced by these factors that is not influenced by IP. Dissemination rates were influenced by all three factors consistent with the presence of a midgut escape barrier. Dissemination rate, body titer, and leg titer were dependent on IP, indicating the need to investigate multiple time points in vector competence studies to elucidate critical events in infection and dissemination.

74. *Chlamydia trachomatis* infection causes mitotic spindle pole defects independently from its effects on centrosome amplification.

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Chlamydiae are gram negative, obligate intracellular bacterial organisms, and *Chlamydia trachomatis* is the etiologic agent of the most commonly reported sexually transmitted disease in the United States. Chlamydial infections have been epidemiologically linked to cervical cancer in patients previously infected by human papillomavirus (HPV). *Chlamydiae* undergo a biphasic life cycle that takes place inside a parasitophorous vacuole termed an inclusion. The inclusion associates very closely with host cell centrosomes, and this association is dependent upon the host motor protein dynein. We have previously reported that this interaction induces supernumerary centrosomes in infected cells, leading to multipolar mitotic spindles and inhibiting accurate chromosome segregation. Centrosome amplification and mitotic spindle defects are characteristic of many human cancers. High risk HPV type 16 oncoproteins E6 and E7 contribute to cervical cancer by cooperating to induce abnormal centrosome numbers; however, pre-cancerous cells subjugate aberrant spindle pole formation by clustering multiple centrosomes to form bipolar spindles. Our findings demonstrate that chlamydial infection causes mitotic spindle defects independently of its effect on centrosome amplification. Chlamydial infection dramatically increased the number of defective mitotic spindles in both the mouse neuroblastoma cell line N1E-115 and a human epithelial cell line transformed with HPV oncoproteins E6 and E7 (End1). Both these cell lines have intrinsic centrosome number defects. We show that chlamydial infection increases centrosome spread and inhibits the spindle assembly check point delay to disrupt centrosome clustering. These data suggest chlamydial infection exacerbates the consequences of centrosome amplification by inhibiting the cell's ability to suppress the effects of these defects on mitotic spindle organization. We hypothesize that these combined effects on mitotic spindle architecture identifies a possible mechanism for *Chlamydia* as a cofactor in cervical cancer formation.

75. Cytokinesis failure causes multinucleation in Chlamydia trachomatis infected cells

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Chlamydiae are obligate intracellular bacteria, which live in a membrane-bound vacuole termed an inclusion. Chlamydia trachomatis is the cause of the most prevalent STD in the United States.
There are often no initial symptoms, leading to chronic infections causing pelvic inflammatory disease and infertility. On a cellular level, infection with Chlamydia trachomatis has been shown to cause changes in host cells such as golgi fragmentation, centrosome over duplication and multinucleation. The two main
mechanisms that can initiate multinucleation are cell fusion and failure in cytokinesis. Our goal was to determine which mechanism was the cause of multinucleation of host cells during chlamydial infection. Using confocal microscopy, we were able to confirm infected cells fail in cytokinesis while eliminating the possibility of cell fusion. Since cytokinesis failure is the main cause of multinucleation, we chose to look at the effects of Chlamydia on the stages of mitosis leading up to cytokinesis. Our findings show that 12 hours after infection, we begin to see a higher ratio of cells in prometaphase than metaphase in infected cells. This suggests Chlamydia is interfering with the cells ability to properly align the chromosomes on the metaphase plate. For these cells to progress through mitosis, the infected cells would need to delay exit from metaphase until proper alignment is achieved. However, we found

that the mitotic index (a measure of the relative time cells take to complete mitosis) of infected cells instead decreased, suggesting that infected cells spent less time in mitosis. We therefore hypothesize infected cells fail to align their chromosomes properly before proceeding to anaphase resulting in a higher proportion of cells failing in cytokinesis leading to multinucleated cells.

76. Ultra-deep Pyrosequencing Captured Low Frequency CXCR4 Virus Populations Co-archived with CCR5 Virus in Peripheral Blood Lymphocytes from HIV-infected Therapy-naïve Children

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Background: Predicted coreceptor biodiversity of HIV-1 Env V3 archived in peripheral blood lymphocytes was profiled using ultra-deep pyrosequencing to test the hypothesis that low-frequency CXCR4 [X4] populations are co-archived with CCR5 [R5] before CD4 decline.
Methods: Diversity of HIV-1 Env V3 in ~400 viral DNA copies was evaluated by 454 GS-FLX pyrosequencing in a cross-sectional, natural history study of 6 HIV-1 subtype B infected children (age 1.5 to 6.1 years) with 2-30% CD4 and 4.0-5.7 $\log_{10}$ HIV-1 RNA copies/ml plasma. Template-specific errors were defined and a novel algorithm involving hierarchical clustering, consensus construction, and fasty alignment for error correction was developed. Biodiversity was evaluated by rarefaction analysis at 3% distance and ACE estimates at sampling level. Coreceptor use was predicted by PSSM. Genetic distances were compared by Random Block model; correlation between frequency of X4 and CD4% was determined by Pearson Correlation Coefficient.
Results: Pyrosequencing error rate was <1% due to position-specific insertions in homopolymeric nucleotide regions. Filtering removed 3.4-10.2% of raw sequence reads, leaving ~9000 to 19000 Env V3 sequences per sample and providing 25-fold coverage of input template. Biodiversity ranged from 28 to 178 operational taxonomic units [OTU]. When sampling was increased 10-fold, OTU increased only 2-fold indicating that sampling was approaching saturation. V3 bioclusters within individuals varied from 2 to >18000 reads/cluster. Frequency of bioclusters containing <100 reads/cluster accounted for 80-93% of diversity, while only 1-8% of bioclusters had >1,000 reads/cluster. Although similar genetic distance within populations was found by 454 or Sanger method (p=0.81), pyrosequencing captured rare X4 and/or intermediate R5 variants at frequencies as low as 0.01-3.6%. No correlation appeared between X4 V3 frequency and CD4% (r=0.25; p=0.63). X4 populations at frequencies from 0.01-99.4% were identified in four subjects with >15% CD4, while R5 viruses accounted for essentially 100% of viruses in one subject with severely suppressed CD4.
Conclusions: Low frequency X4 virus was co-archived with R5 virus well before CD4 decline indicating cause rather than effect for X4 viruses in immune pathogenesis. Pyrosequencing expands robust assessment of pathogen genetic diversity and development of unique computational algorithms to expand molecular epidemiology of viral gene flow in populations.

77. Use of Rolling Circle Amplification to Enable Rapid Completion of Koch's Postulates for New and Emerging Single Stranded Circular DNA Viruses

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In the last decade, viruses belonging to the *Geminiviridae* family have devastated production of important crops such as cassava, cotton, cucurbits, legumes, peppers, and tomato (2,3,4). Geminiviruses have circular single-stranded DNA genomes packaged within small geminate particles. Proving that the geminiviral sequences detected in symptomatic plants are the causal agents of the disease in question has not been done for most geminiviruses. While completing Koch's postulates is difficult for plant viruses in general, it becomes even more complicated when the virus cannot be mechanically transmitted but only transmitted by an insect vector, as is the case for most geminiviruses. Often virologists are forced into the production of infectious clones delivered by *Agrobacterium tumefaciens* which presents many problems, not the least being a highly biased selection of sequences for inoculation.
We present here a new technique for the inoculation of plants with geminiviral DNA that bypasses the need for cloning or insects. Total DNA extracted from begomovirus-infected plants was amplified by rolling circle amplification (RCA) using the bacteriophage Φ29 DNA polymerase (1), and inoculated to plants by particle bombardment. Infection rates of up to 100% were obtained using this technique. This technique successfully inoculated all the geminiviruses evaluated: five bipartite *(Bean golden yellow mosaic virus, Cabbage leaf curl virus, Squash leaf curl virus, Tomato mottle virus, Watermelon chlorotic stunt virus)* as well as one monopartite *(Tomato yellow leaf curl virus)*. The success of the technique was not dependent upon plant species, the four species from three plant families which were tested: *Phaseolus vulgaris* (bean), *Solanum lycopersicon* (tomato), *Cucurbita pepo* (squash), and *Citrullus lanatus* (watermelon-), were all able to be inoculated by this technique. The success of the method was not dependent upon either the type or the age of the source of virus. Infectious DNA was successfully obtained from fresh, frozen or dried plant material, from squashes of plant leaves on FTA cards, as well as from the insect vector. Plant material collected and dried as long as 25 years ago yielded infectious DNA by this method. In summary this method can be used to obtain infectious DNA of single-stranded circular DNA viruses that can be activated for purposes of completing Koch's postulates, for preservation of pure virus cultures, and for many other applications where infectious DNA is required.

78. Larval temperature and nutrition alter the susceptibility of *Aedes aegypti* mosquitoes to chikungunya virus

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In the last five years chikungunya virus (CHIKV) has emerged as an important agent of human arboviral epidemics and a principle vector in the outbreaks was *Aedes aegypti*, the yellow fever mosquito. Fluctuating temperatures and limited food are common features in the aquatic container habitats occupied by immature *Ae. aegypti* and have a strong influence on adult body size and may also influence vector-virus interactions. Here we report how variations in larval temperature and food quantity influence adult *Ae. aegypti* size, development time, and vector susceptibility for CHIKV. We found that larval temperature, but not food quantity, nor the temperature X food level interaction, had a significant effect on chikungunya infection, but that temperature, food quantity, and the interaction had a significant effect on dissemination. We also measured wing length and CHIKV body titer from a subset of freshly engorged mosquitoes from each temperature-food level treatment to determine if the amount of virus ingested from the infectious blood meal was correlated with mosquito body size. We found that wing length was positively correlated with the initial quantity of virus ingested, but significant wing length - infection correlations disappeared after the extrinsic incubation period. This study suggests that larval environmental variables are important in shaping vector-viral interactions and that mosquito size alone may not be a good predictor of viral susceptibility.

79. Effect of two vector species on arbovirus transmission

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Many mosquito-borne arboviruses have more than one competent vector. These vectors are likely to have different spatial and seasonal distributions, and interact with vertebrate hosts differently. The presence of multiple vectors for a particular virus at one location over time will influence the epidemiology of the system, and could be important in the design of intervention strategies to protect particular hosts. A simulation model of West Nile and St. Louis encephalitis viruses and *Culex nigripalpus* was expanded to consider two vector species. If the vectors differed only in their seasonal abundance, the presence of two vectors affected the dynamics and often resulted in multiple epidemic peaks of transmission. The abundance pattern based on *Cx. nigripalpus* dominated the system and was a key factor in generating epidemics in the wild bird population. The day the virus was introduced into the system was critical in determining how many epidemic peaks were observed and when the first peak occurred. Other aspects of vector competence and the differences between the two vector species also affected the likelihood of epidemics. The implications of these results for assessing the relative importance of different vector species are discussed.

80. Sand Fly (*Phlebotomus papatasi*) Research Initiative at USDA Center for Medical, Agricultural and Veterinary Entomology (CMAVE) in Support of the Deployed War Fighter Protection (DWFP) Research Program

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The Deployed War-Fighter Protection (DWFP) Research Program encompasses multiple research directives aimed at reducing morbidity, mortality and irritation by preventing and controlling insect pests and vectors of disease pathogens. With thousands of soldiers deployed in Afghanistan and Iraq, combating sand flies such as *Phlebotomus papatasi* which is a vector of Leishmaniasis has become a military priority. As a result, the Armed Forces Pest Management Board (AFPMB) tasked the Mosquito and Fly Research Unit at CMAVE to establish a colony of *Phlebotomus papatasi* in order to conduct research aimed at protecting deployed soldiers. Acquiring this colony involved development of specific handling and containment protocols, significant upgrades to the USDA-CMAVE Quarantine Facility, and a permit issued by the Florida Division of Agricultural and Consumer Services. Building the insect colony required months of cultivation to achieve a critical mass of insects capable of supporting a research program. The successful colonization of *P. papatasi* facilitates laboratory research projects conducted by USDA-CMAVE scientists to directly address one of the U.S. military's most important vector-borne disease problems.

81. Overview of Deployed War Fighter Protection (DWFP) Research Program Activities at the USDA Center for Medical, Agricultural and Veterinary Entomology.

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The Deployed War-Fighter Protection (DWFP) Research Program is a Department of Defense (DOD)-sponsored program administered by the Armed Forces Pest Management Board (AFPMB). The AFPMB is tasked to develop new management tools against vector and pest species that transmit vector-borne pathogens or annoy deployed soldiers. The DWFP program reinvigorates an historic and mutually beneficial partnership between DOD and USDA by funding Agricultural Research Service scientists to provide support for military preventive medicine. To further advance DWFP objectives, Center for Medical, Agricultural, and Veterinary Entomology (CMAVE) scientists have established collaborations with multiple federal, military, commercial, and university researchers. The three pillars of DWFP research include the development of novel insecticide chemistries or formulations, the development of new personal protection equipment and devices that effectively prevent mosquito and fly bites, and the development of new or improved insecticide application technologies.

82. Activities of the Mosquito Fly Research Unit; Center for Medical, Agricultural and Veterinary Entomology; Agricultural Research Service, USDA, Gainesville, Florida

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The USDA Mosquito and Fly Research Unit is a unique resource in the United States and internationally. For many years, it has had important national and international roles in studying and developing new and innovative methods for controlling blood-sucking insects and flies. This research has resulted in improved management technologies for insects that are pests or transmit disease agents (such as those that cause West Nile fever, dengue, and malaria) to humans and animals worldwide. The Unit's research mission includes the study and development of novel and efficient insect trapping systems, biologically-based control technology, and host protection methods for use against insect pests of medical and veterinary importance. In this research, the Unit has worked closely with mosquito control and public health specialists, researchers, and industry partners to the ultimate benefit of consumers in the U.S. and internationally.

83. Incorporating retrospective clustering into a prospective CUSUM methodology: Evaluating the effects of disease expectation

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The pathogen *Bacillus anthracis*, which is the causative agent of anthrax, is still a problematic zoonotic disease in livestock and humans in many parts of the world, including Kazakhstan. In order to successfully manage biological threats to public and veterinary health such as anthrax it is crucial to identify deviations in a disease status. This study analyzes the retrospective spatial clustering of anthrax in Kazakhstan from historical livestock records, during the time period 1960-2006, using the Local Moran's I statistic and combines this with a CUSUM methodology to examine the affects selecting a baseline rate of disease may have on the ability to successfully detect the emergence of spatio-temporal clusters. Three methods for deriving baseline rates were employed in the CUSUM analysis; a standard z-score calculation, AVG, based on the number of cases in a rayon of Kazakhstan for a given year compared back to the global average, a spatially weighted z-score method, LISA, taken from Local Moran's I calculations for rayons in a given year and a moving window average method, MWA, which derives z-scores by comparing the number of outbreaks in a given year to an aggregation of previous years. The results show that the selection of base line rate of disease in instances where there is unknown population has a significant effect on the ability to detect the onset of clusters both in space and in time.

84. Modeling the potential geographic distribution of Bacillus anthracis under multiple climate change scenarios for Kazakhstan

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Anthrax, caused by the spore-forming bacterium *Bacillus anthracis*, is a zoonotic disease that persists throughout much of the world in livestock, wildlife, and secondarily infects humans. This is true across much of Central Asia, and particularly the Steppe region, including Kazakhstan. While recent efforts have described the potential geographic distribution of environments that can support *B. anthracis* spore survival across Kazakhstan under current climatic conditions, there is no information available on possible changes in the organism's distribution in 2050. This study employed ecological niche modeling to model the current and future geographic distribution of *Bacillus anthracis* in Kazakhstan based on the A2 and B2 IPCC SRES climate change scenarios. Future models suggest large areas predicted under current conditions may be reduced by 2050 with the A2 model predicting 16% loss and the B2 model predicting 32% loss respectively, while very small areas of habitat expansion were predicted by both models. Greater areas of habitat loss are predicted in the southern regions of Kazakhstan by both models, while moderate habitat loss is also predicted in the northern regions by the B2 model. Anthrax disease control relies mainly on livestock vaccination and proper carcass disposal, both of which require adequate surveillance. Interestingly, results also suggest habitat reduction may have the corresponding evolutionary consequence of a loss in genetic diversity of *B. anthracis* within the A1a and A3b genetic clusters by 2050. While speculative, contemplating future changes in livestock distributions and *B. anthracis* spore-promoting environments can be useful for establishing future surveillance priorities.

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