

9th Annual Conference on New & Re-Emerging Infectious Diseases

April 13-14, 2006

Illini Union, Urbana, Illinois

—

Hosted by the Center for Zoonoses Research at the University of Illinois at Urbana-Champaign

and

Co-sponsored by the following units of the University of Illinois at Urbana-Champaign:

The Center for Zoonoses Research

The Department of Pathobiology

The Environmental Council

The Host-Microbe Systems Theme of the Institute for Genomic Biology

The Office of International Programs & Studies

The Program in Arms Control, Disarmament, and International Security (ACDIS)

and by The Conservation Medicine Center of Chicago

CONFERENCE SCHEDULE

Thursday, April 13 - 170 Illini Union A & B

4:00-5:00 pm: Welcome reception

5:15 pm: Welcome and introduction, Dean Herbert Whiteley, College of Veterinary Medicine

5:30 pm: Jonathan Patz, "Deforestation and climate effects on infectious diseases"

Friday, April 14 - Presentations in 407 Illini Union

7:15-8:00 am: Registration and continental breakfast buffet

8:00-8:45 am: Paul Gibbs, "The wind of change: emerging diseases in the 21st century"

8:45-9:30 am: Alison Weiss, "*Bordetella pertussis*; reemergence as a significant health threat despite vaccination"

9:30-10:15 am: Steve Blanke, "*Helicobacter pylori*: persistence mechanisms"

10:15-10:45 am: Break

10:45-11:30 am: R. Mark Buller, "Human monkeypox: a poxvirus zoonoses disease"

11:30-12:15 pm: Ricardo Gurtler, "The eco-epidemiology of Chagas disease in northern Argentina"

12:15-1:30 pm: Lunch & poster viewing **(103 Colonial Room)**

1:30-2:15 pm: May Berenbaum, "Invasion USA: how aliens are changing our world"

2:15-3:00 pm: Nina Marano, "Emerging infectious disease and public policy"

3:00-3:30 pm: Poster Viewing **(103 Colonial Room)**

3:30-5:00 pm: Panel discussion **(314B)**

PRESENTATION ABSTRACTS

DEFORESTATION AND CLIMATE EFFECTS ON INFECTIOUS DISEASES

Jonathan Patz, Associate Professor of Environmental Studies and Population Health Sciences at the University of Wisconsin-Madison, Madison, WI

The WHO estimates that the relative warming that has occurred since the mid 1970s may be causing over 150,000 deaths and millions of cases of diseases annually, with projected risks from climate change expected to double by the year 2030. Many infectious diseases are sensitive to climate, particularly those indirectly transmitted, for example, water- and vector-borne diseases. With stronger warnings now from climatologists studying global warming yet, changing landscapes can significantly affect local weather more acutely than longterm climate change. Land cover change can influence micro-climatic conditions including temperature, evapotranspiration, and surface runoff, key to determinants to the emergence of many infectious diseases. Landcover change can also influence disease vector breeding site characteristics or species composition. In our study site in the Northern region of the Peruvian Amazon, concomitant with new deforestation the area has seen a sharp rise in malaria incidence, increasing fifty-fold from 1987 to 1997, and an invasion by South America's major malaria vector, *Anopheles darlingi*. We compared four different land use categories to determine the relationship between the extent of deforestation and entomological risk factors for malaria. Results show a strong relationship between the extent of deforestation and abundance of *Anopheles darlingi*, and its breeding sites. These ecology-disease relationships demonstrate how decision makers must recognize how much coupled are human and natural systems and that broad-based solutions are required, rather than fractioned or uncoordinated policy changes.

THE WIND OF CHANGE: EMERGING DISEASES IN THE 21ST CENTURY

Paul Gibbs, Professor, College of Veterinary Medicine, University of Florida, Gainesville, FL

The rate at which emerging diseases have surfaced over the last 25 years has shaken, indeed many would say shattered, the complacency of the medical and veterinary communities that emerging diseases of people and animals are under control. In the late 1960's, the Surgeon General of the USA, William H. Stewart, said that "...it was time to close the book on infectious diseases and pay more attention to chronic ailments such as cancer and heart disease." Indeed a measure of that success came towards the end of the 1970's, when the world rejoiced that smallpox had become the first disease to be eradicated from the human species. Such halcyon days from the 1960's to the early 1980's are but sweet memory. The turn of this century has brought with it a succession of disasters, both natural and man made. We now live in a closely interconnected global community; a global society that has survived the "cold war" only to be plunged into a "war on terrorism". The emergence of al Qaeda, the wars in Afghanistan and Iraq, and the natural disasters of the Tsunami in Asia and the hurricanes in Florida and the Gulf Coast have overshadowed the large number of naturally occurring emerging diseases and epidemics that we have faced in the USA, the UK, and elsewhere around the world in recent years. Amidst all the disasters, perhaps we have failed to see the warning signs of possibly bigger epidemics ahead. It was noted that the earthquake that led to the Asian Tsunami in 2004, literally rocked the earth's rotation for a millisecond. In the 21st century, emerging diseases have the potential--- a

possibility, not a probability ---- to devastate the global human population, the effect of which could be to “rock” the earth for a century or more. Avian influenza is such a threat today; a reminder to society of the potential of disease to wreck communities and nations.

This short review analyses the global factors that have lead to the increased emergence of such diseases, sketches several recent epidemics (and where relevant, their relationship to bioterrorism), discusses the lessons learned, and concludes by outlining, in very broad terms, an agenda for action. The epidemics discussed include foot-and-mouth disease, avian and canine influenza, monkeypox, severe acute respiratory syndrome (SARS), bluetongue, bovine spongiform encephalopathy and West Nile encephalitis.

BORDATELLA PERTUSSIS: REEMERGENCE AS A SIGNIFICANT HEALTH THREAT DESPITE VACCINATION

Alison Weiss, Professor, Department of Molecular Genetics, Biochemistry and Microbiology at the University of Cincinnati, Cincinnati, OH

The epidemiology of pertussis is strongly influenced by vaccination. Mortality due to pertussis was very high prior to the introduction of the pertussis vaccine, and even in the 21st century the bulk of the 300,000 yearly deaths due to pertussis occurs in unvaccinated individuals. The United States has been using the pertussis vaccine for decades and experiences few deaths, but over the past 25 years the number of cases of pertussis has been steadily increasing. About 10 years ago, the U.S. switched from the whole cell pertussis vaccine, consisting of killed organisms, to acellular pertussis vaccines, consisting of purified protein components. Contrary to expectations, use of the acellular vaccines has not reversed the upward trend.

It is becoming clear that infection with *B. pertussis* results in a spectrum of disease severity depending on one’s immune status. Severe, life-threatening disease, or classical whooping cough, can occur in non-immune individuals of any age. Both vaccination and recovery from natural infection appear to confer long-term protection from classical whooping cough. In the United States, where vaccination rates are high, severe disease is primarily seen in infants too young to vaccinate. At the other end of the spectrum, partially immune individuals experience non-life threatening disease characterized by a cough that can linger for a month or more.

It is clear that vaccination has successfully targeted the disease, whooping cough, but not the agent, *Bordetella pertussis*. As a strict human pathogen, the survival strategy of *B. pertussis* appears to be immune manipulation, to favor the development of suboptimal responses that make humans susceptible to re-infection, and it is likely that the current pertussis vaccines also promote the development of suboptimal immune responses. Bactericidal immunity, if achievable, would prevent disease as well as carriage of the organisms.

HELICOBACTER PYLORI: PERSISTENCE MECHANISMS

Steven R. Blanke, Department of Microbiology, Institute for Genomic Biology, University of Illinois, Urbana, IL.

Persistent infection with *Helicobacter pylori* is a significant risk factor for the development of gastric ulcer disease and stomach cancer in humans. *H. pylori* carriage is approximately 50% worldwide, and is responsible for significant morbidity and mortality, as well as a negative economic impact, resulting in the loss of billions of dollars annually to afflicted individuals in both medical costs and wage earnings. One of the unusual aspects of *H. pylori* infections is that without medical intervention, the pathogen can persist for the entire lifetime of the host. During the early stages of infection, not only do *H. pylori* circumvent immune clearance, but act to establish a homeostatic state characterized by limited damage of the gastric environment, which, while allowing access to nutrients, also causes significant restructuring of the gastric architecture that may precede the formation of pre-neoplastic lesions. Among the strategies used by *H. pylori* to persist is bacterial remodeling, resulting in fundamental changes resulting in organisms more suitable for colonization of the harsh gastric environment. A primary mechanism of *H. pylori* remodeling is the regulation of the expression of functional proteins, called phase variation. Phase variation in *H. pylori* occurs primarily by slipped-strand mispairing, resulting in genes being entirely “on” or “off,” thereby altering functional properties of individual organisms within a bacterial population. We have begun to explore the hypothesis that phase variation results in altered forms of *H. pylori* able to withstand the rigors of the gastric environment. We have found that phase variants of a single gene may demonstrate remarkably distinct properties in terms of stress resistance, as well as virulence factor expression. Because *H. pylori* may have more than 40 genes that undergo slipped-strand mispairing, phase variation may result in the emergence of functional diversity within a single population. As an adaptation mechanism, phase variation may be important for *H. pylori* persistence within the gastric environment.

HUMAN MONKEYPOX: A POXVIRUS ZONOTIC DISEASE

R. Mark Buller, Professor of Microbiology at Saint Louis University, St. Louis, MO

MPXV causes a smallpox-like disease in humans and may threaten the population either as a zoonotic infection or through a criminal event. The case-fatality rate of human monkeypox in a 1980s prospective study in the Democratic Republic of the Congo (DRC former Zaire) was approximately 10%, compared to variola virus (VARV) smallpox, which ranged from >1% to about 15% in Africa and to approximately 30% in Asia. Unlike smallpox, which had secondary attack rates ranging to 60%, human monkeypox during the 1980s prospective study was about 10%, with interhuman transmission rarely over two or three generations. Recent retrospective studies suggest disease incidence is increasing in part due to encroachment of humans into habitats of animal reservoirs for MPXV. Also the first monkeypox outbreak in the western hemisphere occurred in the U.S. Midwest from April to June of 2003. MPXV entered the U.S. in a shipment of African rodents from Ghana (West Africa) destined for the pet trade. At a pet distribution center prairie dogs became infected and in turn were responsible for 72 confirmed or suspected cases of human monkeypox. Unlike African outbreaks, the U.S. outbreak resulted in no fatalities and there was no documented human-to-human transmission. This less severe epizootic could be due to higher natural resistance of the U.S. population, a healthier patient population lacking background infections, better supportive care for patients and/or strain-specific differences in the virulence of the infecting virus. Evidence will be presented to support the existence of monkeypox virus strains that differ in virulence and transmissibility for humans. Opinions

will be offered on the potential of monkeypox virus to evolve into an endemic human disease agent.

THE ECO-EPIDEMIOLOGY OF CHAGAS DISEASE IN NORTHERN ARGENTINA

Ricardo E. Gürtler, Department of Ecology, Genetics and Evolution, University of Buenos Aires, Buenos Aires, Argentina.

Chagas disease, a complex zoonosis caused by *Trypanosoma cruzi* and mainly transmitted by blood-sucking triatomine bugs, is a major public health problem in Latin America. Dogs, cats, opossums and rodents play important roles in domestic and sylvatic transmission cycles that sometimes are linked by different triatomine species. The control of Chagas disease vectors is mostly based on the residual application of pyrethroid insecticides. However, recurrent domestic and peridomestic reinfestation after community-wide spraying threatens the current elimination attempts of *Triatoma infestans*, the main vector in southern South America. As part of a longitudinal study aimed at modeling the transmission dynamics and control of *T. cruzi* in three rural villages in northwestern Argentina, we monitored regularly bug infestations and infection in all houses jointly with measurements of demography, prevalence and incidence of infection in humans and dogs before and after setting up a community-based surveillance system in 1992. In this presentation I will show the long-term impact of control actions on domestic infestation and transmission of *T. cruzi*; the dramatic decrease observed in the prevalence of infection in sylvatic hosts over two decades; the recent finding of hitherto unknown sylvatic foci of *T. infestans*, and its implications for control.

INVASION USA: HOW ALIENS ARE CHANGING OUR WORLD

May Berenbaum, Professor and Head, Department of Entomology, University of Illinois, Urbana, IL

Ever since the 1950s, the U.S. has been repeatedly invaded on the movie screen by unspeakable aliens from all reaches of the universe. While some were extraterrestrial beings from faraway galaxies (like the cosmic spiders from a collapsing black hole that colonized Wisconsin in "The Giant Spider Invasion"), others, like the Deadly Mantis, which terrorized New York before getting trapped in the "Manhattan tunnel", were creatures from the earth's prehistory. All seemed bent on total destruction of the planet. It's just as well they were largely metaphorical because invading movie aliens rarely display the life history attributes consistent with effective colonization of new habitats. Biological invasions, in contrast with cinematic ones, have long been a part of American history-- our shores have been invaded without interruption since the first European vessels docked in American waters. The reality of biological aliens--nonindigenous species that have invaded the U.S.--couldn't be more different from the movie version. Establishment of nonindigenous species constitutes one of the most profound forms of contemporary global change, effecting enormous economic and ecological costs. Impacts on health are disproportionately large in part because invasive species often owe their success to unique chemical defense and because, without coevolved enemies of their own, they can serve as extraordinarily effective disease vectors. Understanding the processes by which invasive species arrive, colonize, and establish in the United States involves the full range of biological subdisciplines, and, beyond that, encompasses social, behavioral, and economic sciences.

EMERGING INFECTIOUS DISEASES AND PUBLIC POLICY

Nina Marano, Acting Associate Director for Veterinary Medicine and Public Health for the NCID, Centers for Disease Control and Prevention, Department of Health and Human Services

The abstract for Dr. Marano's presentation appears as a separate handout in your packet.

ABOUT OUR SPEAKERS

Jonathan Patz, *Associate Professor of Environmental Studies and Population Health Sciences at the University of Wisconsin-Madison*. Dr. Patz directs a university-wide initiative on Global Environmental Health and is also an Affiliate Scientist of the National Center for Atmospheric Research (NCAR). He has served as Co-chair for the Health Expert Panel of the US National Assessment on Climate Variability and Change, Convening Lead Author for the United Nations/World Bank Millennium Ecosystem Assessment, and Lead author on several United Nations Intergovernmental Panel on Climate Change (IPCC) reports and World Health Organization (WHO) monographs on climate change. He currently is Co-Editor for the journal, *Ecohealth: Conservation Medicine and Ecosystem Sustainability*.

Paul Gibbs, *Professor at the Department of Pathobiology at the College of Veterinary Medicine, University of Florida*. Dr. Gibb's recent research has focused on the epidemiology and control of emerging viral diseases of livestock and the relationship of emerging diseases to human and wildlife populations. The use of pox and herpes viruses as vaccine vectors has been integrated into this work. From 1995-1999, he held the position of Director of the University of Florida's International Center where he provided leadership and vision in helping the Colleges at the University of Florida achieve UF's international goals.

Alison Weiss, *Professor, Department of Molecular Genetics, Biochemistry and Microbiology at the University of Cincinnati*. Dr. Weiss' current research focuses on the interaction of bacterial virulence factors with immune defenses, with a strong emphasis on the role of bacterial toxins. I have had a longtime interest in the toxins produced by *Bordetella pertussis*, the causative agent of whooping cough. Pertussis toxin is essential for virulence. This toxin disrupts cellular communication mediated by GTP-binding proteins, and has a profound influence on the ability to mount an effective immune response.

Steven Blanke, *Associate Professor of Microbiology, University of Illinois at Urbana-Champaign*. Dr. Blanke is also a member of the Host Microbe Systems Research Theme in the Institute for Genomic Biology. His current research explores the fundamental molecular and cellular mechanisms used by pathogenic bacteria to establish infection and persist within a host. Additional areas of expertise include molecular and cellular mechanisms of infectious disease, countermeasures to prevent and treat disease, biodefense and national security issues related to biodefense.

R. Mark Buller, *Professor of Microbiology at Saint Louis University*. Dr. Buller's research revolves around the study of viral pathogenesis, the development of therapeutics for orthopoxvirus infections, and the diagnosis of microbial infections. Pathogenesis is an interplay of the genetic expression of the infecting agent and the host's responses to infection, with the dynamics dictating the severity and the outcome of the disease process. His laboratory's goal is to define the genetic basis for poxvirus virulence in the mouse (ectromelia virus) and humans (molluscum contagiosum virus; MCV), as well as the multifactorial host response to infection, which precedes recovery from disease. MCV has a world-wide distribution causing persistent, benign, skin tumors in children, sexually active adults and is an opportunistic infection of acquired immunodeficiency syndrome (AIDS) patients.

Ricardo Gurtler, *Professor, Department of Ecology, Genetics and Evolution, University of Buenos Aires, Buenos Aires, Argentina.*

Dr. Gurtler is the co-principal investigator at the University of Buenos Aires for the Chagas Project. Dr. Gurtler and his team lead field and laboratory studies to elucidate the processes of reinfestation of houses and peridomestic structures by triatomine bugs and *T. cruzi* in northwestern Argentina.

May Berenbaum, *Professor and Head of the Department of Entomology at the University of Illinois at Urbana-Champaign.* Dr. Berenbaum is interested in the chemical interactions between herbivorous insects and their hostplants, and the implications of such interactions on the organization of natural communities and the evolution of species. Her particular research interests focus on the secondary chemistry of the Umbelliferae (=Apiaceae) and the insect associates of these herbaceous plants. Current research approaches insect/plant coevolution at several levels. In 1984, Dr. Berenbaum initiated The Insect Fear Film Festival which has grown into a nationally recognized event. Below in her words is a history of the festival.

Nina Marano, *Acting Associate Director for Veterinary Medicine and Public Health for the NCID, Centers for Disease Control and Prevention, Department of Health and Human Services.* Dr. Marano began in this newly created position in January of 2004 with the goal of helping to meet the critical need for increased partnerships between the human and veterinary medical, research, and public health communities. She works with organizations such as the AVMA, National Association of State Public Health Veterinarians, and Association of American Veterinary Medical Colleges, to explore ways to more fully integrate veterinarians into efforts to address emerging zoonotic infections.

POSTER ABSTRACTS

1. BORRELIA LUSITANIAE IN LARVAL IXODES RICINUS AND TISSUES FROM PODARCIS MURALIS LIZARDS IN TUSCANY, ITALY.

Giuseppina Amore, Laura Tomassone, Luigi Bertolotti, Charlotte Ragagli, Ambrogi Cecilia, Patrizia Nebbia, Elena Grego, Sergio Rosati, Alessandro Mannelli. Department of Animal Production, Epidemiology, Ecology, University of Turin, Italy.

Borrelia lusitaniae, a genospecies of the *Borrelia burgdorferi* sensu lato (sl) group, was recently isolated from a patient with Lyme borreliosis and it is mostly found in *Ixodes ricinus* ticks in southern Europe and the Mediterranean area. Although *B. lusitaniae*-infected larvae were collected from birds in Switzerland (Poupon et al. AEM 72:976-979, 2006), the role of vertebrates as reservoirs for this agent remains to be clarified. In a study that was carried out by Bertolotti et al. (JME 43:159-165, 2006) in Tuscany, central Italy, *B. lusitaniae* was found in 14% and 34% of host-seeking *I. ricinus* nymphs and adults, respectively. It was suggested that geographical distribution of this genospecies might be associated with environmental conditions and with the role of specific reservoir hosts. Because lizards are a main component of wildlife of the Mediterranean area, we searched the agents of Lyme borreliosis in attached ticks and tissues from these reptiles. Out of 91 *Podarcis muralis* lizards that were captured, 68 (74.8%) were infested by *I. ricinus* larvae, and 26 (28.6%) by nymphs. Prevalence of *B. burgdorferi* sl in larval ticks that were collected from lizards was 18.8% (38 PCR-positive out of 202). To the present date, 22 of these amplicons underwent sequence analysis and were classified as *B. lusitaniae*. Prevalence of infection in nymphs was 52.9% (9/17), and all amplicons were *B. lusitaniae*. The same genospecies was found in 2/12 (16.7%) biopsies (tail) and 2/8 (25%) blood samples from lizards. Conversely, *B. lusitaniae* was not found in immature *I. ricinus* and biopsies that were collected from *Apodemus* spp mice and from a limited sample of passerine birds at the same study area. Based on our results, lizards might play a major role as reservoirs for *B. lusitaniae* and might affect the geographic distribution of this pathogen in Europe.

2. MOLECULAR MECHANISMS THE VACCINIA VIRUS M2L PROTEIN USES TO INHIBIT NF-KB SIGNALING PATHWAYS

Olivia Hinthong, Xiao-Lu Jin, Roderick Gedey, and Joanna Shisler.
Department of Microbiology, University of Illinois at Urbana-Champaign.

Nuclear factor-kappa B (NF- κ B) is a pivotal eukaryotic transcription factor that activates the proinflammatory response by upregulating the transcription and expression of anti-viral immune and inflammatory molecules. Inhibition of the NF- κ B pathway is a strategy utilized by many viruses to favor their survival and replication within the host cell. Wild-type strains of vaccinia virus such as Western Reserve (WR) or Ankara inhibit the action of host cell NF- κ B. In contrast, Modified Vaccinia Ankara (MVA), an attenuated vaccinia strain, activates NF- κ B during viral infection. MVA possesses six large deletion regions that account for the loss of many of its immunomodulatory genes. NF- κ B inhibition is restored in MVA when a 5.2kb region of wild type Ankara DNA is re-inserted into the MVA genome. One of the genes present in this region is the M2L ORF. Our lab has created a recombinant MVA/M2L

virus, in which the WR M2L ORF was stably inserted into the Del III region of the MVA genome. This virus allows for investigation of the ability of the M2L protein to inhibit NF- κ B activity. Indeed, the ability of M2L to inhibit NF- κ B activity was demonstrated via electromobility shift and luciferase reporter assays. Previous work by Roderick Gedej demonstrated that MVA infection triggers the MAPK (mitogen-activated protein kinase) signaling pathway, specifically through phosphorylating and hence activation of ERK1/2 (extracellular-signal-regulated kinase 1/2) proteins. Furthermore, activation of the MAPK pathway is *required* for MVA-induced NF- κ B activation. Since M2L expression inhibits virus-induced NF- κ B activation, I hypothesized that M2L inhibited signaling molecules within the MAPK pathway as its mechanism for inhibiting NF- κ B activation. Preliminary data show that the vaccinia virus M2L protein does indeed inhibit in the MAPK signaling pathway: the ERK1 and ERK2 proteins, while phosphorylated in MVA-infected cells, were not phosphorylated in MVA/M2L-infected cells. A plasmid which allows M2L to be expressed independently of infection will also be used in future studies to determine if M2L inhibits ERK1 and ERK2 activated by other reagents. Future studies will determine which step(s) M2L inhibits in the MAPK pathway.

3. SEQUENCE VARIATION WITHIN THE PRION PROTEIN GENE FROM WHITE-TAILED DEER (*ODOCIOLEUS VIRGINIANUS*) OF NORTHERN ILLINOIS

Emily Jewell and Amy Kelly

Illinois Natural History Survey, University of Illinois, Champaign, IL

Chronic Wasting Disease (CWD), a cervid transmissible spongiform encephalopathy (TSE), was first identified in Northern Illinois in 2002. CWD is a neuro-degenerative disease characterized by the accumulation of an abnormal form of the prion protein. In addition to cervids, TSE's are found in sheep (scrapie), cattle (Mad Cow Disease), and humans (Creutzfeldt-Jakob disease). In sheep, certain genotypic variants of the prion protein gene (*Prnp*) offer resistance to scrapie allowing domestic breeders to select for resistant genotypes in attempts to control the disease. Though the efficacy of selection strategies in wild populations is unknown, our goal was to identify possible allelic variants related to CWD disease susceptibility. We sequenced *Prnp* from numerous white-tailed deer in the CWD affected areas of Illinois to determine allelic variation. Tissues were obtained from the Illinois Department of Natural Resources CWD Surveillance program. We obtained DNA sequences by isolation of DNA followed by amplification of the *Prnp* gene locus via PCR. 32 CWD-positive and 118 CWD-negative deer displayed *Prnp* polymorphisms within the range of published sequences. No differences in allele frequencies between positive and negative groups could be associated with susceptibility. Deer sampled were from a restricted geographical area, and all deer within this region display low variation in genotypes at the *Prnp* locus. We continue to analyze data and are increasing sample size and geographic region represented.

Additional animals have been added to the sample pool, bringing the total number of deer sequenced to 157 (37 positive and 120 negative). Results with increased sample size suggest that CWD-positive deer have lower than expected frequencies of polymorphism compared to CWD-negative animals. Four of the nine polymorphic nucleotide loci exhibit

significant linkage between genotype and disease status, but only half of these loci code for changes in amino acid sequences.

4. COLIFORM LEVELS AND ANTIBIOTIC RESISTANCE PATTERNS OF *E. COLI* COLLECTED FROM RIVERS, ANIMAL, AND HUMAN POPULATIONS IN THE CHESAPEAKE BAY WATERSHED RISK FACTORS AND SOURCES OF FECAL CONTAMINATION

¹Johnson, Yvette; ¹Myint, Maung; ²Kaneene, John, ³Donohoe Janice, and ³Steele Stephanie

¹ Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL

² Michigan State University, East Lansing, MI

³ University of Maryland, College Park, MD

The Chesapeake Bay watershed is an economically important but fragile ecosystem. Several sites along the Nanticoke and Pocomoke Rivers have been identified as impaired sites affected by local agriculture. The objective of this study is to compare and contrast antibiotic resistance patterns observed from *E. coli* isolates obtained from wildlife, domestic animal, human, environmental and surface water sources. The hypotheses are that the *E. coli* isolates obtained from wildlife, domestic animals, and human-sourced fecal samples have statistically significant differences in their patterns of antibiotic resistance; and these patterns may be analyzed using multivariate statistical techniques to identify the most probable sources of fecal contamination of *E. coli* obtained from surface water samples.

Ten sites along each of the rivers were selected for quarterly sampling. Standard protocols were followed to determine total coliform and fecal coliform counts. Biochemically confirmed *E. coli* isolates were tested for antibiotic sensitivity using the disc diffusion method. Univariate analyses and multivariable regression modeling techniques have been applied to the dataset to control for potential confounders.

There was no significant difference between total coliform levels in the 2 rivers. However, the Pocomoke River (mean = 70.4 cfu) had a significantly greater fecal coliform level than the Nanticoke River (mean = 31.8 cfu). Antibiotic resistance patterns were similar in both rivers. Low levels of resistance (0-5%) were found for all of the antibiotics except Cephalothin 30, to which 74% of the Nanticoke River isolates and 50% of the Pocomoke River isolates were resistant. Increased risk of elevated coliform levels is associated with medium density residential land-use and increased population density.

Overall, *E. coli* isolates from domestic poultry and livestock displayed to several of the antibiotics tested. Approximately 12% of isolates were resistant to neomycin, 20% were resistant to streptomycin, 50% to tetracycline, 20% to ampicillin, 30% to cephalothin, and 30% to sulfisoxazole. Human-sourced isolates displayed the greatest resistance to cephalothin and tetracycline (48% for each). Wildlife isolates were the least resistant with approximately 25% of the isolates resistant to cephalothin but sensitive to all the other antibiotics tested.

5. A COMPARISON OF WEST NILE VIRUS DETECTION METHODS: TAQMAN RT-PCR AND VECTEST ANTIGEN ASSAY

Nina M. Krasavin, Richard L. Lampman, and Robert J. Novak
Illinois Natural History Survey, Medical Entomology Program, Champaign, IL

Mosquitoes identified as female *Culex* (*Culex*) species, primarily mixtures or uniform batches of *Culex pipiens* Linnaeus and *Culex restuans* Theobald, were collected from gravid traps by two mosquito abatement districts (MADs) in Cook County, Illinois from May or June to September or October in 2002 through 2004. Pools (batches) of mosquitoes were tested by the MADs for West Nile virus (WNV) using VecTest™ WNV antigen assays and later tested for WNV-RNA by the TaqMan™ reverse transcriptase-polymerase chain reaction (RT-PCR). The combined percentage of TaqMan-positive pools from the two mosquito abatement districts (MAD) was 38.7% (n=791 pools) in 2002, 13.7% (n=1676 pools) in 2003, and 28.2% (n=1486) in 2004. The VecTest assays detected about 57% and 69% of the TaqMan RT-PCR-positive pools from Des Plaines Valley MAD (DVMAD) and Northwest MAD (NWMAD) in 2002, but only about 40% and 46% in 2003 and 36% and 55% in 2004, respectively. The gap between the two assays tended to decrease as the transmission season progressed, except in 2002. In that year, the two assays were most alike in August when infection rates indicated a high likelihood of more than one infected mosquito per pool. Based on the 2004 data, the VecTest assay became less effective detecting WNV as TaqMan cycle thresholds increased between 18 and 28. The 95% confidence intervals of annual and monthly infection rate estimates from the two detection methods did not always overlap and the pattern of overlap varied between MADs. Presumably, the gap between assay methods partially reflects the proportion of the vector population in the early stages of the extrinsic incubation period, especially during amplification of transmission within an area. Although the highest infection rates occurred when monthly temperatures were above average in 2002, transmission of WNV continued to be detected in 2003 to 2004, despite cooler monthly temperatures.

6. WEST NILE VIRUS TRANSMISSION IN EAST-CENTRAL ILLINOIS: MOSQUITO INFECTION RATES AND BIRD SEROPOSITIVE RATES

Richard L. Lampman, Emily Wheeler, Mike Ward, Tara Beveroth, Nina Krasavin, Ken Kunkel, Joel Morris, Brendan Heffron, and Robert J. Novak
Illinois Natural History Survey, Medical Entomology Program, Champaign, IL

To better understand local West Nile virus (WNV) transmission cycles, a multi-year study in Urbana-Champaign examined the spatial and temporal distribution of mosquito infection rates and bird seropositive rates. The main West Nile virus (WNV) vectors were *Culex pipiens* and *Culex restuans*, outnumbering detections from all other species by 30 to 100 fold. *Culex salinarius* and *Cx. tarsalis* (two species implicated as major vectors in coastal northeast US and in states west of the Mississippi River, respectively) were present in low abundance and were only rarely positive for WNV. The other WNV-positive mosquito species included *Aedes vexans*, *Anopheles* spp., *Aedes (Ochlerotatus) trivittatus*, *Uranotaenia sapphirina*, and *Orthopodomyia signifera*. The temporal pattern of infection rates in *Culex* from gravid traps and abundance of *Culex* species in from oviposition traps varied annually. *Culex pipiens* and *Culex restuans* have different temporal patterns of abundance with the former peaking in August and the latter in July. Degree-day and maximum temperature exceedance models (threshold of 27°C) successfully predicted *Cx. pipiens* crossover in 2005 to within a day of observed crossover. The peak in *Culex*

infection rates occurred within two weeks of *Cx. pipiens*-*Cx. restuans* crossover in 3 of the 4 study years. The largest number of WNV-antibody positive birds were House Sparrows, Northern Cardinals, American Robins, Mourning Doves, and Gray Catbirds. A bloodmeal analysis of *Culex* specimens was consistent with the avian seropositive rates, verifying the role of *Culex* and urban passerine birds as the primary amplification vectors and hosts. Several of the seronegative bird species were captured in adequate numbers to suggest either their exposure rate was low or their mortality rate was high. The temporal patterns of infection rates in mosquitoes and seropositive rates in birds did not parallel each other, except with juvenile birds. This probably reflects the confounding nature of seasonal bird dispersal patterns and year-to-year survivorship of antibody positive birds. Transmission rates declined in CU since 2003 and there was no 2005 resurgence, as seen in northeastern Illinois (i.e., Cook and DuPage counties). Meteorological variables appear to be critical regulators of WNV transmission.

7. DETERMINING THE MOLECULAR MECHANISM OF THE VACCINIA VIRUS PROTEIN K1L IN INHIBITION OF NF- κ B ACTIVATION.

Kristen Lee, Samir Patel, Xiao-Lu Jin and Joanna Shisler
Department of Microbiology, University of Illinois, Urbana IL.

Poxviruses encode many proteins that interfere with the host cell immune response. One common target for interference by viral proteins is NF- κ B, a transcription factor that activates proinflammatory genes. Infection of cells with wild-type vaccinia virus strains inhibits NF- κ B activation, while infection with the attenuated vaccinia virus strain Modified Virus Ankara (MVA) activates NF- κ B. Since MVA lacks the majority of the immunoevasion genes that are present in the wild-type vaccinia virus strains, our hypothesis is that wild type vaccinia viruses possess genes whose products inhibit virus-induced NF- κ B activation. Our lab identified a 5.2 kb region of DNA from a wild-type vaccinia virus strain, that when inserted into the MVA genome, inhibited NF- κ B activation. One ORF present in this 5.2 Kb region of DNA, encoded a host range protein called K1L. Stable insertion of only the K1L gene into the MVA genome resulted in a recombinant virus that inhibited NF- κ B activation in infected cells. We are interested in elucidating the mechanism of K1L inhibition of NF- κ B activation. Several different cellular proteins activate NF- κ B including PKR, NIK and MAVS. Previous work done in our lab showed that K1L expression in virus infected RK13 cells correlated with decreased phosphorylation of the alpha subunit of the eukaryotic translation initiation factor eIF2. Since eIF2 α phosphorylation is a downstream event of PKR activation, we hypothesize that K1L is inhibiting NF- κ B activation via inhibition of PKR.

8. EXAMINING MODIFIED VACCINIA VIRUS ANKARA INDUCED NF κ B ACTIVATION.

Stefani Martin, Department of Microbiology, University of Illinois, Urbana, IL

NF κ B is a conserved transcription factor that controls proinflammatory and apoptotic genes. Its activation and release from the I κ B inhibitory proteins is dependent upon I κ B kinase (IKK) activation, which in turn is activated by ligand-receptor interactions, including Tumor Necrosis Factor Receptor 1 and Toll-like receptors. Vaccinia virus (VV), the prototypic poxvirus member, inhibits NF κ B activation due to the expression of viral immune evasion proteins. Modified Vaccinia Virus Ankara (MVA) is an

attenuated vaccinia strain that is missing most of its immunomodulatory genes. Therefore, MVA infection induces host NF- κ B activation. Whether this activation is due to 1) a lack of immune evasion proteins in MVA or 2) the expression of a virally encoded protein is unknown. Previous experiments have implicated an early viral gene involved in the Extracellular Receptor Kinase (Erk) pathway may be responsible for the observed activation. To examine the possible viral proteins involved with NF- κ B activation, a VV library has been scanned via luciferase assays. 293T cells were transfected with three plasmids: pNF- κ B-luc, which contains the firefly luciferase gene under the control of an inducible κ B promoter, pRL-null, which contains the sea pansy luciferase gene under a constitutive promoter, and a plasmid from the VV library. If NF- κ B is activated by an overexpressed VV protein, then an increase in the expression of the firefly luciferase protein should be observed. Indeed, screening of the 270 plasmid library yielded approximately thirty VV encoded genes whose products induced luciferase activity at least 5-fold above control cells. One of these genes, B8R, is mutated in MVA. Currently, the mutated form has been cloned into an expression vector, and is being tested via luciferase assay. Identifying the protein(s) responsible for MVA induced NF- κ B activation would lead to a better understanding of the mechanisms behind this pathway during poxvirus infection.

9. METABOLOMIC DIVERSITY IN THE SPECIES *ESCHERICHIA COLI* AND ITS RELATIONSHIP TO GENETIC POPULATION STRUCTURE

Ram P Maharjan and Thomas Ferenci. School of Molecular and Microbial Biosciences, The University of Sydney, Australia

The genomic richness and intra-species heterogeneity of the prokaryotic world is suggestive of extensive biochemical diversity. In this study, metabolomic profiling permitted a phylogenetic assessment of metabolic diversification amongst environmental, medical and laboratory strains of *Escherichia coli*. Strikingly, no two *E. coli* isolates exhibited the same metabolite pool profile. Only 27% of detected metabolite spots in 2-dimensional high-performance thin layer chromatography (2DHPTLC) were found in all strains, indicating that a relatively small core of metabolism is conserved across a species. The population structure determined using metabolomics exhibited clustering of strains in parallel to genetic relatedness, as established by multi-locus DNA sequencing. On the other hand, metabolome patterns did not cluster in parallel with the pathogenicity or environmental origins of strains, but some unique spots were found in most bacteria. These results suggest that great metabolic diversity, to the point of individuality, is likely to be characteristic of a bacterial species. Furthermore, the high resolving power of 2DHPTLC metabolite fingerprinting provides an economic and powerful means of using metabolomics for the analysis of evolutionary relationships and the precise typing of organisms.

10. PACKAGING AND PROCESSING AT THE RETAIL GROCERY OUTLET AS RISK FACTORS FOR BACTERIAL CONTAMINATION OF MEAT

Myint, Maung San and Johnson Yvette Joyce

Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL

Since implementation of the HACCP system for pathogen reduction in processing plants the US Food Safety Inspection Service (FSIS) reports reductions in the prevalence of bacterial pathogen contamination at processing plants. Routine surveillance at retail grocery outlets however, is not conducted by FSIS. Recent studies have found that retail meat and its external packaging may be contaminated with antibiotic resistant bacterial pathogens. The objective of this study was to identify risk factors for bacterial pathogen contamination of raw meat obtained from retail grocery stores.

A cross-sectional study was conducted to estimate the prevalence of contamination by *Salmonella spp.*, *E. coli*, and *Campylobacter spp.* in uncooked chicken, pork, and beef products from retail grocery stores in Maryland and Illinois and to determine whether products processed and packaged for final sale at the processing plant is at a lower risk for contamination than products that are handled and re-packaged at the retail grocery outlet. Preliminary findings indicate that out of 90 poultry meat samples, collected, the overall prevalence of *Salmonella* contamination was 27.8% (C.I 17.97-37.59). Among non-ground products, store packaged products were 26 times more likely to be contaminated with *Salmonella* than processor packaged products (CI 4.6 – 148.1). The *Salmonella* serotypes in the positive samples were: *S. Heidelberg* 56% (14/25); *S. Kentucky* 20% (5/25); and *S. Typhimurium* (Copenhagen) 24% (6/25). Results obtained from beef, and pork samples and antibiotic sensitivity profiles of the isolates will also be presented.

11. METABOLOMIC DIVERSITY IN THE SPECIES *ESCHERICHIA COLI* AND ITS RELATIONSHIP TO GENETIC POPULATION STRUCTURE

Ram P Maharjan and Thomas Ferenci. School of Molecular and Microbial Biosciences, The University of Sydney, Australia

The genomic richness and intra-species heterogeneity of the prokaryotic world is suggestive of extensive biochemical diversity. In this study, metabolomic profiling permitted a phylogenetic assessment of metabolic diversification amongst environmental, medical and laboratory strains of *Escherichia coli*. Strikingly, no two *E. coli* isolates exhibited the same metabolite pool profile. Only 27% of detected metabolite spots in 2-dimensional high-performance thin layer chromatography (2DHPTLC) were found in all strains, indicating that a relatively small core of metabolism is conserved across a species. The population structure determined using metabolomics exhibited clustering of strains in parallel to genetic relatedness, as established by multi-locus DNA sequencing. On the other hand, metabolome patterns did not cluster in parallel with the pathogenicity or environmental origins of strains, but some unique spots were found in most bacteria. These results suggest that great metabolic diversity, to the point of individuality, is likely to be characteristic of a bacterial species. Furthermore, the high resolving power of 2DHPTLC metabolite fingerprinting provides an economic and powerful means of using metabolomics for the analysis of evolutionary relationships and the precise typing of organisms.

12. THE MC160 PROTEIN EXPRESSED BY THE DERMATOTROPIC POXVIRUS MOLLUSCUM CONTAGIOSUM VIRUS PREVENTS TUMOR NECROSIS FACTOR ALPHA-INDUCED NF- κ B ACTIVATION VIA INHIBITION OF I KAPPA KINASE COMPLEX FORMATION

Daniel Nichols, Department of Microbiology, University of Illinois, Urbana, IL

The pluripotent cytokine tumor necrosis factor alpha (TNF- α) binds to its cognate TNF receptor I (TNF-RI) to stimulate inflammation via activation of the NF- κ B transcription factor. To prevent the detrimental effects of TNF- α in keratinocytes infected with the molluscum contagiosum virus (MCV), this poxvirus is expected to produce proteins that block at least one step of the TNF-RI signal transduction pathway. One such product, the MC160 protein, is predicted to interfere with this cellular response because of its homology to other proteins that regulate TNF-RI-mediated signaling. We report here that expression of MC160 molecules did significantly reduce TNF- α -mediated NF- κ B activation in 293T cells, as measured by gene reporter and gel mobility shift assays. Since we observed that MC160 decreased other NF- κ B activation pathways, namely those activated by receptor-interacting protein, TNF receptor-associated factor 2, NF- κ B-inducing kinase, or MyD88, we hypothesized that the MC160 product interfered with I kappa kinase (IKK) activation, an event common to multiple signal transduction pathways. Indeed, MC160 protein expression was associated with a reduction in in vitro IKK kinase activity and IKK subunit phosphorylation. Further, IKK1-IKK2 interactions were not detected in MC160-expressing cells, under conditions demonstrated to induce IKK complex formation, but interactions between the MC160 protein and the major IKK subunits were undetectable. Surprisingly, MC160 expression correlated with a decrease in IKK1, but not IKK2 levels, suggesting a mechanism for MC160 disruption of IKK1-IKK2 interactions. MCV has probably retained its MC160 gene to inhibit NF- κ B activation by interfering with signaling via multiple biological mediators. In the context of an MCV infection in vivo, MC160 protein expression may dampen the cellular production of proinflammatory molecules and enhance persistent infections in host keratinocytes.

13. GIANT FRONTAL SINUS MUCOCELE WITH SECONDARY *Aspergillus niger* INFECTION

Obiageli Ntukogu, MS3, University of Illinois College of Medicine at Urbana-Champaign, Urbana, IL.

John Brockenbrough, M.D. Dept. of Surgery, Otolaryngology/Head & Neck Surgery Carle Hospital, Urbana IL.

Aspergillus niger is a ubiquitous fungi found in soil, etc and often a contaminant in food and water. Despite this organisms prevalence, in the genus of *Aspergillus*, it is one of the least likely candidates to cause disease in an immunocompetent host. With the observed increase in immunocompromised individuals, *Aspergilliosis* infection has become increasingly common. Here we report a clinical case of an immunocompetent 58-year old man who succumbed to an *Aspergilliosis* infection causing chronic sinusitis after an incomplete removal of mucosa during a procedure to obliterate the nasal sinus. This case emphasizes the need for complete sinus obliteration to remove mucosa forming mucoceles. Incomplete removal may provide a rich environment for pathogen growth and this may be detrimental to an immunocompromised patient.

14. DOMAIN EXCHANGE BETWEEN HUMAN TOLL-LIKE RECEPTORS 1 AND 6 REVEALS A REGION REQUIRED FOR LIPOPEPTIDE DISCRIMINATION

Katherine O. Omuetti^{1,3}, John M. Beyer², Christopher M. Johnson^{2,3}, Elizabeth A. Lyle² and Richard I. Tapping^{2,3}
Dept. of Biochemistry¹, Microbiology,² and the College of Medicine³, UIUC, Urbana, IL.

Among the ten human TLRs, TLR2 appears to be unique in its requirement for cooperation with other TLRs, namely TLR1 and TLR6, to mediate cell signaling. Through reconstitution experiments we have more precisely defined the function of these human TLRs. Human colonic epithelial cells cotransfected with TLRs 1 and 2 preferentially respond to a synthetic triacylated bacterial lipopeptide (Pam₃CSK₄). However, examination of a wide variety of lipopeptide derivatives indicate that recognition by human TLRs 1 and 2 does not strictly correlate with the number or position of the acyl chains on the modified cysteine residue. Conversely, human TLRs 2 and 6 exclusively respond to lipopeptides possessing a diacylglycerol group. Surprisingly, we have found that an R-stereoisomer of diacylated macrophage activating lipopeptide-2 (MALP-2) exclusively activates epithelial cells through TLRs 6 and 2, but not through TLR 1 and 2. These results suggest that the chirality of the central carbon of the diacylglycerol group of these agonists is a structural determinant for human TLR recognition. Examination of chimeric receptors, generated by domain exchange between TLRs 1 and 6, has revealed that leucine rich repeats (LRRs) 9-12 of the extracellular domain enable these receptors to discriminate between structurally similar lipopeptides. However, additional chimeric constructs reveal that this region alone is not sufficient to generate receptors that can functionally cooperate with TLR2. Our results support the idea that TLR1 and TLR6 diverged during evolution to differentially recognize natural lipoprotein structures and that this function has been conserved with respect to the human receptors.

This work was supported by start up funds from the University of Illinois as well as NIH grant AI052344 (to RI Tapping).

15. CLASS1 INTEGRON DETECTION AND GENOTYPING OF SALMONELLA SPP. ISOLATED FROM SWINE PRODUCTION SYSTEMS

Sangeeta Rao, Carol W. Maddox, Patricia Hoen-Dalen, Sara Lanka, Ronald M. Weigel
University of Illinois, Urbana, IL

The main objective of this study was to determine if Class1 Integron typing could serve as a screening tool for molecular typing of *Salmonella* isolates. This study was conducted on 520 *Salmonella* isolates collected from environmental drag swabs from 3 swine production units located in Illinois to determine 'on-farm' transmission of *Salmonella* by locating their genotypic clusters based on Rep-PCR using 3 primers: ERIC, BOX and REP. The clusters were considered on dendrogram by taking a cut-off of 85% similarity using Hierarchical cluster analysis. The information of Class 1 integron sizes and antibiotic resistance was depicted on the dendrogram to determine if antibiotic resistance genes (Class 1 Integrons) were associated with the genotype. The Class 1 integron patterns in a separate analysis showed a very high association (p<0.01) with certain phenotypic antibiotic resistances. For the genotypic clusters, each farm was analysed separately. 'Farm R' consisted of 40 isolates with 6 genotypic clusters and no integrons. Farm 'S' consisted of 120 isolates with 4

genotypic clusters, all possessing 1000 bp integron. Farm 'D' consisted of 360 isolates representing 23 clusters distributed across 3 integron patterns of 1000, 1000 & 1200 and 1600 bp. A Fisher exact test was performed to determine the association between the whole genome clusters and the Integrons patterns in 'Farm D'. Thirteen (56.52%) clusters showed a significant ($p < 0.05$) positive association with the presence of a particular pattern of integron and one cluster showed a significant ($p < 0.01$) positive association with 2 integron groups. Other clusters were all non-significant in depicting any association and were all representative of very few isolates. 62-69% of isolates belonging to an integron pattern were predictive of a genotypic cluster. Therefore, the Integron based PCR have been demonstrated to be associated with *Salmonella* genotype and can be used as a preliminary screening technique for the antibiotic resistance patterns.

16. INTRACELLULAR BINDING PARTNERS OF *PASTEURELLA MULTOCIDA* TOXIN (PMT)

Tana L. Repella, Mengfei Ho, Brenda A. Wilson
Department of Microbiology, University of Illinois, Urbana IL

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 intracellular binding partners of PMT. A library screening approach using the BacterioMatch[®] II Two-Hybrid System is being used to identify intracellular target proteins of PMT that are responsible for interacting with PMT and causing the consequent cellular responses. Results from initial library screens have identified a number of signaling proteins as potential intracellular binding targets of the N-terminus or C-terminus of PMT. In addition, probing ProtoArray[™] human and yeast protein microarrays with full-length PMT identified additional signaling proteins as potential intracellular binding targets. Several of the putative PMT-interacting proteins obtained from the two-hybrid assay were verified by co-elution of the proteins via column chromatography. The binding partners of PMT identified so far using these methods include proteins known to be involved in calcium, protein kinase, G-protein, and protein trafficking signaling pathways. Now that potential interactors have been verified using genetic and biochemical methods the NFAT-bla CHO-K1 cell line and the Dual-Luciferase Reporter Assay System are being used to investigate the significance of these interactions inside mammalian cells.

17. A MULTIPLEX TAQMAN REAL TIME RT-PCR FOR IDENTIFICATION AND QUANTIFICATION OF CRYPTIC SPECIES OF THE CULEX PIPPIENS COMPLEX IN NORTH AMERICA

Y O Sanogo, R Lampman, B Danzer, and R J Novak
Illinois Natural History Survey, 607 E Peabody Dr, Champaign, IL 61820, USA

Mosquitoes of the *Culex pipiens* complex and their cryptic species (e.g. *Cx. salinarius*, and *Cx. restuans*) represent the major enzootic vectors of West Nile and SLE viruses in North America. Members of the complex differ in their ecology, physiology, feeding behavior and vector competency, yet they are difficult to discriminate based upon their morphology. Since they are found in sympatry in many ecological niches, field collected mosquito pools used in

arboviral surveillances are frequently co-contaminated with representatives of the group. This can significantly hinder arbovirus surveillance efforts and obscure the prediction of transmission dynamics of mosquito-borne arboviruses. In this study, we developed a TaqMan based multiplex RT-PCR assay to simultaneously detect and quantify sibling species of West Nile virus and SLE virus vectors. Primers and Taqman probes were designed to specifically amplify and determined the relative ratio of each mosquito species in various pools using TaqMan relative quantification assay with the 18S rRNA gene as an invariant internal control. This is the first report of the use of a relative quantification assay in mosquito vector identification. Furthermore, this technique can be used to detect both the vectors and viruses in the same mosquito pool. Our finding represents a step toward the elucidation of the specific role of different members of the *Culex pipiens* complex in the transmission of emerging pathogens, a topic that is still being debated.

18. TOLL-LIKE RECEPTOR 2 AND THE PROXIMAL ADAPTOR TIRAP/MAL CO-LOCALIZE IN MEMBRANE ASSOCIATED LIPID RAFTS IN RESPONSE TO MICROBIAL AGONISTS

Vitaly A. Stepensky^{1,3}, Elizabeth A. Lyle², Richard I. Tapping^{2,3}.

¹Department of Cell and Developmental Biology,

²Department of Microbiology and the

³College of Medicine, University of Illinois, Urbana-Champaign

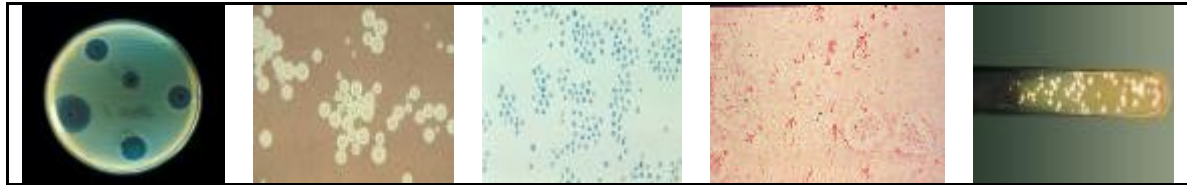
Toll-like receptors (TLRs) are a family of cell surface molecules that activate innate immune responses through direct recognition of microbes. Two intracellular adaptor molecules known as MyD88 and TIRAP/Mal are required for transducing signals from TLR2 receptor complexes. However, our current understanding of the mechanism of TLR mediated signaling in response to microbial agonists, and subsequent recruitment of these adaptor molecules, remains poor. To explore these questions we have generated functional CFP and YFP fusion proteins of TLRs and adaptors and have utilized high resolution confocal microscopy to directly visualize their movement in transfected COS-7 cells. Our data reveals a model whereby TLR2 and TIRAP/Mal are located in separate compartments, the Golgi apparatus and cytosol respectively, in resting cells. Stimulation of cells with synthetic bacterial lipoproteins (TLR2 agonists) induces dramatic rapid movement of TLR2 and TIRAP/Mal into discrete 1-2 μ m domains on the surface of the plasma membrane. These microdomains associate with filamentous actin and also co-stain with cholera toxin indicating that constitute discrete lipid rafts. The movement of TLR2 and TIRAP/Mal into lipid rafts appears to be required for intracellular signaling as the cholesterol depleting agent Nystatin abrogates the formation of these microdomains and completely disrupts cell activation. Dominant negative and siRNA mediated knock down experiments reveal that MyD88 is required for the recruitment TIRAP/Mal to these receptor complexes. Through staining of endogenous TIRAP/Mal and TLR2, and the use of a Rhodamine labeled synthetic lipopeptide agonist, we have verified that all of these molecules colocalize on the surface of primary human monocytes.

19. THE IMPACT OF WEST NILE VIRUS ON BIRDS: A COMPARISON OF SEROPREVALENCE, MOSQUITO BLOODMEAL ANALYSIS, AND AVIAN POPULATION TRENDS

Michael Ward, Tara Danner, Richard Lampman, Nina Krasavin, and Robert Novak. Illinois Natural History Survey, Medical Entomology Program, Champaign, IL

The population trends of 16 species were estimated using three different bird censuses (Breeding Bird Survey, Christmas Bird Count, and Spring Bird Count) before and after the arrival of West Nile virus (WNV) in Illinois. Of the 16 birds whose population trends were estimated three parids, two corvids, and a fringilid showed increasing or stable population before WNV, and significant declines after the arrival of WNV. However, just because a species is declining since the arrival of WNV does not confirm that WNV is the cause. In order to determine the exposure rates of these species we conducted a serology study. We also determine which bird species were most often feed upon by *Culex* mosquitoes (the species of mosquito responsible for WNV). This was done by sequencing blood extracted from the abdomen of mosquitoes. The species that were most often seropositive and whose blood was most often retrieved from mosquitoes were mourning doves, northern cardinals, American robins, and house sparrows. Current data from Illinois suggest perennial outbreaks of WNV in certain locations, and this cycle may lead to long-term declines and local extinction of species most affected by WNV. In contrast, the species that are probably the most important species in the enzootic cycle are unaffected at the population scale and continue to produce susceptible young allowing the epizootic to continue.

CENTER FOR ZOOSES RESEARCH AT A GLANCE



CZR LEADERSHIP

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

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

OBJECTIVES

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.

CAMPUS WIDE MEMBERSHIP

The CZR brings together faculty from 13 departments/units in the Colleges of Veterinary Medicine, Engineering, Applied Life Sciences, Agricultural, Consumer and Environmental Sciences (ACES)

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

9th Annual Conference on New and Re-Emerging Infectious Diseases (April 13-14, 2006)
Veterinary Student Education in Infectious Diseases (Summer Training Program, 2006 - funded by NIH and Merck-Merial)
Collaboration with IDPH on WNV surveillance and emergency preparedness
Global Infectious Disease Research Training Program (NIH, Jan. 24, 2003)
Regional Biocontainment Facility (NIH, Feb. 10, 2003)
Dedication of new biomedical research laboratory space at CVM Veterinary Medicine Basic Sciences Building, (NIH, Nov. 1, 2003)



Center for Zoonoses Research
College of Veterinary Medicine
University of Illinois
at Urbana-Champaign
3515 Basic Sciences Building
2001 South Lincoln
Urbana, Illinois 61802
217/265-8511
www.cvm.uiuc.edu/czr



OUR SPONSORS

ACDIS (Arms Control, Disarmament and International Security)

Established at the University of Illinois at Urbana-Champaign in 1978, the Program in Arms Control, Disarmament, and International Security (ACDIS) is composed of over a dozen core faculty, numerous faculty associates and affiliates, and students—all drawn from over twenty different disciplines. These resident scholars collaborate with visiting researchers such as U.S. Air Force National Defense Fellows and Fulbright Visiting Scholars to pursue progressive and relevant academic research and discuss important issues in international security.

Dramatic changes in the world have brought new challenges for national and international security. The shifts in international relations brought on by the end of the cold war and the "global war on terrorism" demand cutting-edge research. ACDIS pursues innovative and influential research on subjects of global importance.

Fueled by world-renowned University of Illinois faculty from more than twenty different disciplines, ACDIS is one of the most comprehensive international security studies programs in the United States. Whether concerned with nuclear deterrence, conflict management, or energy security, ACDIS faculty, students, and other participants are united in the belief that the university community makes important contributions to understanding and preventing war and other forms of mass violence.

ACDIS enables a constructive public dialogue by addressing some of today's most relevant international security problems. Through regular lecture series and periodic current affairs forums, ACDIS brings together academic experts, students, policy makers, and informed members of the community to examine significant world issues.

ACDIS faculty and graduate students consult frequently with governmental and non-governmental organizations on international security issues. Through conferences, publications, and media events, ACDIS shares the knowledge gained through rigorous study to inform the

The Conservation Medicine Center of Chicago (CMCC)

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 term "conservation medicine" is relatively new, but physicians, veterinarians, public health professionals and ecologists have been independently exploring the concept for the past decade.

It is now common knowledge that people and animals can suffer from some of the same diseases. Those infectious diseases that can be transmitted between animals and humans

are known as zoonoses. The CMCC has set up a core laboratory to investigate potential zoonotic diseases. This Infectious Disease Diagnostic Laboratory is funded by a \$900,000 grant from the U.S. Department of Health and Human Services, Health Resources and Services Administration. The CMCC is applying for more grants to coordinate research and support education.

The Department of Pathobiology at the College of Veterinary Medicine

The Department of 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.

Initiation of lifelong learning skills is the goal of departmental teaching at the professional, graduate, and undergraduate levels. The department is responsible for teaching in both the basic science and clinical portions of the veterinary curriculum, providing veterinary students with the basic knowledge required for their clinical years and skills in diagnostic medicine.

The department has a strong graduate program that attracts students with BS, MS, or DVM degrees. Graduate degrees can be obtained in conjunction with the MD degree (Medical Scholars Program), the DVM degree (Veterinary Medical Scholars Program) or pathology residency. Virtually all students receive tuition waivers and stipends. Graduate students participate in both the teaching and research functions of the department. The ultimate goal of the graduate program is to produce leaders in biomedical research and education for the 21st century. Continuing education courses, such as Molecular Biology and Industrial Toxicology and Pathology are also offered. The department also participates in undergraduate teaching, but to a lesser extent. Computer technologies are used to enhance instruction and develop distance learning, which is critical to education today and in the future.

The Environmental Council at the University of Illinois

Appointed by the Provost, members of the Environmental Council serve three-year terms guiding environmental activities at the University of Illinois. Representing all campus departments, Environmental Council members focus on attracting interdisciplinary talent to advance environmental initiatives. Providing research and educational opportunities for students and faculty, the Environmental Council capitalizes on the University of Illinois' intellectual strengths and resources to achieve and lead environmental excellence.

Our Objectives

Create citizen environmental leaders who understand basic relationships among ecology, human behavior, technology and scientific discovery and can make informed decisions about business, management, and politics.

Encourage "Citizen Science" to grapple with the most important environmental problems that affect people, neighborhoods, communities, governments, nations and the world.

Focus on national security as it relates to the environment by dealing with long term, complex environmental systems that can improve political and cultural stability.

Shape future leaders of all professions so that they can go forward into the world with an understanding of how our environment intersects with the choices that are made both personally and professionally.

Promote interdisciplinary engagement by bringing together the best minds in their respective fields to build consensus about how to handle the environmental problems facing real people in the Great Lakes Region and around the world.

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

Faculty: Brenda A. Wilson (Theme Leader, Microbiology), Steven Blanke (Microbiology), H. Rex Gaskins (Animal Sciences), Lois L. Hoyer (Veterinary Pathobiology), Gary J. Olsen (Microbiology), Abigail A. Salyers (Microbiology), James M. Slauch (Microbiology), Richard I. Tapping (Microbiology), John L. Xu (Microbiology), Cheng-Xiang Zhai (Computer Science)
Affiliates: Jiawei Han (Computer Science), Steven R. Leigh (Anthropology), Lawrence B. Schook (Animal Sciences), Rebecca M. Stumpf (Anthropology)

What is the relationship between humans and the abundant, complex, and dynamic microbial populations that live inside of us? Many of these microbes exist as commensals that, by definition, live in association with the host without causing disease. Indeed, the relationship is often mutually beneficial. Yet the dynamic interactions between a host and its microbiota in the presence of a host immune response are poorly understood. This is ironic, in that these interactions greatly affect human health. The study of microbial diseases has focused traditionally on single, clearly defined interactions between the host and frank pathogens. This approach, however, discounts the complexity of the interactions present, as the normal microbiota is a key player in this system.

The overall goal of the Host-Microbe Systems (HMS) Theme is to exploit genomic technologies to study the dynamic interactions between the host and its commensal as well as pathogenic microbes. The HMS 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 HMS 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, their relationship to development of immune responsiveness to normal and abnormal microbiota, and the immune system's inability to prevent or resolve these infections. Researchers will study the role of normal vaginal microbiota in preventing genital tract infections and the impact of microbiota composition on

susceptibility to certain groups of pathogens, including those responsible for sexually transmitted diseases, bacterial vaginosis, yeast vaginitis, and pelvic inflammatory diseases.

The HMS Theme has established collaborations with Carle Hospital and several National Primate Research Centers to obtain vaginal samples from both overtly healthy and symptomatic women and nonhuman primates. Modern genomic, proteomic and metabolomic technologies will be applied to survey the genetic, metabolic and immunologic content (such as cytokines, antibodies, antimicrobial peptides) of the human and nonhuman primate vaginal ecosystem. The evolutionary and comparative biological components of the proposed project have the potential to address contemporary human biomedical questions at a level that is unprecedented, offering a unique window on both biological and cultural issues that affect women. The broad scope offered by inclusion of other primates could address human biomedical questions by establishing an evolutionary and comparative biology context that considers environments, microbes, and host immune systems.

Other goals include:

- Identifying the components of the microbial population in the vagina
- Studying the population dynamics of the vaginal ecosystem and the conditions under which they change
- Understanding the overall physiology and metabolism of both the microbes in the vaginal ecosystem and the host
- Enhancing our understanding of vaginal host-microbe interactions, especially those interactions that involve susceptibility to and pathogenesis of polymicrobial infections
- Defining animal model systems that are most ideal for studying human disease

The research will address several questions of profound biomedical significance with broad applications in women's health, evolutionary biology, conservation, global population dynamics, and animal models of human disease. Theme research will have significant commercial implications by providing new screening and diagnostic technologies, developing novel intervention strategies, and identifying new targets for drug development, improved vaccines, and alternative treatment modalities.

International Programs and Studies

Complementing the University of Illinois' role as a leader in the nation, International Programs and Studies (IPS) coordinates, promotes, and supports international activities on campus. Through its various offices and units, IPS fosters international expertise by facilitating faculty and student research abroad, coordinating international exchanges and study abroad, supporting international scholarly activities on campus, and leading public engagement in international affairs.

Our Vision

To become a recognized academic institutional leader of quality international education and innovation committed to the development and dissemination of knowledge and enhancing global citizenship.

Our Mission

International Programs and Studies coordinates and promotes the international dimension of teaching, research, and public engagement at the University of Illinois at Urbana-Champaign through:

- Advancing International Research.
- Enhancing the resource base for international activities.
- Facilitating international exchange.
- Fostering the internalization of the curriculum.
- Leading the campus efforts to internalize public engagement.
- Promoting multidisciplinary international studies.