



COLLEGE OF VETERINARY MEDICINE
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

Welcome to the Tenth Annual Conference on New & Reemerging Infectious Diseases

April 19-20, 2007

College of Veterinary Medicine at the University of
Illinois at Urbana-Champaign

*Celebrating ten years of
infectious disease research excellence*

SCHEDULE

THURSDAY, APRIL 19, 2007

Opening Address: - 5:30 pm - 100 Large Animal Clinic (FREE and Open to the Public)

Marta Guerra, DVM, MPH, Ph.D., Dipl. ACVPM (opening speaker)

Senior Staff Veterinary Epidemiologist, Centers for Disease Control and Prevention

Presenting: *"Investigations and responses at CDC: epidemiology in action"*

FRIDAY, APRIL 20, 2007 - 2271C Veterinary Medicine Basic Sciences Building

8:00-8:30 am - Registration in main lobby of Veterinary Medicine Basic Sciences Building

8:30-8:45 am - Welcome and opening remarks

8:45-9:30 am - Douglas Goodin, Ph.D.

Director, Remote Sensing Research Laboratory, Department of Geography, Kansas State University

Presenting: *"Landscape hierarchy and spatial scale: a framework for the ecology of zoonotic disease"*

9:30-10:15 am -Uriel Kitron, Ph.D.

Professor, Department of Pathobiology, University of Illinois at Urbana-Champaign

Presenting: " Environment, Change and Disease: Vector-borne Zoonoses

10:15-10:30 am - Break

10:30 - 11:15 am - Helen Jost, Ph.D.

Department of Veterinary Science and Microbiology, University of Arizona

Presenting: *"The unusual toxins of Arcanobacterium haemolyticum"*

11:15-12:00 pm - J. Stephen Dumler, MD

Division of Medical Microbiology, Department of Pathology, The Johns Hopkins Medical Institutions

Presenting: *"Novel pathogens, novel pathogenetic mechanisms: Anaplasma phagocytophilum"*

12:00-1:30 pm - Lunch and poster viewing - Atrium, second floor Veterinary Medicine Basic Sciences Building

1:30-2:15 pm - Tamara Maier, Ph.D.

Department of Microbiology and Molecular Genetics, Medical College of Wisconsin

Presenting: *"Analysis of Francisella tularensis Himar1-based transposon mutants defective for replication in macrophages"*

2:15-3:00 pm - Roberto Docampo, MD, Ph.D.

Sanford Orkin Eminent Scholar, Department of Cellular Biology, University of Georgia

Presenting: *"Novel targets for the treatment of Trypanosomiasis"*

3:00-4:00 pm - Poster Reception

ABOUT THE CONFERENCE

The Annual Conference on New and Re-emerging Infectious Diseases brings together national and international experts whose research interests range from avian influenza to West Nile virus. This two day, on-campus event features lectures, posters and a keynote presentation that is free and open to the public. <http://www.cvm.uiuc.edu/idc>

Because of the interdisciplinary nature of the field of emerging infectious diseases and microbial pathogenesis, the Conference organizers strive to include a broad spectrum of topics presented by a diverse selection of experts in the fields of Biochemistry, Cell Biology, Epidemiology, Immunology, Microbiology and Molecular Genetics. Speakers are a balanced mix of established and young investigators from national and international institutions. Previous conferences included speakers from Argentina, Belgium, Germany, Italy, Spain and Brazil, as well as researchers who have conducted fieldwork in Asia, Africa and South America.

The Conference brings together a large diverse group of scientists from the University of Illinois, other Midwest universities, as well as other universities in this country and abroad, fostering scientific and academic interactions to further our understanding of microbial pathogenesis and emerging infectious diseases. Because of the recognition that this conference has achieved at the national level, supporting this Conference once again at the University of Illinois will reaffirm our role as leaders in the field. It is an ideal opportunity to showcase the University and its effort in global health.

THE CENTER FOR ZOOSES RESEARCH

CZR LEADERSHIP

Dean Herbert E. Whiteley; Associate Dean Edwin Hahn, ex-officio; Co-directors, Dr. Uriel Kitron and Dr. Brenda Wilson; Scientific Steering Committee; Campuswide Advisory Board

Contact: Debra Domal, Program Coordinator at 217/265-8511 or domal@uiuc.edu or visit <http://www.cvm.uiuc.edu/czr>

HIISTORY OF CZR

- Established by the Board of Trustees of the University of Illinois on January 20, 1960
- Vibrant research and collaboration focus for several years through the 1970's
- Rejuvenated in the late 1990's by establishment of an annual New and Re-Emerging Infectious Disease Conference and funded research projects
- Reorganized in 2002 - Venture tech funds, advisory board, web site, GIS lab

CZR TODAY

Worldwide attention to infectious diseases, esp.: emerging diseases, many of them zoonoses; food borne pathogens, food safety and antibiotic resistance; Biodefense and bioterrorism; Emergency preparedness

MISSION

To promote and develop:

- collaborative work among faculty from CVM, rest of UIUC and other institutions worldwide in an integrated dynamic program.
- synthesizing approach to zoonoses and infectious disease research based on the unique expertise in veterinary and medical research from the molecular to the ecosystem level.
- dissemination of information concerning zoonoses research through organization of conferences, seminars, and publications in various media
- training grants to attract top graduate students, post-doctoral trainees and visiting scientists.
- collaborative efforts and service to the Illinois Departments of Public Health and Agriculture.
- interest and awareness from UIUC faculty and administration about ongoing research on infectious diseases and food safety and building of biocontainment facility
- recognized research and training center by international organizations.

STATEWIDE MEMBERSHIP

Illinois Department of Public Health (IDPH), Illinois Department of Agriculture (IDA), Illinois Natural History Survey (INHS)

INTERNATIONAL COLLABORATION

Argentina (Chagas), Brazil, Canada, Chile, Finland, France, Germany, Great Britain, Mexico, Italy. Kenya (malaria, schistosomiasis), Sweden, Trinidad (dengue, malaria), Uganda (viral disease in primates), Venezuela

RECENT AND UPCOMING ACTIVITY

- Co-sponsor of the NCSA Infectious Disease Informatics Workshop, September 7-8, 2006
- Tenth Annual Conference on New and Re-Emerging Infectious Diseases, April 19-20, 2007
- 2007 Veterinary Student Education in Infectious Diseases Summer Training Program, funded by NIH and Merck-Merial)

SPEAKER ABSTRACTS

MARTA GUERRA, DVM, MPH, PhD, Dipl. ACVPM

Investigations and Responses at CDC: Epidemiology in Action

The Centers for Disease Control and Prevention has as its mission to promote health and quality of life by preventing and controlling disease, injury, and disability. It partners with local and state health departments, other federal agencies, international organizations, and ministries of health and agriculture of countries to surveil and investigate health problems, and conduct research on diseases and conditions of public health importance. Since 2000, CDC has been involved in investigations of emerging and reemerging diseases such as Ebola, Severe Acute Respiratory Syndrome (SARS), monkeypox, and avian influenza. CDC participates in ongoing programs such as the eradication campaign against polio involving numerous global partners, and health evaluations of immigrant and refugee populations. Disaster response, whether natural or man-made, is also a priority of CDC's mission, and has included conducting syndromic surveillance after the 9/11 terrorist attacks and assisting states in many public health capacities after Hurricane Katrina. These investigations and responses provide the opportunities to develop and advocate sound public health policies, implement prevention strategies, and foster safe and healthful environments for the future.

DOUGLAS GOODIN, Ph.D.

Landscape Hierarchy and Spatial Scale: A Framework for the Ecology of Zoonotic Disease

Landscape epidemiology, the study of spatial patterns of disease and disease risk arising from underlying environmental causes, has benefited greatly from the theoretical perspective of landscape ecology, another discipline that attempts to understand the relationship between spatial pattern and ecological process. For example, landscape epidemiologists have made use of ecological concepts such as fragmentation and to understand the geographical distribution and spatial dynamics of disease vectors. One theoretical aspect of landscape ecology that is increasingly being used in disease applications is the concept spatial hierarchy. Hierarchy in ecology is a multifaceted theory incorporating elements of non-linear dynamics and complexity. The fundamental concept of hierarchy theory is that processes occurring at finer scales (i.e. "lower" in the spatial hierarchy) are constrained by processes at higher levels. Hierarchical levels can also be distinguished by the rates at which processes occur – faster at finer scale, slower at coarser ones. Hierarchy theory in ecology arose as a response to the need for a rigorous method of handling middle-number systems – that is, systems whose components are too few to treat statistically but too many to address with classical Newtonian mathematics. Hierarchy provides a framework by which these middle number systems can be decomposed into a series of manageable units, whose environmental drivers can be characterized by the scale (and thus the rate) at which they occur. Such a framework is amenable to the study of landscape epidemiology, since the linkages between environmental factors and disease are neither univariate nor confined to a specific spatial scale. In this paper, I will discuss some aspects of spatial hierarchy in the context of an ongoing project studying hantavirus dynamics in Paraguay. I will discuss how hierarchical concepts have been used to interpret observed results, and how hierarchy theory is being used to frame new research questions.

B. HELEN JOST, Ph.D.

*The unusual toxins of *Arcanobacterium haemolyticum**

Arcanobacterium haemolyticum is an emerging pathogen, and is a significant cause of bacterial pharyngitis in adolescents and young adults. It is under-recognized as an etiologic agent, due to clinician and diagnostic laboratory unfamiliarity with the organism, and its slow growing, fastidious nature. The

initial pharyngitis can progress to more invasive disease, such as osteomyelitis, meningitis and endocarditis, however, little is known about the disease pathogenesis of *A. haemolyticum* infections. *A. haemolyticum* expresses an unusual phospholipase D (PLD) with amino acid similarity to recluse spider venom. We have cloned and purified recombinant, HIS-tagged PLD. Addition of HIS-PLD to HeLa cells results in significant remodeling of the host membrane architecture, as measured by lipid raft formation. Furthermore, a *pld* knockout mutant is 51% reduced in its ability to adhere to and invade HeLa cells. We have also identified a member of the cholesterol dependent cytolysin (CDC) family, arcanolysin (ALN), expressed by *A. haemolyticum*. ALN is a unique member of the CDC family, in that purified, recombinant toxin has a pronounced human cell preference and is only partially inhibited by cholesterol. In addition, ALN has an N-terminal extension, similar to the translocon domain seen only in the CDC streptolysin O. An *aln* knockout mutant has a significant growth defect compared to the wildtype, which is related to the ability to acquire nutrients from within red blood cells. An *aln* knockout mutant is also significantly reduced for adhesion to HeLa cells as compared to the wildtype. Both PLD and ALN are membrane active toxins and contribute to the adhesion and invasion of *A. haemolyticum* to host cells. Therefore, we hypothesize that these molecules contribute to the pathophysiology of invasive *A. haemolyticum* infections.

J. STEPHEN DUMLER, M.D.

Novel pathogens, novel pathogenetic mechanisms: *Anaplasma phagocytophilum*. J. Stephen Dumler,

Anaplasma phagocytophilum is a tick-transmitted obligate intracellular bacterium that infects neutrophils and myeloid cells in the mammalian host. Since discovery as a human pathogen in 1990, it has emerged to become the 3rd most common vector-borne infection in North America. In humans, the infection results in a febrile condition varying from mild to fatal, mostly as a result of inflammation. Since the neutrophil is an exceedingly uncommon host for intracellular bacterial parasites, we hypothesized that *A. phagocytophilum* uses novel mechanisms to subvert host cell function, and that these changes ultimately contribute pathogen survival and disease. In vitro studies have clearly shown that neutrophil infection by *A. phagocytophilum* induces significant functional changes including deactivation of antimicrobial mechanisms (phox dysregulation, down regulation of antimicrobial proteins), loss of endothelial cell binding and transendothelial cell emigration, and promotion of persistent proinflammatory responses (degranulation, chemokine expression, and delayed apoptosis). The net effect is continued inflammatory recruitment of new hosts and lack of microbicidal mechanisms, rendering a higher proportion and prolonged presence of pathogen accessible to tick bites. Surprisingly, many of the alterations have their basis in altered transcription in the host. We discovered a novel protein call AnkA in *A. phagocytophilum* that is translocated from the bacteria within a host vacuole into the host nucleus, where it complexes with heterochromatin. Emerging investigation shows that AnkA is largely responsible for many of the host transcriptional changes by directly binding to regulatory regions of the DNA. This binding leads to altered eukaryotic histone structure, such as histone acetylation, and potentially recruitment of some transcriptional activators or repressors to multiple loci in the myeloid cell chromosomes. These early findings illustrate how the investigation of agents that are adapted to unusual niches provides a comprehensive understanding of how pathogens condition hosts to become permissive to their needs. As with those who seek potential therapeutics from microbes in hot springs, the arctic, or the jungle, the study of novel intracellular pathogens could provide important clues for treatment of the infection and for new therapies for even non-infectious diseases.

TAMARA MAIER, Ph.D.

Analysis of Francisella transposon mutants defective for replication in macrophage

Francisella tularensis is the etiologic agent that causes tularemia in humans and animals. It is recognized as a potential agent of bioterrorism due to its low infectious dose and multiple routes of entry. *F. tularensis* replicates within several cell types including macrophages, hepatocytes and epithelial cells, eventually causing cell death by inducing apoptosis. In this study, we developed genetic approaches to screen for virulence factors as potential targets for therapeutic or vaccine development. A *Himar1*-based transposon system was constructed, optimized and used to mutagenize *F. tularensis* strain LVS. A library of ~7000 insertion mutants was screened in J774A.1 macrophages for a reduction in the cytopathic effects based on a simple cell retention assay. Stage 1 screening was performed in 96 well format with an estimated MOI of ~50-200. Mutants (441) with significant cell retention compared to the parent were retested in 24 well format with a defined MOI of 100. The 140 candidates that retained a significant macrophage monolayer 2 days post-infection were chosen for further study. To focus the mutant pool towards genes related to pathogenesis, the remaining candidates were assessed for growth in complex Mueller Hinton (MH) and Chamberlain's defined broth medium (CDM). Analysis of data from combined *in vitro* and cellular assays narrowed the field to 92 strains. We rescued and sequenced flanking DNA corresponding to 77 of these insertions. Our screen identified genes involved in a variety of processes, including transport, metabolism, and cell wall and membrane biogenesis, that could potentially encode required gene products for the replication of *Francisella* in murine macrophages.

ROBERTO DOCAMPO, M.D., Ph.D.

Novel targets for the treatment of Trypanosomiasis

Chemotherapy of trypanosomiasis is unsatisfactory because of toxicity and lack of efficacy of existing drugs, and it is important to identify structures and metabolic processes in trypanosomes that might be potential targets for drug development. In the last few years we have been investigating two structures (the acidocalcisome and the contractile vacuole) and one metabolic pathway (the isoprenoid pathway) for target identification. Acidocalcisomes are dense, acidic organelles with a high concentration of phosphorus present as pyrophosphate and polyphosphate complexed with calcium, and other cations. The acidocalcisome membrane contains a number of pumps (Ca²⁺-ATPase, V-H⁺-ATPase, H⁺-PPase), exchangers (Na⁺/H⁺, Ca²⁺/H⁺), and channels (aquaporins), while its matrix contains enzymes related to pyrophosphate and polyphosphate metabolism (1). Acidocalcisomes are functionally linked to the contractile vacuole complex in *T. cruzi* (2, 3). Ablation by RNAi of several acidocalcisome proteins of *Trypanosoma brucei* has demonstrated an essential role of acidocalcisomes in osmoregulation and intracellular pH regulation (4-6).

Isoprenoids are an extensive group of natural products with diverse structures consisting of various numbers of five carbon isopentenyl pyrophosphate (IPP) units. Beginning with dimethylallyl diphosphate (DMAPP), successive additions of IPP, give geranyl diphosphate (GPP, 10 carbons), farnesyl diphosphate (FPP, 15 carbons), geranylgeranyl diphosphate (GGPP, 20 carbons) and longer chain products. The enzyme farnesyl diphosphate synthase (FPPS) plays a central role by producing FPP, an important precursor of sterols, dolichols, ubiquinones, and prenylated proteins. FPPS forms FPP by the sequential condensation of DMAPP with two molecules of IPP. Bisphosphonates, analogous of pyrophosphate used in the treatment of osteoporosis and other bone diseases, target the mevalonate pathway by inhibiting FPPS. In previous work we cloned sequenced, and expressed the genes encoding the FPPS of *Trypanosoma cruzi* (7) and *T. brucei*, (8) and demonstrated the inhibition of their protein products by bisphosphonates as well as their 3D structure bound to different bisphosphonates (9, 10). More recently we identified and characterized a solanesyl diphosphate synthase involved in the synthesis of the solanesyl group necessary for the synthesis of

ubiquinone 9 (11). This enzyme is also potently inhibited by bisphosphonates and represents a novel target for the treatment of trypanosomiasis.

References

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POSTER ABSTRACTS

Calcineurin-independent inhibition of 3T3-L1 Adipogenesis by *Pasteurella multocida* toxin: Suppression of Notch1, stabilization of β -catenin and Pref1*

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Pasteurella multocida toxin (PMT) is a potent mitogen and a specific activator of Gq-dependent signaling pathways. PMT impairs osteoblast differentiation and causes bone loss and fat reduction *in vivo*. We examined the effect of PMT on cell signaling pathways involved in 3T3-L1 adipocyte differentiation. We demonstrate that PMT treatment prior to or together with differentiation induction factors inhibits adipogenesis and prevents upregulation of important adipocyte markers – PPAR α and C/EBP α . Moreover, PMT completely downregulates PPAR α and C/EBP α expression in mature adipocytes. Differentiation of preadipocytes into adipocytes requires the suppression of Pref1 and Wnt signaling, along with the degradation of β -catenin. PMT prevents downregulation of Pref1 and β -catenin under differentiation-inducing conditions. In addition, PMT treatment downregulates expression of Notch1, a protein responsible for cell fate decision and implicated in regulation of adipogenesis in 3T3-L1 cells. PMT action on adipogenesis was not reversed by cyclosporin A, an inhibitor of G β q-PLC-calcium-dependent calcineurin activation. Our study demonstrates that the effect of PMT on Pref1/PPAR α /C/EBP α expression and adipogenesis does not occur just through activation of the G β q-calcium-calcineurin pathway, but involves Wnt/ β -catenin and Notch1 signaling pathways, two signaling pathways strongly linked to cancer predisposition, neurological and immunological dysfunctions, and fat and bone development.

Spatial and temporal analysis of St. Louis and West Nile Virus encephalitis in greater Chicago, Illinois, in 1975 and 2002.

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Until recently, St. Louis encephalitis (SLE) and LaCrosse encephalitis were the most common arboviral infections reported annually in United States. Since 1999, West Nile Virus (WNV) has spread from New York west and has rapidly become the predominant arboviral agent of encephalitis in the U.S.

In Illinois, two major outbreaks of SLE in 1975 and WNV in 2002 have occurred in Cook and Dupage counties, encompassing the City of Chicago and surrounding suburbs. To compare the epidemiology of these two diseases, we analyzed the spatio-temporal clustering of human cases of SLE in 1975 and WNV in 2002 using a geographic information system (GIS) and spatial statistics. Similarities and differences in environmental and demographic features were also studied. Case data were obtained from the Illinois Department of Public Health and mapped as points for the two counties. Spatial analyses were performed using human cases both as point data and summarized for 1.8 km hexagons.

Second-order spatial analysis of the case distribution detected clustering at all distances for both the SLE (1975) and the WNV (2002). For grouped data, global Moran's I detected a significant cluster

for both diseases, with a higher value for WNV. Local spatial statistics (Getis/Ord $G_i^*(d)$ and Anselin's Local Moran test) identified one overlapping hot spot in southern Cook county for both the SLE (1975) and WNV (2002) outbreaks and two additional clusters in northern Cook county for WNV only.

SLE cases in 1975 occurred between July 18th and October 1st with the mode of the epidemic curve at the beginning of September. The WNV outbreak in 2002 started earlier (July 10th) and ended one week later, with the mode in the last week of August. Significant space-time interaction between cases was detected (using Jacquez's k-nearest neighbor test) only for SLE cases. This space-time clustering was evident primarily during the last three weeks of the outbreak (September 11th - October 1st, 1975).

These are the initial results of a larger study to evaluate the similarities and differences between SLE and WNV to better understand their epidemiology and provide tools for prediction of future patterns of potential WNV outbreaks.

Molecular determinants of *Pasteurella multocida* toxin entry and translocation into host cells

Yuka Bannai*, Mengfei Ho, Tracy Chong, Leila Aminova, and Brenda A. Wilson
University of Illinois

Atrophic rhinitis (AR) is a disease of the upper respiratory tract found most commonly in pigs and other domestic animals. AR is associated with toxigenic strains of *Pasteurella multocida* that produce a major virulence factor, an intracellularly-acting mitogenic toxin called PMT. Most PMT research has focused on understanding the involvement of the molecular intracellular events, however the entry determinants of the toxin action (receptor binding, entry pathway and translocation) are not yet known. Functional and structural studies of PMT suggest that the N-terminus contains the receptor binding domain and the C-terminus contains the intracellular activity domain. In addition, PMT is thought to have multiple binding determinants. Our preliminary studies indicate that an N-terminal deletion mutant enters and remains in the endosome, which suggests it lacks a signal(s) to translocate into the cytosol or to travel further down the entry pathway. Other preliminary results suggest that full length PMT goes as far as the ER. Studies presented here are designed to address the intracellular trafficking pathway of PMT. To determine if PMT is an AB toxin that utilizes retrograde transport to enter the cytosol of mammalian cells, a series of deletion mutants of PMT, as fusions with GFP, will be used for fluorescence microscopy studies where each cellular organelle is marked with red fluorescence and colocalization with toxin is observed.

Identification of virulence factors encoded in the genome of the human pathogen *Arcanobacterium haemolyticum*.

Stephen J. Billington, Erynn A. Lucas and B. Helen Jost. Department of Veterinary Science and Microbiology, University of Arizona.

The Gram positive bacterium *Arcanobacterium haemolyticum* is a underrecognized cause of human pharyngitis and skin infections. In addition, this organism can invade into deeper tissues resulting in more severe infections, such as meningitis, pneumonia and osteomyelitis. As an initial step toward understanding the pathogenesis of *A. haemolyticum* infections, a draft genome of *A. haemolyticum* ATCC 9345 was determined by pyrosequencing to >20X coverage. GenomeWalker experiments are being used in an attempt to close the genome, which is currently represented by 64 contigs. The *A. haemolyticum* ATCC 9345 genome is ~1.95Mb with a 53.1% G+C content and contains no plasmids. Automated annotation identified ~1,870 open reading frames, including those for two unusual toxins; a previously identified phospholipase D (PLD) and a novel cholesterol-dependent cytolysin, arcanolysin (ALN). ALN has a high degree of similarity to pyolysin, the CDC of the related animal pathogen *Arcanobacterium pyogenes*, but is divergent from the rest of the CDC family. In addition, ALN contains an N-terminal amino acid extension which is not part of the four domain structure of CDCs. This extension which contains a PEST-like sequence, may be important for the function of this toxin. Both PLD and ALN affect the ability of *A. haemolyticum* to adhere to host epithelial cells, possibly through the actions of these toxins on the host cell membrane. Genes for several putative adhesins have also been identified in the genome sequence, including those for two collagen-binding proteins, two neuraminidases, and three fimbrial gene operons, all of which may play a role in disease pathogenesis. Determination of the draft genome of *A. haemolyticum* allows a more targeted approach of studies aimed at better understanding the pathogenesis of infections caused by this organism.

***Bacillus anthracis* *racE1* and *racE2* encode functional glutamate racemases with predicted differences in their fine active site features.**

Dylan Dodd¹, Joseph G. Reese², Craig R. Louer¹ Jimmy D. Ballard³, M. Ashley Spies², and Steven R. Blanke¹

From the ¹Department of Microbiology and the Institute for Genomic Biology and the ²Department of Biochemistry, University of Illinois, Urbana, Illinois, USA and the ³Department of Microbiology and Immunology, The University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA.

For many bacteria, the enzyme glutamate racemase is essential for viability, and thus represents an attractive target for the development of new chemotherapeutic agents. *Bacillus anthracis* harbor two putative glutamate racemase genes, *racE1* and *racE2*, but little is known about the importance or role of these genes. Here, we investigated whether both *racE1* and *racE2* encode functional glutamate racemases, and, if so, whether the proteins are similar or highly divergent targets for inhibitor development. To probe the biochemical properties of RacE1 and RacE2 in a highly defined cell-free system, we cloned and expressed *racE1* and *racE2* in *E. coli*. Analysis of purified recombinant RacE1 and RacE2 revealed that both proteins catalyzed the reversible racemization of L-glutamate to D-glutamate with similar, but not identical, steady state kinetic properties. Analysis of the pH-dependence of L-glutamate racemization suggested that RacE1 and RacE2 both possess two titratable active site residues important for catalysis. Moreover, directed mutagenesis of predicted active-site residues, identified by homology modeling, resulted in complete attenuation of enzymatic activity in both RacE1 and RacE2, suggesting that both enzymes share common active site features. However, a detailed comparison of RacE1 and RacE2 homology models (based on *B. subtilis* RacE-

D-glutamate) suggests that several conserved active site residues may be positioned differently relative to bound D-glutamate. The predicted differences in the fine active site features suggest that effective attenuation of glutamate racemase activity in *B. anthracis* may require chemotherapeutics capable of inhibiting two enzymes with similar but slightly divergent active site features.

Environmental Regulation of the SPI1 Type Three Secretion System in *Salmonella enterica* serovar Typhimurium

Jeremy R. Ellermeier¹, James M. Slauch^{1,2}

Department of Microbiology¹ and College of Medicine² University of Illinois, Urbana

Salmonella enterica serovar Typhimurium invades non-phagocytic intestinal epithelial cells using a Type Three Secretion System (T3SS) encoded on *Salmonella* Pathogenicity Island 1 (SPI1). HilC, HilD, and RtsA are members of the AraC family of transcriptional activators, each of which can independently activate expression of SPI1 genes by binding upstream of the master regulatory gene *hilA* to induce its expression. HilA, in turn, activates the SPI1 T3SS structural genes. We recently proposed a model in which HilC, HilD, and RtsA form a feed forward regulatory loop controlling expression of *hilC*, *hilD*, and *rtsA*. Thus, production of the SPI1 T3SS is controlled by the combined action of HilC, HilD and RtsA. Our goals are to characterize the role of HilC, HilD, and RtsA in SPI1 regulation and to understand the overall regulatory circuit that controls this important virulence function. Current focus is on understanding how environmental signals feed into the system. We show that high levels of iron induce expression of *hilA* and a deletion of *fur* (*ferric uptake regulator*) causes a 6 to 7 fold decrease in *hilA* expression. Fur regulation of SPI1 is dependent on HilD and controls *hilD* at the post-translational level. Fur “activation” of *hilD* is presumably via repression of an inhibitor of HilD. This regulation is not mediated through the Fur regulated small RNAs FrrA (RyhB) and FrrB, which redundantly control expression of *sodB*, or the known SPI1 repressor Hile. Thus, iron is potentially an important trigger in the regulation of SPI1.

Comparative microbial ecology of gastrointestinal bacteria in wild primates from Kibale National Park, Uganda

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Limited knowledge is available about the gastrointestinal biota of wild animals or how diet affects the genetic and phenotypic characteristics of their commensal gastrointestinal bacteria. Populations of non-human primates in western Uganda that exhibit differences in gut physiology and diet were studied. *Escherichia coli* and *Enterococcus* spp. were targeted for collection because these bacteria are part of the normal flora of the animals yet they differ in cellular composition and have the potential to be pathogenic. Bacterial isolates were collected non-invasively from populations of red colobus monkeys (*Ptilocolobus tephrosceles*), black-and-white colobus monkeys (*Colobus guereza*), red-tailed guenons (*Cercopithecus ascanius*), chimpanzees (*Pan troglodytes*) and people (*Homo sapiens*) living in forest habitats in and near Kibale National Park. DNA fingerprints of bacterial isolates were generated using rep-PCR, which can discriminate among closely related bacterial isolates. The “disk diffusion” method was used to test for resistance to antibiotics commonly used by the local human population.

Tannin-tolerance is being evaluated by growing bacteria in the presence of varied concentrations of black wattle tannin. Comparison of bacterial genotypes showed that colobus monkeys, which have ruminant-like stomachs and eat a folivorous diet, had less diverse bacteria than guenons or chimpanzees, which are hind-gut fermenting omnivores that primarily consume fruit. Antibiotic resistance testing showed that monkeys living in disturbed forest fragments encroached upon by local people have more antibiotic resistant isolates than do monkeys and apes that live deep within the park. Ongoing studies will determine whether tannin-tolerant bacteria are isolated more frequently from primates that consume a high level of tannins in their diet. Further research will be required to identify whether a molecular and/or ecological link exists between antibiotic resistance and tannin tolerance in these species.

Assembly and Activity of Heterologous A and B Fragments of Cytotolethal Distending Toxin

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The cytolethal distending toxins (CDTs) comprise a family of genotoxins that, analogous to other intracellular-acting bacterial toxins, possess an “AB” architecture, where the B fragment facilitates the entry of the catalytic A fragment into sensitive cells. Although CDTs are generated by several Gram-negative pathogens that colonize seemingly unrelated niches within the host, the mechanism by which each toxin recognizes and binds to sensitive cells within its specific colonization niche has not been investigated. To explore the functional interrelatedness between CDTs, we generated hybrid toxins comprised of heterologous combinations of the A and B fragments produced from *Haemophilus ducreyi* (Hd), *Actinobacillus actinomycetemcomitans* (Aa), and *Campylobacter jejuni* (Cj). Each of these hybrid toxins was tested for the capacity to assemble into a stable ternary complex, as scored by size exclusion FPLC. In addition, each hybrid toxin was assessed for the capacity to intoxicate HeLa cells resulting in the induction of G2/M cell cycle arrest. These experiments revealed that heterotoxins formed from the A and B fragments generated from the closely related CDTs of Hd and Aa assembled into stable ternary complexes. Additionally, these hybrid toxins bound and entered cells, localized to the nucleus, and induced G2/M cell cycle arrest. In contrast, the A and B fragments of Cj formed detectable ternary complexes to differing degrees with heterologous A and B fragments from the more distantly related CDTs of Hd and Aa, indicating a possible instability of the heterotoxins in solution. Nonetheless, the B fragment from each toxin demonstrated the capacity to increase the sensitivity of cells to heterologous A fragments by approximately 2-4 orders of magnitude. These results indicate that the A and B fragments of different CDTs are able to functionally interact and intoxicate cells, even in the absence of detectable solution ternary complexes. These results suggest that the interactions between the A and B fragments during assembly of CDTs are complex, and imply a model where interactions with the surface of target cells might stabilize subunit interaction and promote toxin assembly.

Sphingomyelin: A novel VacA receptor important for cellular binding and intoxication.

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The *Helicobacter pylori* vacuolating cytotoxin (VacA) binds and enters host target cells by a poorly understood mechanism. In this study, we explored the relationship between VacA interactions with cell surface components and downstream steps of intoxication. We have identified that vacuolation activity of VacA is modulated specifically by sphingomyelin, since exogenous supplementation of sphingomyelin potentiates the activity of VacA and concurrently depletion of plasma membrane sphingomyelin using sphingomyelinase enzyme inhibits toxin activity. Further cell surface association and internalization of VacA into host cells are influenced by sphingomyelin in a manner correlating to its vacuolation activity thus suggesting the role of sphingomyelin on host cellular intoxication. Previous studies have identified receptor protein tyrosine phosphatases (α , β) as putative VacA receptors. VacA binding studies carried out using different cell lines, categorized on the basis of receptor identity, showed no correlation between receptor identity and extent of cell surface association of VacA. However sphingomyelinase pretreatment to these cell lines drastically reduced cell surface association of VacA independent of the presence of VacA receptors, thus highlighting role of sphingomyelin as VacA receptor/co-receptor. Sphingomyelinase pretreatment also decreases VacA partitioning into lipid rafts and internalization into host cells, independent of receptor protein tyrosine phosphatases (α , β). Collectively our data supports a model where plasma membrane sphingomyelin functions as a cellular receptor for VacA, which influences VacA partitioning into lipid rafts, internalization and subsequent cellular intoxication.

Characterization of the inhibitory effects of nisin against Bacillus anthracis.

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Lantibiotics, specifically nisin, have been utilized in the food industry world-wide since the 1970's as a preservative, preventing growth of spore-forming bacteria. Nisin has been shown to induce pore formation and inhibit cell wall biosynthesis via lipid II targeting in vegetative bacilli, and to inhibit outgrowth via an unknown mechanism in spores. Our lab is interested in elucidating the mode of action of nisin, specifically against *Bacillus anthracis*, in the inhibition of spore outgrowth and identify the target that is utilized for this inhibition. In characterizing the effects of nisin against *B. anthracis*, the minimum inhibitory concentrations (MIC) and minimum bacteriocidal concentrations (MBC) for nisin, a less active form of nisin, and nisaplin (a food-grade form of nisin) have been determined. The effect of nisaplin on the overall growth of the bacteria has been studied utilizing growth curves. Additionally, the effects of nisaplin on both the establishment of a membrane potential during germination and the propagation of a membrane potential during vegetative growth have been investigated. Fluorescence microscopy was utilized to study the association of rhodamine-labeled nisin to spores during the course of germination. These initial studies have shown that nisin has the ability to inhibit the growth of *B. anthracis* at micromolar concentrations, and nisaplin has the ability to inhibit both the establishment and propagation of a membrane potential. With the current threat of the use of pathogens as biological weapons and the onset of widespread antibiotic resistance among pathogens, new forms of antimicrobial agents must be utilized, and nisin provides a safe and effective option.

***Helicobacter pylori* VacA induced activation of cellular apoptosis in AZ-521 gastric epithelial cells**

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Helicobacter pylori VacA enters gastric epithelial cells, induces mitochondrial dysfunction, and causes cell death. However, the mechanistic relationship between VacA-induced mitochondrial dysfunction and cell death remain unclear. To better define the mechanisms underlying VacA induced cell death, we investigated the temporal consequences of intoxication. Within human-derived AZ-521 gastric epithelial cells, VacA induced extensive membrane remodeling leading to vacuolation and loss of mitochondrial membrane potential resulting in mitochondrial dysfunction, within 4 h of intoxication. Within 8 h, cell death markers were clearly absent, suggesting that vacuolation and mitochondrial dysfunction preceded cell death. Cell membrane permeabilization and phosphatidyl serine (PS) exposure significantly increased by 14 h, and continued to increase at 24 h and 48 h. However, significantly lower cytolysis compared to cells induced to undergo active necrosis, even after 48 h of intoxication, suggesting regulated and limited cellular damage. Caspase-3 activation and DNA fragmentation was apparent 24 h following intoxication. Membrane permeabilization and PS inversion was VacA dose dependent and significantly increased with VacA activation and the presence of NH₄Cl. A partial reduction in membrane permeabilization and PS inversion was observed with inhibition of caspase activity, suggesting the involvement of caspases in VacA induced cell death. Collectively, these data suggest that, VacA induces an early disruption of mitochondrial function, followed by activation of apoptotic machinery, resulting in caspase-3 activation, DNA fragmentation and changes in membrane properties, leading to cell death by caspase-dependent and independent programmed cell death mechanisms.

Brucellosis As A Major Emerging Disease In Korea.

I: Comparative Serology Of Human Brucellosis Cases

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Brucellosis is a recent emerging zoonosis in Korea, first diagnosed in cattle about 5 decades ago (Park *et al.*, 1959) and in humans (Park *et al.*, 2003). It had been previously predicted that this disease could turn into a major epidemic as food animal farming expands without any major regulatory change mandating an integrated approach to bovine brucellosis (Baek *et al.*, 2003), as opposed to the current official policy of “test and slaughter”. We advocate that Korea should take advantage of and emulate successful brucellosis eradication initiatives from Australia and New Zealand and to a limited extent, the USA. These countries found that incorporating vaccination into the eradication program was essential to a long-term national brucellosis control policy. Regulatory agencies in Korea have been struggling with the reality that the “test and slaughter” policy has had no significant impact on the prevalence of bovine brucellosis and human cases have escalated. More than 300 human cases have been reported between January 2005 and August 2006. In addition, during the same interval, more than 50,000 cattle have been slaughtered due to bovine brucellosis.

The socio-economic impact of this disease is particularly felt among the farmers, many of whom have been bankrupted by the epidemic. The scientific community appears frustrated by the apparent lack of political will to adopt control methods that exploit the availability of *Brucella abortus* strain RB51 vaccine. This strain does not confound serologic testing or cause any abortions when used in pregnant cattle (Poester *et al.*, 2006). Moreover, it is safe for humans who may become exposed to it during cattle vaccination campaigns.

We have evaluated various tests for their diagnostic value (specificity, sensitivity and predictive value). The key tests used are the tube agglutination test (TAT), Enzyme Linked Immunosorbent Assay(ELISA) and the 2-mercaptaethanol Agglutination Test (2-MAT). Maximum efficiency is achieved when these tests are performed in combination to varying degrees as may be indicated.

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Brucellosis as a major emerging disease in Korea.

ii: western blot analysis of human sera

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Brucellosis is a systemic zoonotic disease caused by the gram-negative bacilli of the genus *Brucella*. Severe complications including meningitis, meningoencephalitis, brain abscess, epidural abscess, meningovascular syndromes have been reported (Young 2000).

The most specific diagnostic method for brucellosis is the by the culture method, isolation, and identification of the causative organism. The literature indicates that almost all available serologic assays have been used for diagnosing brucellosis, illustrating that the ideal test has not yet been found.

We report on our efforts to evaluate the applicability of Western Blot Analysis to the diagnosis of human brucellosis in Korea. Prior to examination, test sera were analyzed by the tube agglutination test (TAT) and 2-mechaptoethanol test agglutination (2-ME-AT) in conjunction with clinical evaluation of the patients. Data from our observations in Korea demonstrated that the 58 Kd, 56 Kd, 45Kd, 43Kd, 36 Kd, and 3Kd are consistently observed in untreated cases. Following treatment, the 52 Kd, 45 Kd, and 43 Kd were found to persist even without any clinical symptoms consistent with brucellosis. Severe disease tended to be associated with the presence of very strong reactions to the 90 Kd, 62 Kd, 26Kd and 18 Kd. These data are preliminary but lay a foundation for a more extensive field evaluation study in Korea.

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Ecological and anthropogenic influences on patterns of parasitic prevalence in free-ranging howler monkeys

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Parasites play a central role in ecosystems, affecting the ecology and evolution of species interactions, host population growth and regulation, and community biodiversity. Howler monkeys are the most widespread non-human primates in South America, with 8 of 10 *Alouatta* species living in South America. We took a meta-analysis approach, integrating data from studies on wild *Alouatta caraya*, *A. seniculus*, *A. guariba*, and *A. belzebul*, to examine how various factors such as latitude, altitude, annual precipitation, continuous or fragmented forests, and degree of contact with human settlements affected parasite prevalence. We included in the analysis data on the prevalence of gastrointestinal parasites, blood parasites and ectoparasites. When all parasite types were analyzed together we found that type of human contact affected the prevalence of different parasites (Kruskal Wallis test, $P < 0.05$); the same trend was found when comparing only gastrointestinal parasites (Kruskal-Wallis test, $P < 0.05$). Levels of gastrointestinal parasite prevalence also varied according to degree of human contact ($\chi^2 = 18.53$, $df = 1$, $P < 0.05$). We found a negative relationship between prevalence and latitude, both considering all parasite species together ($N = 91$, $r = -0.26$, $P < 0.05$), and only gastrointestinal parasites ($N = 65$, $r = -0.33$, $P < 0.05$). Logistic regression models suggested latitude and altitude were mediators of the likelihood of having high or low parasitic prevalence (either higher or lower than 20%) ($P < 0.05$). Our general analysis suggests that the prevalence of parasites did not vary across fragmented and continuous forests ($P > 0.05$). Approximately 83% of gastrointestinal parasites, and 100% of blood-borne parasites found in howlers are found in humans. In sum, the results presented indicated that latitude, precipitation, altitude, and degree of human contact affected the prevalence of certain parasite species in howlers across South America. Our results provide a baseline for understanding causative factors for patterns of parasitic infections in wild primate populations and may alert us to eminent threats to primate conservation.

Bacterial toxin targeting mitochondria: The case of *Helicobacter pylori* VacA

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Helicobacter pylori, the causative agent of acute gastritis and duodenal ulcers, secretes a vacuolating cytotoxin (VacA) that induces cell death in mammalian cells by a mitochondrial dependent mechanism. We have previously shown that VacA enters cells and localizes to the mitochondria, suggesting that VacA may induce apoptosis as a result of directly interacting with this organelle. *Helicobacter pylori* vacuolating cytotoxin (VacA) localizes with mitochondria within intoxicated cells, and induces changes associated with permeabilization of mitochondrial membranes. However, the mechanisms underlying the capacity of VacA to interact with and modulate mitochondrial properties are poorly understood. Using cell free systems developed from gastric tissue and gastric cell lines; we demonstrated that VacA alone is sufficient to cause a reduction in transmembrane potential ($\Delta\psi_m$) of isolated mitochondria, suggesting that VacA interactions with mitochondria induce changes in mitochondrial function. Mitochondrial import assays revealed that a fraction of VacA is rapidly internalized (within 5 min) into mitochondria beyond the outer membrane. However, a fraction of VacA also remains on the outer membrane, indicating that VacA partitioning into mitochondria is complex. Fractionation experiments revealed that VacA is able to associate both peripherally and

integrally with mitochondrial membranes. Initial characterization of mitochondrial outer membrane components important for VacA interactions revealed that VacA binds both specifically and non-specifically to components on the surface of the mitochondrial outer membrane, including one or more proteinaceous components. Studies with mitochondria isolated from mutant forms of yeast *Saccharomyces cerevisiae* revealed that one or more import receptors on the surface of the mitochondrial outer membrane may facilitate VacA binding and internalization into mitochondria. Structure-function studies with recombinant VacA fragments revealed that a VacA amino terminal fragment comprising residues 1-311 is important and sufficient for binding and internalization into mitochondria. In contrast, a carboxyl-terminal fragment comprising residues 312-821 binds, but is not internalized into mitochondria. These data support a model where VacA binding and import into mitochondrial inner membrane facilitates the elaboration of a VacA intracellular activity. Importantly these results suggest that that some pathogens and in particular, *H. pylori* may directly target the mitochondria as a strategy to modulate cell death.

Antibiotic resistance in humans, primates, and livestock in Kibale National Park, Uganda

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Relatively little is known about prevalence, incidence, or risk of transmission of zoonotic diseases in primates, or about how anthropogenic changes to primate habitats affect rates and patterns of disease transmission. Humans, primates, and livestock living in and around the forests of Kibale National Park in Uganda were surveyed for the presence of antibiotic resistant *Escherichia coli* and *Salmonella sp.* We recovered a *Salmonella* isolate from a human living near the park that was resistant to six antibiotics and had the same resistance pattern as human *E. coli* isolates collected in the region, indicating there may have been an exchange of genes encoding multi-resistance between bacterial species. We found 29 different resistance patterns in 624 *E. coli* isolates with resistance to at least one antibiotic in 84.7% of humans, 25% of livestock, 8% of red-tailed monkeys, 6.5% of chimpanzees, 4% of black and white colobus, and 0% of red colobus. Humans in this region harbor a high prevalence of *E. coli* that are resistant to antibiotics frequently used in the region. Monkeys with multi-resistant *E. coli* all lived in forest fragments associated with human villages. Chimpanzees harboring multi-resistant *E. coli* lived in forest where they come in close contact with research personnel and tourists, and often venture into villages to raid crops. This study provides evidence that high contact rates between humans and non-human primates enhance the transmission of multi-antibiotic resistant bacteria or resistance-conferring genes from humans to wild primates.

***Pasteurella multocida* toxin (PMT) down-regulation of $G\alpha_{q/11}$ proteins from lipid rafts**

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We have shown that *Pasteurella multocida* toxin (PMT) causes transient activation of the G alpha q subunit ($G\alpha_q$) that is coupled to phosphatidylinositol-specific phospholipase C-beta 1 (PLC β 1) in *Xenopus oocytes* [B. A. Wilson, et al. *J. Biol. Chem.* 272:1268-1275, 1997]. However, the molecular mechanism of PMT action on $G\alpha_q$ and the resulting cellular effects in mammalian cells are not

clearly understood. In the present study, we examined the mechanism of PMT action on $G\alpha_q$ in HEK-293T and Swiss 3T3 cells. Immunoblotting and immunoprecipitation experiments showed that PMT treatment for 16 hours did not induce significant down-regulation of total $G\alpha_q$ content in HEK-293T cell lysates. We then examined whether PMT affected signaling molecules in lipid rafts. Detergent-treated HEK-293T cell lysates were separated into fractions by OptiPrep density gradient centrifugation, and signaling molecules in the fractions were determined by immunoblotting. Compared to $G\alpha_s$, $G\beta$ subunits or flotillin-1, a lipid raft marker in detergent-resistant membrane (DRM) fractions, treatment of cells with PMT resulted in a significant decrease in $G\alpha_q$ levels in DRMs. These results suggest that PMT treatment causes down-regulation of $G\alpha_q$ in HEK-293T and Swiss 3T3 cells through loss of $G\alpha_q$ protein from lipid rafts.

Prevalence of *Escherichia coli* in Retail Beef, Chicken, Pork, Turkey, Fish, and Shellfish from Champaign County, Illinois

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Escherichia coli is one of the most common causes of food borne disease in the United States. The CDC has estimated that food borne diseases cause around 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States each year. The objectives of this study were to estimate the prevalence of *E. coli* of raw retail meats (beef, chicken, turkey, pork, fish, and shellfish) within the Champaign County, IL area, and to evaluate the risk factors for increased contamination in retail meat products. A total of 240 raw retail meat samples were collected from 14 random stores in Champaign County from May through July of 2006. Store location, condition of sample (ground vs. non-ground), brand of sample, and type of packaging (pre- packaging vs. store packaging) data was collected for risk analysis. These samples were then tested for *E. coli* by standard culturing methods. Overall prevalence of *E. coli* was 30% with a prevalence of 36.7% in beef, 41.2% in chicken, 33.3% in pork, 48.3% in turkey, 2.9% in fish, and 4.3% in shellfish. There was no statistically significant difference of *E. coli* prevalence between samples from Champaign-Urbana and samples from areas in the county outside Champaign- Urbana. Ground meat products were three times more likely to be contaminated with *E. coli* when compared to non-ground meat products (95% C.I., 1.76 - 5.54). Non-store brand products were 2.5 times than store brand products (95% C.I., 1.31 - 4.57) and pre-package products were 4 times than store packaged products (95% C.I., 1.78 - 7.78) to be contaminated with *E. coli*. Based on these results it can be inferred that fish and shellfish have a lower prevalence of *E. coli* contamination when compared to beef, chicken, pork, and turkey. It can also be inferred that ground, non-store brand, and pre-packaged meats have a higher risk associated with *E. coli* contamination compared to non-ground, store brand, and store packaged meats. Estimating the prevalence of *E. coli* in meats at the retail level can be a better indicator of the public health risk associated with meat consumption when compared to the prevalence found at the processing plant.

Detection of *Salmonella* in retail raw meat at grocery stores in Champaign County, Illinois

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The United States Department of Agriculture assesses pathogen presence in raw meat samples at processing plants, but there is a lack of testing meat after further handling at retail outlets. Sampling

was conducted to estimate the prevalence of *Salmonella* in raw beef, chicken, turkey, pork, fish, and shellfish at retail stores in Champaign County, Illinois. This estimation provides information for the evaluation of hazardous control points to manage pathogen related food-borne disease in raw meat at retail grocery outlets. A total of 240 samples between beef, chicken, turkey, pork, fish and shellfish samples were randomly collected from fourteen retail grocery stores in Champaign County, Illinois from May through July 2006. Store location, brand of meat, packaging location, and ground or non-ground meat status was evaluated for contamination risk. The samples were tested for *Salmonella* by culture method. The overall presence of *Salmonella* in raw meat samples from retail stores in Champaign County, Illinois was 6.3%. Of the poultry (chicken and turkey) samples, 22.2% tested positive for *Salmonella* presence, 71.4% of which were ground samples. Beef tested positive for *Salmonella* in 1.6% of the samples. *Salmonella* was not isolated from pork, fish, or shellfish samples. Ground meat products were 5 times more likely than non-ground meat products to have *Salmonella* contamination with a (95% C.I. 0.0583 - 0.6139). Non-store brand meat products were 28 times more likely to have *Salmonella* contamination than store brand meat (95% C.I. 6.1572 - 130.2715). Stores outside of Champaign-Urbana were 4 times more likely to have *Salmonella* positive meat samples than Champaign-Urbana stores (95% C.I. 1.2506 – 13.1118). According to personnel from the Champaign County Public Health District responsible for the inspection of retail grocery stores in Champaign County, this increase in risk may be due to historical differences in the frequency of inspection at stores outside of the Champaign-Urbana city limits.

Detection of Antisera Recognizing Vaccinia and Monkeypox Virus Proteins in Wild Small Mammals in Uganda

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Concomitant with the loss of vaccine-based immunity to smallpox, monkeypox virus (MPXV) has become an emerging pathogen in Central and West Africa. Outbreaks in humans have involved hunting, butchering, and consuming monkeypox-infected mammals. Interestingly, monkeypox has not been reported in East Africa. Whether this is due to lower levels of human contact with mammals or absence of a suitable MPXV reservoir is unknown. To test the latter possibility, in May-July 2006, we live-trapped small mammals along forest-agricultural ecotones in Kabarole and Semuliki Districts, Uganda. Blood was collected from 69 anesthetized animals, including a diversity of rodents and insectivores. Sera were assayed using an ELISA for *Orthopoxvirus* antibodies (vaccinia substrate > 97% genetically identical to MPXV). Presence of reactive antisera is strongly suggestive of previous *Orthopoxvirus* infection. Sera samples were assayed for reactivity to viruses. Each sample was tested in duplicate, and a final absorbance (O.D.) value was calculated. Samples with a final O.D. value > 0.100 over \geq two dilutions were scored reactive. One serum sample, from the only African dormouse collected, met this criterion. To ensure that the serum was specifically reacting with vaccinia virus, an ELISA was performed using sucrose-purified vaccinia. The Ugandan dormouse sample yielded high absorbencies at all dilutions. Serum from captive dormice without previous exposure to orthopoxviruses yielded very low absorbencies. To assess specificity of these antibodies for monkeypox virus, an ELISA was performed using sucrose-purified monkeypox virions. The serum was strongly reactive with monkeypox virions, yielding higher O.D. values than obtained from an assay utilizing sucrose-purified vaccinia virions tested in parallel. Sera from two

captive dormice yielded low absorbance values when tested concomitantly. These results are the first to describe *Orthopoxvirus* antisera in mammals in East Africa. Future work expanding on these efforts will help determine public health risk of human *Orthopoxvirus* illness.

The Role of Periplasmic Cu/Zn Superoxide Dismutase of *Salmonella* Typhimurium in Protection against Phagocytic Superoxide

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Salmonella enterica serovar Typhimurium strain 14028 produces two Cu/Zn cofactored superoxide dismutases in the periplasm, SodCI and SodCII. Only SodCI contributes to virulence in the mouse model of infection by combating phagocytic superoxide; SodCII has no role in virulence, even in the absence of SodCI. We have previously shown that this phenomenon is primarily attributable to differences in the two proteins rather than regulation. Although SodCI and SodCII are 60% identical at the amino acid level, we have noted two significant differences: SodCI is a dimer and is not released from the periplasm by osmotic shock; SodCII is monomeric and is quantitatively released by osmotic shock. Our analyses have shown that this “tethering” of SodCI in the periplasm is via a non-covalent interaction. Our goals are to determine the features of SodCI that allow tethering and to test the role of tethering in pathogenesis. We constructed, using site-directed mutagenesis, a monomeric, fully active SodCI. This mutant protein is entirely released by osmotic shock. Thus, dimerization is critical for tethering. Moreover, upon recombination of the monomeric allele into the normal chromosomal *sodCI* locus, we have shown the resulting strain is equally avirulent to a *sodCI* deletion mutant in a mouse model of infection. These results are consistent with the hypothesis that tethering of SodCI is critical for its role in virulence. Analysis of related SodCs from other pathogens is being used to further test this hypothesis and to correlate functional characteristics and virulence. How is tethering related to growth in macrophages? We have shown that treatment of *Salmonella* with antimicrobial peptides, likely to be occurring in the macrophage phagosome, causes release of SodCII but not SodCI from the bacterial cell. This provides a rationale for the role of tethering in pathogenesis. Finally, we have been addressing how phagocytic superoxide damages bacterial cells. By taking advantage of the genetic concept of synthetic interactions, we show that there is a direct extracytoplasmic target of superoxide and that damage to cytoplasmic targets, including DNA, is not relevant to the antimicrobial actions of phagocytic superoxide.

Core genomes and signature genes that define *Streptococcus pyogenes*

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The signature genes tool developed for the SEED and implemented at the National Microbial Pathogen Data Resource, www.nmpdr.org, has been used to compare the translated genomes of all completely sequenced strains of *Streptococcus pyogenes* to define a core genome for GAS. The tool allows the user to select a reference genome to compare with any number of genomes selected in a comparison set. The commonality factor is set to 80% by default, but this may be reset by the user. For example, the 80% common core of GAS with respect to the strain with the largest genome (MGAS 10750) contains 1,476 proteins that have bidirectional, best BlastP hits (BBH), at an E-value of 1×10^{-10} or less, in 10 of the 12 available genomes. Increasing the stringency of the analysis to 100% reduces the number of core proteins to 1,368. One may also select a second set of genomes to contrast with the first set. This results in the set of proteins that are shared by all genomes in set 1, e.g. both M3-type genomes, which are not present in set 2, e.g. the other 10 GAS genomes. The result constitutes a signature set of proteins that distinguishes the M3-type strains. The information generated by this genome comparison could be used to design a microarray for the simultaneous analysis of the core GAS genome as well as signatures for each sequenced strain or M-type. The bioinformatics analysis reveals interesting consistencies and inconsistencies which generate hypotheses for testing on microarrays. Protein functions are organized in subsystems in NMPDR and SEED, so it is possible to infer functional differences imparted by gene signatures. Subsystems annotation is used for metabolic reconstruction, analysis of central machinery and signaling pathways, finding missing genes, integrating regulatory networks, detection of horizontally transferred genes, and prediction of the functions of hypothetical proteins. We demonstrate the use and applications of the NMPDR tools and the SEED subsystems in understanding the pathogenesis of *S. pyogenes*, the evolution of its virulent strains, and the study of horizontally transferred toxins.

Comparison of DGGE and T-RFLP Profiling for Evaluation of Vaginal Microbiota

Noriko Nakamura^{1,2}, Mengfei Ho^{1,3}, Kathy M. Yeater^{1,4}, Corrin McCann⁵, Suzanne R. Trupin⁶, Lois L. Hoyer^{1,4}, H. Rex Gaskins^{1,2,4,7}, and Brenda A. Wilson^{1,3}

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The vaginal microbial ecosystem is highly complex and is considered to play a role in the etiology of vaginal infections, such as bacterial vaginosis. It has been shown recently that the vaginal microbiota is highly diverse in clinically diseased women as well as healthy individuals. However, it remains to be determined whether a single bacterium, a specific consortium of bacteria, or a population shift in the vaginal microbiota causes disease. The ability to reliably identify the highly diverse microbial populations in the human vaginal tract is critical for understanding its relationship to the etiology of vaginal infections. Microbial fingerprinting methods have been used widely to study the microbial ecosystem of various environmental ecosystems including the vaginal microbiota. In this study, we used PCR-denaturing gel electrophoresis (DGGE) and terminal restriction fragment length polymorphisms (T-RFLP) to analyze the vaginal microbiota of 23 women suffering from current or recent vaginal infections at the time of sampling. Both methods confirmed high diversity of microbial

communities among the subjects, with subjects falling into loosely defined groups. However, results showed no clear correlation of these samples with the demographic information. General agreement in clustering by these two methods was found for some of the samples. Extensive analysis of data revealed that although T-RFLP provided greater resolution of diversity, the use of DGGE or T-RFLP alone may not be adequate in assessing the overall complexity of the microbial community, but combined they provide complementary information that provides a more comprehensive assessment.

The polymorphism P315L of human Toll-like receptor 1 impairs innate immune sensing of microbial cell wall components.

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As a pattern recognition receptor, Toll-like receptor 1 (TLR 1) mediates innate immune responses to a variety of microbial cell wall components including bacterial lipoproteins. We have previously shown that the central region of the extracellular domain of human TLR1, comprising leucine rich repeat (LRR) motifs 9 through 12, is required for sensing of bacterial lipopeptides. Here, we have investigated three nonsynonymous single nucleotide polymorphisms (SNPs) located in this region of TLR1 by generating these variants and examining receptor function. We have found that a variant of TLR1 based upon the SNP P315L, located in the loop of LRR 11, is greatly impaired in mediating responses to lipopeptides and a variety of other bacterial agonists for this receptor. Despite normal cell surface expression, the P315L variant also fails to bind to GD2.F4, a commonly utilized anti-TLR1 monoclonal antibody. While a number of amino acid substitutions at position 315 impair receptor function, the leucine substitution has the strongest deleterious effect. GD2.F4 inhibits agonist-induced activation of TLR1 supporting a crucial role for the loop of LRR 11 in receptor function. These results also suggest that the P315L SNP may predispose certain individuals to infectious disease where sensing of microbial cell components by TLR1 is critical to innate immune defense.

Botulinum Neurotoxin A: Cleavage of SNAP-25 and Delivery of Cargo Proteins to Neurons.

Melissa Pires-Alves¹, Mengfei Ho¹, Karla Kieser¹ and Brenda A. Wilson¹

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Botulinum neurotoxin type A (BoNT/A) targets peripheral cholinergic neurons, where it selectively cleaves synaptosome associated protein of 25 kDa (SNAP25) causing flaccid paralysis. BoNT/A is synthesized and released as a single-chain protein that is activated by proteolytic cleavage to form a catalytic light chain (LC, 50 kDa) and a receptor binding and translocation heavy chain (HC, 150 kDa). Truncated SNAP-25 (residues 141-206) was expressed as a fusion protein with green fluorescent protein (GFP) at the N-terminus, or GFP at the N-terminus and red fluorescent protein (RFP) at the C-terminus or as a fusion protein with an N-terminal cyan fluorescent protein (CFP) and a C-terminal yellow fluorescent protein (YFP). Cleavage of GFP-SNAP or GFP-SNAP-RFP by BoNT/A-LC could be detected by gel shift using SDS-PAGE. Cleavage of CFP-SNAP-YFP abolished fluorescence resonance transfer between CFP and YFP, providing an assay to detect toxin activity in real-time *in vitro* for high throughput screening for potential inhibitors. Kinetic studies

showed that BoNT/A-LC efficiently cleaved all of the SNAP-25 constructions with K_m and K_{cat} values similar to those reported in published data for native SNAP-25. BoNT/A-HC (residues 544-1296) was also expressed as a fusion protein with GFP at the N-terminus. Purified GFP-BoNT/A-HC was tested as a potential cytosolic delivery vehicle for neurons. After incubation with NG108-15 cells fluorescence microscopy showed effective transport of GFP-toxin into the cells. These results provide opportunity to replace GFP with other potential peptides or inhibitors to block BoNT/A-LC activity inside cells and enable the design of novel post-exposure anti-toxin therapeutics.

Analysis of *in vitro* Infection Models for *Bacillus anthracis* Spore Uptake Using Flow Cytometry

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Spores of *Bacillus anthracis*, the etiologic agent of anthrax, are inhaled into the alveolar spaces of the lungs and believed to be phagocytosed by alveolar macrophages. The early events encompassing the interaction of the spore with phagocytic cells has not been well characterized. In this study, we have evaluated the interaction of *B. anthracis* spores with primary human alveolar macrophages (huAV) relative to the commonly used macrophage cell lines (RAW264.7, J774A.1), a mouse alveolar macrophage cell line (MHS), and a human monocyte cell line (THP-1) to determine the cell line that most closely mimics the host response. We hypothesize that interactions between spores and macrophages will vary depending on the source of the macrophage. huAV were harvested by bronchial lavage from volunteers and utilized in our infection model. Macrophages were infected with fluorescently-labeled spores derived from non-capsulated *B. anthracis* Sterne 7702 and analyzed for uptake by flow cytometry. These experiments revealed for the first time quantitative differences in the capacity of primary macrophages and macrophage cell lines to interact with spores, suggesting fundamental differences in the cell surface composition of the macrophages derived from different sources. Specifically, huAV take up a similar number of spores as compared with the RAW264.7, but significantly less than J774.1A macrophages. We also quantitatively demonstrated at the single cell level that spore uptake correlates with cell death as determined by propidium iodide (PI) staining. We observed that RAW264.7 macrophages were not affected by the presence of spores, regardless of spore number or time. The huAV, however, were susceptible to the presence of spores over time as compared with the uninfected population, and subpopulation analysis revealed that the number of PI positive cells was similar throughout the population regardless of the number of spores endocytosed. These studies suggest fundamental differences in the interaction of *B. anthracis* with macrophage cell lines compared to primary human alveolar macrophages.

The role of Rab GTPases in intracellular trafficking of *Pasteurella multocida* Toxin (PMT)

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The dermonecrotic toxin produced by the gram-negative coccobacillus *Pasteurella multocida* (PMT) is associated with several diseases including atrophic rhinitis, respiratory diseases in both cattle and rabbits, and dermonecrosis and bacteremia associated with animal bite wounds or exposure to animals. PMT is an intracellularly acting protein toxin that exerts its effects on host cells by activating phospholipase C, calcium, cytoskeletal, and mitogenic signaling pathways to cause actin

rearrangements and cellular proliferation. Our studies reported here are aimed at identifying Rab GTPase-associated PMT trafficking pathways within the cell. Rab GTPases are small G-proteins that regulate vesicle trafficking. Rab GTPases are associated with a variety of different vesicle trafficking pathways including ER to Golgi transport (Rab1a), Golgi to ER transport (Rab2a and Rab6a), late endocytic traffic (Rab7), trans-Golgi network to plasma membrane transport (Rab8 and Rab3a), late endosome to trans-Golgi network transport (Rab9a), plasma membrane to early endosome transport (Rab5a), early endosome to plasma membrane transport (Rab4a), and endocytic recycling traffic (Rab 11a). The Dual-Luciferase assay for serum response element (SRE) activation is being used to measure the effect of expression of dominant negative, constitutively active, and wild-type Rab GTPases on PMT activity. Cells that are expressing a dominant negative, constitutively active, or wild-type Rab are treated with PMT to determine whether expression of the Rab mutant has an effect on PMT signaling through SRE pathways.

Effects of forest fragmentation and livestock contact on the prevalence of *giardia sp.* And *cryptosporidium sp.* Among wild primates in western uganda

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² Department of Zoology, Makerere University, Uganda

In June 2005, 205 fecal samples were collected from domestic livestock and three species of wild primates in and near Kibale National Park, western Uganda. Fecal samples were examined for zoonotic protozoal organisms, *Cryptosporidium sp.* and *Giardia sp.* Primate fecal samples were collected from undisturbed areas of forest, as well as from three highly disturbed forest fragments outside of the park proper. Livestock samples were collected from areas surrounding the disturbed forest fragments. Red colobus (*Ptilocolobus tephrosceles*), red-tailed guenons (*Cercopithecus ascanius*), sheep (*Ovis aries*), goats (*Caprus hircus*) and cattle (*Bos indicus*) harbored *Cryptosporidium sp.* or *Giardia sp.*, but black-and-white colobus (*Colobus guereza*) and pigs (*Sus scrofa*) did not. All non-human primate samples from undisturbed forest were negative for both parasites. Seven of 35 (20%) red colobus samples collected from forest fragments and one of 20 red-tailed guenons (5%) were infected with either *Cryptosporidium sp.* or *Giardia sp.* Seven of 90 (8%) domestic animals living near the forest fragments were infected with either *Cryptosporidium sp.* or *Giardia sp.* The higher prevalence of *Cryptosporidium sp.* and *Giardia sp.* in primates living in forest fragments suggests that habitat disturbance plays a role in the transmission or persistence of these pathogens. The presence of both *Cryptosporidium sp.* and *Giardia sp.* in the domestic livestock living near the forest fragments suggests that these species may be involved in the transmission of *Cryptosporidium sp.* and *Giardia sp.* to wild primates. Habitat disturbance and exposure to domestic livestock may play a role in transmission or persistence of these pathogens among wild primates.

Disruption of the *Wolbachia* surface protein gene *wspB* by a transposable element in mosquitoes of the *Culex pipiens* complex (Diptera, Culicidae)

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Culex pipiens quinquefasciatus Say and *Culex pipiens pipiens* Linnaeus are sibling species incriminated as important vectors of emerging and re-emerging infectious diseases worldwide. The two forms differ little morphologically and are differentiated mainly based upon ecological, behavioral, physiological and genetic traits. Within the North American zone of sympatry, populations of *Cx. p. quinquefasciatus* and *Cx. p. pipiens* undergo extensive introgression and hybrid forms have been reported in nature. Both *Cx. p. quinquefasciatus* and *Cx. p. pipiens* are infected with the endosymbiotic bacteria *Wolbachia pipientis*. Here, we report the presence of a transposable element belonging to the IS256 family (IS256wPip) associated with *Wolbachia* in both *Cx. p. quinquefasciatus* and *Cx. p. pipiens* populations. Using reverse transcriptase PCR and sequences analysis, we show that IS256wPip has disrupted the wspB locus, a paralog of the *Wolbachia* outer membrane protein (wspA) gene. The inactivation of the wspB appears to be specific to *Cx. p. quinquefasciatus* and to hybrids of the two forms, and was not observed in the surveyed *Cx. p. pipiens* mosquitoes. Our results support the hypothesis of a different origin of North American *Cx. p. quinquefasciatus* and *Cx. p. pipiens* populations. The flux of mobile genetic elements in the *Wolbachia* wPip genome could explain the high level of crossing types observed between different *Culex* populations. The insertion of IS256wPip into wspB may comprise a genetic candidate for discriminating *Wolbachia* symbionts in *Culex*.

Interaction of *B. anthracis* with macrophages

Bojana Stojković, Eric M. Torres, Angie M. Prouty, Hetal K. Patel, Theresa M. Koehler†, Jimmy D. Ballard●, and Steven R. Blanke‡. ‡Department of Microbiology, University of Illinois at Urbana-Champaign, 302 Burrill Hall, Champaign, IL 61801. †Department of Microbiology and Molecular Genetics, University of Texas-Houston Health Science Center, Houston, Texas 77030, USA. ●Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104.

Bacillus anthracis is the causative agent of anthrax. The most serious form of this disease, inhalational anthrax, has been subjected to rigorous study during the last five years due to its potential use as a bioterrorist weapon. Studies done to date have been limited to microscopy observations as well as live cell counting, both of which have its limitations in elucidating early events of the macrophage infection. Using infection system developed in our laboratory, we are able to study interaction of Raw 264.7 macrophages with *B. anthracis* at the level of single cell using flow cytometric analysis. This method allows for discrimination of the macrophage and spore population based on differences in forward and side-scatter properties. In addition, infected macrophage population can be distinguished based on the level of macrophage-associated fluorescence. The extent of macrophage-associated fluorescence increased in relation to the number of recoverable spores. Moreover, one can distinguish between the extracellular spores from intracellular by quenching fluorescence, providing percentage of infected macrophage population. The effects of common variables within *in vitro* infection models (MOI, serum, medium) were scored for the first time. In addition, quantitative comparisons of spore interaction and uptake by different macrophage cell lines, Raw 264.7 and J774A.1 were demonstrated for the first time.

Detection of Spotted Fever Group (SFG) Rickettsiae and changes in tick distribution patterns of *Ixodid* ticks in the natural park of the Tosco-Emilian Appennine (Italy)

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Rickettsiae belonging to the Spotted Fever Group (SFG) are agents of emerging tick-borne zoonosis in Europe. In order to evaluate the risk for humans, we are investigating the presence of these pathogens in ticks in the natural park of the Tosco-Emilian Appennine, Italy.

Dermacentor marginatus nymphs and larvae collected from small mammals in the area in 1994-95 by Mannelli et al. (1997) were tested by *Polymerase Chain Reaction* (PCR). The genes *gltA* and *ompA* of SFG rickettsiae were amplified from 10% of larvae and 54.2% of nymphs. Positive samples were sequenced and showed high similarity with *R. slovaca*, a SFG species that is considered to be the causative agent of the “tibola” (*tick-borne lymphadenopathy*) syndrome in humans.

In August 2006, ticks were collected by dragging in the same area. The presence of *Haemaphysalis punctata*, *H. sulcata*, *Ixodes ricinus*, and *D. marginatus* was recorded. These results were compared to the tick collection of 1994-95 (Mannelli et al., 1997), and an increase of host-seeking ticks was observed. Moreover, *I. ricinus* was found at higher altitudes. This could be due to a greater ungulate population in the area and to climatic changes.

The Normalized Difference Vegetation Index (NDVI; LANDSAT-7) didn't show a correlation with tick abundance; this is probably due to the landscape features of the park and to the prevalence of *H. punctata* and *H. sulcata*, two tick species that are characteristic of barren zones.

Investigation of myxoma virus as an oncolytic agent in canine neoplastic cells

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Myxoma virus (MYX) is a rabbit-specific poxvirus, which is non-pathogenic for all other vertebrate species tested, although it has been shown to productively infect cells from diverse species *in vitro*. Recently published studies have shown MYX has oncolytic properties against malignant human glioma cells *in vivo*. This is likely due in part to the fact that several neoplastic cell lines are deficient in their interferon (IFN) responses (Lun, McFadden, et al., 1995), making them ideal targets for oncolytic virus therapy. In addition, some tumor cells have aberrant tumor necrosis factor (TNF)-alpha signaling, which may play a role in the susceptibility of the tumor to MYX infection and to apoptosis (Sedger, McFadden, et al., 2006). SERP2 is a serine proteinase inhibitor encoded by MYX that attenuates inflammatory response to infection in rabbits and may inhibit granzyme-mediated apoptosis of infected cells (MacNeill, et al, 2006); therefore, deletion of SERP2 from MYX should increase intensity of the immune response to a tumor and the rate of apoptosis in infected cells. This study examines the effectiveness of MYX infection in several canine neoplastic cell lines. It is also designed to determine if permissive canine neoplastic cells have the same signaling pathway defects that have been reported in permissive human cell lines.

Canine (Bliley: transitional cell carcinoma; Abrams: osteosarcoma; Den: hemangiosarcoma) and human (MG-63: osteosarcoma; U-118MG: glioma) tumor cell lines were evaluated to observe

cytopathic effects, determine susceptibility of cell lines to infection, and evaluate potential cell-to-cell spread of MYX. Rabbit kidney epithelial cells (RK-13s) are fully permissive to MYX infection and served as a positive control for these experiments. The following viruses are being used: MYX (Lausanne strain) wild-type (MYX WT), MYX with SERP2 deletion (MYX Δ SERP2::lacZ), and MYX expressing green fluorescent protein (MYX gfp). Cytopathic effects were documented with images of infected cells taken via phase microscopy and fluorescence. Each cell line was infected in six-well plates at an MOI of 0.1 and 10 plaque-forming units per cell for multi-step and one-step growth curves, respectively. The rate and extent of apoptosis was investigated with a fluorescence-release terminal caspase assay using Ac-DEVD-AMC as a substrate after quantification of protein concentration via Bradford assay.

Significant cytopathic effect was observed at both MOIs in all cell lines except MG-63 as early as 24 hours post-infection. One-step growth curves indicated logarithmic viral replication in all tumor cell lines except Bliley and U-118MG. Multi-step growth curves demonstrated some cell-to-cell spread of virus in canine but not in human tumor cell lines, although virus growth was not sustained in any cell line tested except RK-13 cells. Apoptosis is accelerated in Den but decreased in Bliley after MYX Δ SERP2::lacZ infection; this decrease in apoptotic rate exhibited by Bliley will be further investigated by collecting earlier time points for caspase assay.

MYX appears to be an efficient oncolytic virus in a canine osteosarcoma cell line (Abrams) and a canine hemangiosarcoma cell line (Den). Evaluation of the rate and extent of apoptosis in selected cell lines during viral infection is ongoing. Further studies will investigate cytokine production and viral protein production in these tumor cell lines and primary cell cultures.

ABOUT OUR SPEAKERS

MARTA GUERRA, DVM, MPH, Ph.D., Dipl. ACVPM

Senior Staff Veterinary Epidemiologist, Centers for Disease Control and Prevention

Growing up in the multicultural environments of Havana, Cuba and Washington D.C., Marta Guerra developed a keen interest in international health. Guerra is a veterinarian and holds a Master's degree in Public Health and Ph.D. in tropical medicine. Today, she is an Epidemic Intelligence Service Officer in CDC's viral and rickettsial disease program.

Immediately after being selected as an EIS officer, she was called to Uganda to help control an Ebola hemorrhagic fever outbreak. EIS officers must be prepared to leave their homes indefinitely with short notice if there is an outbreak, but it is a sacrifice they readily make. Such was the case with Guerra. After only two days' notice, she found herself on a plane to Gulu.

Guerra calls her stint in Uganda her most significant experience to date. "It is very rewarding to be able to put into practice what you have been studying," Guerra recalls. Because the strain of Ebola was not as virulent as those found in past outbreaks in other countries, relief teams were able to ensure the survival of more victims. Still, due to the highly infectious nature of the disease and the high death rate (58%), the need to counter the outbreak was dire. Guerra stated that certain Ugandan customs may facilitate the spread of the disease, as families often care for the sick at home and in hospitals, handle the bodies and many villagers attend the funerals of the victims.

DOUGLAS GOODIN, Ph.D.

Professor and Director, Remote Sensing Research Laboratory, Department of Geography, Kansas State University

Dr. Goodin's research interests include spatial complexity and patterning in landscapes, biosphere/climate interactions, ecology of infectious disease, remote sensing and geospatial analysis. Since 2004, he has been part of a multidisciplinary team studying the impact of rapid anthropogenic land cover change on hantavirus ecology in Paraguay. This team has been involved in creating an ecological database for Paraguay. At present, our database focuses on rodent biology in the Interior Atlantic Forest in Eastern Paraguay. Over the course of this project, they will collect information on landscape ecology, land use, human ecology, climate, and hantavirus prevalence in the rodent communities.

URIEL KITRON, Ph.D.

Professor, Pathobiology
Co-Director, Center for Zoonoses Research

Current research efforts funded, among others, by NIH, NSF and CDC, include a large-scale collaborative study of Lyme disease and ticks in the Midwest, a study of the urban ecology of West Nile virus in Chicago, and international studies of malaria and schistosomiasis in Kenya and of Chagas disease in Argentina. His lab also collaborates with the Illinois Department of Public Health on mapping the spread of West Nile Virus.

Teaching interests include epidemiology of infectious diseases, spatial epidemiology and ecological parasitology. Because of the applied nature of some of his research, he is also interested in the transmitting of scientific information. Beyond the teaching of courses this is manifested in work with

the public, state, national and international public health agencies and in communication with the media. He teaches a seminar course entitled, Epidemiology and the Media, where such issues are discussed.

HELEN JOST, Ph.D.

Associate Professor, Department of Veterinary Science and Microbiology, University of Arizona

Dr. Jost's primary research interest is bacterial adhesion to the host, with a specific emphasis on *Arcanobacterium* spp.-host cell interactions. The arcanobacteria are non-motile, Gram positive bacteria related to the corynebacteria and actinomycetes.

Currently, her research focuses on how *Arcanobacterium* spp. are able to adhere to and/or colonize the host, which includes mechanisms such as fimbriae, neuraminidase activity, the ability to bind extracellular matrix proteins such as collagen, fibronectin and fibrinogen, and biofilm formation. In addition, I am also interested in the mechanisms of antimicrobial resistance, and the role toxins play in disease pathogenesis in these organisms.

J. STEPHEN DUMLER, M.D.

Associate Professor, Department of Pathology, Division of Medical Microbiology,
The Johns Hopkins Medical School

Dr. Dumler's current research focuses upon the host-pathogen interactions of obligate intracellular tick-borne rickettsial bacteria of the genus Ehrlichia and Anaplasma, and interactions with the spirochete Borrelia burgdorferi that causes Lyme disease. Most work focuses on Anaplasma phagocytophilum that causes human granulocytic anaplasmosis (HGA). The bacterium that causes this increasingly recognized disease has successfully adapted to an endosomal compartment of neutrophils. The bacterial cellular and molecular mechanisms of adherence and entry, and the mechanisms by which the bacteria manipulate the host cell are major areas of study.

His laboratory uses a multidisciplinary approach to investigation, melding new molecular and cellular biology tools with standard microbiologic, histopathologic, and immunologic studies to discern mechanisms of disease by these unique bacterial pathogens.

TAMARA MAIER, Ph.D.

Postdoctoral Fellow, Department of Microbiology and Molecular Genetics, Medical College of Wisconsin

Dr. Maier's recent accomplishments include a GLRCE Postdoctoral Training Program Fellowship (9/1/04 – 8/31/06), NIH/NIAID Region V Great Lakes Regional Center of Excellence for Biodefense and Emerging, Infectious Diseases Research and development of genetic tools and identification of virulence genes in *Francisella* (P.I.)

ROBERTO DOCAMPO, M.D. Ph.D.

Sanford Orkin Eminent Scholar, Department of Cellular Biology, University of Georgia

Dr. Docampo's lab's strategy is to search for metabolic pathways in parasites that may be essential for their survival but may not find an equivalent counterpart in the host. Currently their efforts are concentrated on the mechanisms by which pH and calcium homeostasis are maintained by different trypanosomatids (*T. cruzi*, *T. brucei*, and *Leishmania* sp.) and malaria parasites (*P. falciparum*, *P. berghei*) and more specifically in the biochemical and molecular characterization of a new organelle

that we have discovered and named the acidocalcisome [see figure]. This organelle is acidic due to the presence of a vacuolar H⁺-ATPase and a vacuolar H⁺ -pyrophosphatase. In addition, it has a Ca²⁺-ATPase, for Ca²⁺ uptake, and a Ca²⁺ channel, for Ca²⁺ release. In addition, we are studying the signaling mechanisms that occur in the parasites and in the host cells during their interaction. Recent developments in the study of the basic biochemistry of these parasites have resulted in the discovery that bisphosphonates, drugs widely used in the treatment of benign and malignant diseases characterized by increased bone resorption, could have a role as lead antiparasitic agents.

ABOUT OUR SPONSORS

Special thanks to our 2007 sponsors:

The Conservation Medicine Center of Chicago

The Conservation Medicine Center of Chicago (CMCC) is a collaboration among the Chicago Zoological Society, which operates Brookfield Zoo; Loyola University Chicago Stritch School of Medicine; and the University of Illinois College of Veterinary Medicine. The Center, which uses facilities at the three institutions, brings together a unique team of physicians, veterinarians, researchers and clinicians in many disciplines.

The goal of the CMCC is to study the relationship among animals, people and the environment: how animals and people each affect the ecosystem and how changes in the ecosystem affect the health of all species.

The Department of Pathobiology, University of Illinois at Urbana-Champaign College of Veterinary Medicine

The Department of Veterinary Pathobiology is one of three departments in the College and plays a central role in the University of Illinois' three-part mission of teaching, research and service. In this land-grant research university, our educational mission is pursued in concert with our research mission. The department encompasses the disciplines of Epidemiology and Preventive Medicine, Microbiology and Immunology, Parasitology, and Comparative Pathology.

Earth and Society / The Environmental Council at the University of Illinois at Urbana-Champaign

The mission of Earth and Society is to use the University's unique geography and academic strengths to make significant contributions to earth and society in the areas of discovery, learning, public engagement, policy, and civics.

The *Earth and Society* initiative will provide seed money to stimulate cross-cutting scholarship and grant writing activity in areas *such as*:

- Infectious Disease & Environment
- Nutrient Dynamics in Urban & Rural Landscapes
- Freshwater Ecosystem Restoration
- Agroecology at the Rural-Urban Fringe
- Urban Design & Healthy Communities
- Energy Use and Reliance on Fossil Fuels
- Conservation, Population, & Ecosystem Health
- Reinventing the use of materials

Dr. Doug Goodin's presentation is co-sponsored by Earth and Society.

The Host-Microbe Systems Theme of the Institute for Genomic Biology, University of Illinois at Urbana-Champaign

The overall goal of the Host-Microbe Systems Theme is to exploit genomic technologies to study the dynamic interactions between the host and its commensal as well as pathogenic microbes. The theme will focus initially on the vaginal microbiota, a complex ecosystem in which the composite microbes, their relative abundance, and their interactions with and effects on cellular and immunological responses of the host are critical indicators of the state of a woman's health. Despite the important roles they play in maintenance of vaginal health, there is a profound gap in our knowledge of both the vaginal microbiota and the local immune system.

The theme will explore the various aspects of the role of normal vaginal microbiota in obstetrical and gynecological infectious diseases and how shifts in the composition of the microbiota influence the healthy or diseased state. A central goal will be to understand the pathogenesis of vaginal infections and the immune response to normal and abnormal microbiota. Researchers will study the role of normal vaginal microbiota in preventing vaginal infections and the impact of microbiota composition on susceptibility to certain pathogens, including those responsible for sexually transmitted diseases, bacterial vaginosis, yeast vaginitis, and pelvic inflammatory diseases. Other goals include:

- Identifying the microbial population and immunological components of the vagina
- Studying the population dynamics of the vaginal ecosystem
- Understanding the physiology and metabolism of the microbes and host in the vaginal ecosystem
- Enhancing our understanding of vaginal host-microbe interactions, especially those interactions that involve susceptibility to and pathogenesis of polymicrobial infections

The Program in Arms Control, Disarmament, and International Security (ACDIS), University of Illinois at Urbana-Champaign

The Program in Arms Control, Disarmament, and International Security (ACDIS) is an interdisciplinary research, teaching, and public service program at the University of Illinois at Urbana-Champaign devoted to advancing and disseminating knowledge about the problems of war and peace. Established in 1978, the program's primary areas of focus include: energy uses of technology and energy security; South Asian regional security issues; arms control and nuclear nonproliferation policy; conflict management; globalization; democratization in Russia and Eastern Europe; human rights; and military history.

ACDIS is a unit within International Programs and Studies and receives funding from the State of Illinois, private foundations, and federal government agencies. ACDIS maintains a research library, organizes seminars, workshops, conferences, and other public events, and produces and distributes a variety of publications featuring faculty, student, and visiting scholar research. It participates in the U.S. Air Force National Defense Fellows program, annually hosting one or more officers for a year of advanced research and training.

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