EIGHTH ANNUAL CONFERENCE ON NEW AND RE-EMERGING INFECTIOUS DISEASES

Hosted by

THE CENTER FOR ZOONOSES RESEARCH

UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

APRIL 21-22, 2005

The Levis Faculty Center

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Our Friday afternoon combined panel on avian influenza and rabies is co-sponsored by CZR and the Conservation Medicine Center of Chicago.

CONFERENCE SCHEDULE

Thursday, April 21, 2005 - Levis Faculty Center	
5:00 PM	Welcome Reception (2nd floor)
6:00 PM	Welcome by Dean Herbert Whiteley, CVM (3rd floor)
6:15 PM	Dr. Lynn Enquist, Professor and Chair, Department of Microbiology, Princeton University. Presenting: *"***Visualizing directional movement of an alphaherpesvirus in neurons."**
Friday, April 22, 2005 – Levis Faculty Center	
8:00 AM	Conference Registration & Poster Set-up
8:30 AM	Conference Introduction & Welcome
8:45 AM	Dr. David Williams, Associate Professor, Biological Sciences, Illinois State University, presenting: "**Redox biochemistry and drug development for schistosomiasis"**
9:30 AM	Dr. Ana Calvo, Assistant Professor, Department of Biological Sciences, Northern Illinois University, presenting: "**The velvet gene, veA, is necessary for normal fungal development and biosynthesis of natural products"**
10:15 AM	Coffee Break/Poster Viewing (4th floor)
10:45 AM	Dr. Marcia Castro, Assistant Professor, Department of Geography, University of South Carolina, resenting: "**Environmental change and malaria: a comparative analysis of Dar es Salaam, Tanzania and the Brazilian Amazon"**
11:30 AM	Dr. Carmel Ruffolo, Assistant Professor, Molecular Microbiology and Bioinformatics , University of Wisconsin-Parkside, presenting: **"The Nudix hyrodlase of *Pasteurella multocida* and its role in pathogenesis."**
12:15 PM	Lunch and poster viewing (4th floor)
1:30 PM	Dr. Alessandro Mannelli, College of Veterinary Medicine, Universita' degli Studi di Torino, presenting: "**Epidemiology and control of avian influenza in the main poultry production area of Italy."**
2:15 PM	Dr. Peter Clyne, Assistant Director, Wildlife Conservation Society's Asian Program, presenting: **"Conservation aspects of the avian influenza outbreak in Asia."**
3:00 PM	Poster viewing and break (4th floor)
3:30 PM	Dr. Thomas Muller, Institute for Epidemiology, WHO Collaborating Centre for Rabies Surveillance and Research, Federal Research Institute for Animal Health, presenting: **"Fighting rabies - a European track record."**
4:15 PM	Dr. Stan Gehrt, Assistant Professor in Wildlife Ecology and Wildlife Extension Specialist, School of Natural Resources, The Ohio State University, presenting: **"The movement of raccoon rabies in wild populations and the effect of control measure."**

PRESENTATION ABSTRACTS

VISUALIZING DIRECTIONAL MOVEMENT OF AN ALPHAHERPESVIRUS IN NEURONS

L. W. Enquist, Professor and Chair, Department of Molecular Biology
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The alphaherpesviruses have evolved to enter the peripheral nervous systems of their natural hosts and establish a quiescent infection (latency) in peripheral ganglia that can be reactivated. The spread of infection is through chains of synaptically connected neurons. The biology of pseudorabies virus (PRV), a swine herpesvirus with a broad host range, is of some interest because certain attenuated mutants can spread only in one direction and are used in tracing neural circuitry. The viral genome inside capsids is moved long distances in axons in a controlled fashion on microtubules. Entry requires movement from axon terminals to cell bodies and, after reactivation, egress requires movement back to axon terminals in the periphery (and only rarely to the central nervous system). In particular, I will discuss on-going imaging experiments to visualize the dynamics of long distance axonal transport during entry and egress of individual PRV virions tagged with GFP or mRFP in cultured peripheral nervous system neurons.

REDOX BIOCHEMISTRY AND DRUG DEVELOPMENT FOR SCHISTOSOMIASIS

David Williams, Associate Professor, Department of Biological Sciences,
Illinois State University

Schistosomiasis is an important, debilitating disease affecting ~250 million people in more than 70 countries. The annual mortality of this disease is estimated to be ~280,000 in sub-Saharan Africa, while 20 million individuals suffer from extreme disability. In the coming years the Bill and Melinda Gates Foundation and endemic country programs will treat tens of millions of people with the single anti-schistosomiasis drug in widespread use. There is already clinical and laboratory evidence for the existence of praziquantel resistance parasites and widespread use is expected to generate strong selective pressure for drug resistant. There is an urgent need for new anti-schistosome drugs.

Schistosoma mansoni, a causative agent of schistosomiasis, resides in the bloodstream of their host for up to 30 years without being eliminated by the host immune attack. One proposed survival mechanism is the production of an

antioxidant “firewall” that neutralizes the oxidative assault of the host’s immune attack. Our recent work strongly supports the hypothesis that this antioxidant firewall is a potential weakness of the organism. Peroxiredoxins (Prx) appear to be the main parasite enzymes for reduction of hydrogen peroxide. Our recent work indicates that parasite Prx proteins are biochemically distinct from human Prx and are essential parasite enzymes. Moreover, schistosomes lack two enzymes essential for redox metabolism in mammals, glutathione reductase and thioredoxin reductase. Instead, schistosomes utilize a single, novel enzyme, which provides both reductase functions as well as a significant portion of the glutaredoxin activity in the worm. Our recent work indicates that this parasite enzyme is essential for worm survival. Because parasite redox proteins are essential for survival and are substantially different from host redox proteins we propose that schistosome redox proteins are valid targets for the development of drugs to treat schistosomiasis.

THE VELVET GENE, VEA, IS NECESSARY FOR NORMAL FUNGAL DEVELOPMENT AND BIOSYNTHESIS OF NATURAL PRODUCTS

Ana Calvo, Assistant Professor, Department of Biological Sciences, Northern Illinois University

The detrimental impact of *Aspergillus* spp. on health includes opportunistic infections, allergic reactions and mycotoxin contamination of food. Immunosuppressed patients are especially vulnerable to opportunistic infections. The most common species causing infection are *Aspergillus flavus, Aspergillus terreus*, and *Aspergillus fumigatus*. Primarily these *Aspergillus spp*. can cause infection in the respiratory track that can later become systemic. The mortality by pneumonia caused by *Aspergillus* is approximately 85%. Outbreaks in hospitals are associated with contaminated ventilation systems. Additionally, *Aspergillus* antigens are fungal allergens and may initiate allergic bronchopulmonary aspergillosis. Besides constituting a threat to immunocompromised patients, *Aspergillus flavus*, as well as *Aspergillus parasiticus* produce the most carcinogenic natural product yet described, aflatoxin. These species are found to contaminate diverse oil seed crops worldwide. Additionally, these species form resistant structures called sclerotia that allow the fungus to survive for years until conditions are favorable again for growth. Our goal is to find possible molecular strategies to control these detrimental effects. We have found a genetic link, velvet or veA, between mycotoxin biosynthesis and resistant structure production in *Aspergillus flavus*, *Aspergillus parasiticus* and in the model fungus *Aspergillus nidulans*. *veA* is conserved not only in the genus *Aspergillus* but across fungal genera. Furthermore, *veA* appears to be unique to fungi. For this reason elucidating the *veA* mechanism of action could be useful to reduce harmful effects and to enhance beneficial properties of a variety of filamentous fungi.

ENVIRONMENTAL CHANGE AND MALARIA: A COMPARATIVE ANALYSIS OF DAR ES SALAAM, TANZANIA AND THE BRAZILIAN AMAZON

Marcia Castro, Assistant Professor, Department of Geography, University of South Carolina

The study characterizes and compares the interrelationships between environmental change and malaria transmission in two different socio-cultural and ecological settings. The first portrays a scenario of urban expansion, while the second illustrates the opening of new human settlement areas in the tropical forest with accompanying rapid urban development.

The urban area of Dar es Salaam, Tanzania was selected as a case study for the first setting. The city started as a small trading center established during the European colonization, and became the most densely populated area in Tanzania. Moreover, it is the city with the most rapid population growth in east Africa. Since 1965, it is estimated that approximately 69% of the population growth was caused by rural-urban migration. Regarding malaria, the disease is the leading cause of outpatients in hospitals and clinics, deaths of hospitalized people, and admissions of children under 5. It represents one of the most important obstacles to economic development and investment in Tanzania.

Machadinho, a settlement project located in the Northwestern portion of the Brazilian Amazon, in the State of Rondônia (RO), was selected as a case study for the second setting. Machadinho was opened for occupation in late 1984 and shortly thereafter severe outbreaks of malaria were observed. Four waves of data collected in the area show that malaria was epidemic in the early years of occupation, when severe disturbances to the forest were happening. Ten years after the occupation malaria is endemic, revealing a unique pattern of malaria transition over time.

The comparison of those two settings reveals important differences regarding the level of malaria rates and the strategies for disease control. However, at a deeper level, there are remarkable similarities mainly driven by the types of environmental changes that each area observes.

THE NUDIX HYUDROLASE OF PASTEURELLA MULTOCIDA AND ITS ROLE IN PATHOGENESIS

Carmel Ruffolo, Assistant Professor, Molecular Microbiology and Bioinformatics, University of Wisconsin-Parkside

Nudix hydrolases are a super-family of enzymes which all contain a distinctive catalytic Nudix motif. The motif is thought to be involved in the hydrolysis of nucleoside diphosphate derivatives. The PnhA enzyme of *Pasteurella multocida* is a Nudix hydrolase which belongs to the subfamily of dinucleoside oligophosphate pyrophosphatases. *P. multocida* is a Gram-negative bacterial pathogen that causes a variety of respiratory and haemorrhagic diseases in a broad range of hosts. *P. multocida* is the aetiological agent of fowl cholera in avian species, haemorrhagic septicemia in cattle, atrophic rhinitis in swine, and pneumonia in sheep and cattle. The PnhA enzyme hydrolyzes diadenosine *tetra*-, *penta*-, and *hexa*-phosphates with a preference for diadenosine *penta*-phosphate, from which it forms ATP and ADP. The enzyme requires a divalent metal cation Mg^{2+} or Mn^{2+} and prefers an alkaline pH of 8 for optimal activity.

PnhA shares strong similarity to two other members of the Nudix hydrolase family, IalA of *Bartonella bacilliformis* and *ygdP* gene product of *Escherichia coli.* Both proteins are required for conferring an invasive phenotype, allowing each respective bacterium to invade their particular host cell type. The *pnhA* gene, which encodes for PnhA, was mutated in the *P. multocida* strain ACP13 and resulted in 60% reduction in cell size. Wild-type and ACP13 strains were tested for virulence by using two models, the chicken embryo-lethality model and the amoebal co-infection model. When compared to the wild type strain ACP13 was found to be up to 1000-fold less virulent in both models. This study was the first study to use an animal model to assessing the virulence of a bacterial strain that lacked a dinucleoside oligophosphate pyrophosphatases. The results suggest that PnhA which catalyzes the hydrolysis of diadenosine *penta*-phosphates may also play a role in facilitating *P. multocida* pathogenicity in the host.

EPIDEMIOLOGY AND CONTROL OF AVIAN INFLUENZA IN THE MAIN POULTRY PRODUCTION AREA OF ITALY.

A. Mannelli, Capua I., Dalla Pozza M., Ferrè N., Toson M., Guberti V., Marangon S.
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Avian influenza virus are maintained in wild fowls. Following the transmission to domestic poultry, the virus can mutate and provoke disease of varying severity: low pathogenic avian influenza (LPAI) and highly pathogenic avian influenza (HPAI). Moreover, the zoonotic potential of the infection became most evident in recent years. Northern Italy, were the majority of the country's poultry production is concentrated, was struck by HPAI in 1999 – 2000 causing the destruction of 13 million birds. Subsequently, LPAI was repeatedly reported. Control measures included vaccination. Changes in the structure of poultry industry and surveillance on wild fowl population are presently implemented.

AVIAN INFLUENZA – A WILDLIFE CONSERVATION PERSPECTIVE

Peter Clyne, Assistant Director, Wildlife Conservation Society's Asian Program

The widespread fear of an avian influenza pandemic poses a serious threat to the conservation of wildlife. First, there are signs that the public is beginning to view wild birds as an enemy. Second, discussions amongst government officials continue about culling wild bird populations. Finally, there remains the possibility of avian influenza depleting vulnerable bird populations.

However, this same fear also represents an opportunity for improving wildlife conservation. A clear understanding by the public and government sectors of the role of the "wet markets" of SE Asia in the genesis and dissemination of avian influenza may lead to stronger regulation of these markets, and thereby increase the cost of wildlife trading.

FIGHTING RABIES – A EUROPEAN TRACK RECORD

Thomas Müller, Thomas Selhorst, Friedrich-Loeffler-Institut, Federal Research Institute for animal Health, WHO Collaborating Centre for Rabies Surveillance and Research, D-16868 Wusterhausen, Germany,

In Europe, oral vaccination of foxes against rabies (ORV) has been developed into the preferred method to control and ultimately eradicate fox rabies. During the first field studies with ORV in Switzerland at the end of the 1970s the feasibility of ORV was clearly demonstrated. Hence, in the early eighties and nineties oral rabies vaccination programs were implemented in west and middle European countries, respectively. During the last decade more and more eastern European countries have also launched field trials.

Next to efficient vaccines a suitable baiting strategy is essential. A baiting strategy and, hence, its success is defined by several parameters such as the bait density, the mode of bait distribution, the seasonal timing of vaccination campaigns, the selection and size of vaccination areas, and their mode of enlargement. Because only few scientifically validated recommendations concerning the partial effect of the above mentioned influential parameters were available at that time, most decisions had to be made on the basis of assumptions: Approximately 15 baits per km² were distributed twice a year, in spring when the fox density was at their lowest levels, and in autumn when the juveniles started to disperse. The initial success of the campaigns supported the concept of a spring and autumn campaign and was copied by many other countries. The rapid enlargement of vaccination areas favored the implementation of aerial distribution supported by ongoing technical improvements. Unfortunately, in the following years set backs were observed in several countries and the complete eradication of vulpine rabies in the European countries practising ORV was protracted. The increased fox population density was often blamed for the difficulties encountered during the final eradication phase. It was assumed that to control rabies with ORV in areas with an increased fox density, the overall vaccination coverage had to increase as well. Several studies had found a positive relationship between the number of baits distributed and bait-uptake. Consequently, in order to increase the vaccination coverage the number of baits was often increased in areas were rabies persisted. Nowadays, it is widely accepted that an increased bait density does not necessarily result in a higher bait uptake by the target species. As a result of the setbacks encountered, baiting strategies were adapted or even completely new concepts were introduced; e.g. additional vaccination campaigns, double baiting, and distribution of baits at fox dens. All these concepts aimed at maximizing the overall vaccination coverage. This was not only achieved by increasing the number of baits and/or campaigns, but also by optimising the timing of the campaigns (e.g. avoiding the effects of maternal immunity) and bait distribution pattern (e.g. flight line spacing). The experience gained after 25 years of ORV in Europe has clearly demonstrated that it is without any doubt the most (cost-)effective way to control and eradicate wildlife rabies but also that there is no single universal approach. Baiting strategies have to be adjusted to the local situation.

Oral vaccination is without any doubt one of Europe’s success stories. Rabies could be eliminated in large parts of Western Europe and Germany and as a result, several European countries have become rabies-free: Belgium, Luxembourg, France, Italy, Switzerland, Finland and the Netherlands. Even in middle European countries big progress in rabies control has been made due to this modern and powerful method of rabies control. In contrast to other European countries, Germany is facing rabies in urban settlements in the final phase of eradication. The local increase in the number of rabies cases and the resulting spread of rabies in a limited area in recent years are mainly due to (i) increased fox densities (ii) the persistence of rabies in areas with a extremely high density of settlements in which ORV is severely hindered, (iii) inconsistent vaccination,

e.g. missing complementary distribution of baits per hand in non-flying zones and (iv) other priorities giving rabies control not the awareness it needs especially in the final phase of eradication. Corrective actions have been taken to adapt the vaccination strategy to peculiar topographical features of a fragmented landscape.

THE IMPORTANCE OF HOST POPULATION DYNAMICS FOR UNDERSTANDING AND MANAGING WILDLIFE DISEASE

Stan Stanley Gehrt, Assistant Professor in Wildlife Ecology and Wildlife Extension Specialist, School of Natural Resources, The Ohio State University

Ecological aspects of host populations are important components of the wildlife and parasite relationship, yet host dynamics are often underappreciated in attempts to understand and control wildlife disease. Herein, I will discuss the role of our field research on host ecology and it's relevance to the understanding and control of raccoon rabies. Raccoon-strain rabies is an emerging infectious disease that has spread across the Atlantic coast with tremendous health and economic costs. Our research has focused on population dynamics of raccoons as they relate to rabies. Factors such as density, movement patterns, and habitat use, were used to construct a spatially based dynamic model of raccoon rabies. Our field research and modeling efforts also focused on a common control strategy for raccoon rabies, which is the distribution of oral rabies vaccines for raccoons. This type of control relies heavily on the distribution, abundance, and movement of raccoons, and much design in bait distribution and their resulting efficacy has not been thoroughly evaluated. As an interesting side note, our collaborative modeling efforts have also provided the incentive for new research in raccoon behavior. The rabies example illustrates the role that ecology can play in disease control.

POSTER ABSTRACTS

1. USE OF *BRUCELLA ABORTUS* STRAIN RB51 IN KOREA: EFFICACY AND SAFETY TRIAL IN DAIRY AND NATIVE BEEF CATTLE

Byeong Kirl Baek [1], DVM, Ph D., Matsuda Kiku [1], DVM, Ph D., Gae Myung Jung[1], DVM, MS., Sung Ill Lee[2], DVM, Ph D., Chang Hyun Kim[3], MS. and Ibulaimu Kakoma[3], DVM, Ph D.

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A study was carried out to evaluate the efficacy of *Brucella abortus* strain RB51 vaccine(RB51) under Korean field conditions as an alternative or complementary strategy to the current official eradication program that is based solely on 'test and slaughter'. One dairy herd(273 animals) and one indigenous Korean cattle farm(29 animals) known to be infected with *B. abortus* were selected for experimental vaccination using *B. abortus* RB51 vaccine. A comparison was made between these two farms and a third farm(373 animals) where there was no prophylactic intervention in Korean indigenous cattle(Han Woo) farm.

RB51 (4.0×10^9 cfu) was subcutaneously inoculated into 255 Holstein cattle and 19 animals of the Han Woo breed. These herds were screened by tube agglutination test (TAT) and plate agglutination test (PAT) using the routine surveillance system according to the official brucellosis protocol. The herds were monitored until they were verified to be brucellosis-free. Animals that were serologically positive or suspicious were condemned to slaughter and farmers were compensated accordingly.

Prior to vaccination, the prevalence rate was 10-20%. By 16 weeks post vaccination there was no evidence of brucellosis based on the PAT and TAT results. In certain farms where there was no intervention with RB51 the seroprevalence remained unchanged or continued to increase until all animals were slaughtered and the farm closed.

These results demonstrate that RB51 is a suitable vaccine strain for prophylaxis against bovine brucellosis in both dairy and indigenous Korean cattle based on significant differences in seroprevalence/slaughter between vaccinated and non-vaccinated farms.

2. HUMAN BRUCELLOSIS IN KOREA: AN EMERGING ZOONOSIS OF SIGNIFICANT PROPORTION

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Bovine brucellosis was first reported in Korea in 1956 and was traced to an infected imported animal. The population of brucellosis-infected cattle rose from 1,088 in 2003 to 4,076(4-fold) in 2004. These figures are based on surveys on female animals (≥1 year old) only since male animals are not mandated in the government campaign. Human brucellosis was first suspected in 1999 and definitively diagnosed in 2002 with 1 case. In spite of the 50 year program of "test and slaughter" of infected cattle, the human disease has continued to increase dramatically over the last 3 years with 16 cases in 2003, 46 cases in 2004 and it is speculated that higher numbers of cases will be reported for 2005, given the trend so far. There is no brucellosis vaccine suitable for human use.

The majority of the afflicted individuals were male farmers, veterinarians and veterinary assistants. A significant risk factor, as expected, is close contact with ruminant animal fluids or ingestion of unpasteurized milk. The etiologic agent involved is so far considered to be *Brucella abortus* but the possibility of involvement of other species cannot be precluded.

To-date there has not been any human fatality attributed solely to brucellosis and patients appear to respond well to the recommended treatment with rifampicin-doxycycline combination over a 6 week period as recommended by the Korean Ministry of Health.

The trend of human brucellosis in Korea is suggestive of an important emerging disease. It is reasonable to assume that the origin of human brucellosis is largely from farm animals and dogs and that controlling bovine brucellosis by vaccination offers the best option for eradicating the disease in human populations.

3. HIGH PREVALENCE OF BORRELIA LUISTANIAE AND SPOTTED FEVER GROUP RICKETTSIAE IN HARD TICKS IN TUSCANY, ITALY

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In Italy, the risk of tick borne zoonoses (TBZ), such as Lyme Borreliosis and Mediterranean Spotted Fever varies within short distances, and factors affecting the dynamics of pathogen transmission are largely unknown. Different species of spirochetes belonging to the Borrelia burgdorferi sensu lato group differ in their ecology and in the capability of causing disease in people. Moreover, other, newly recognized pathogens can be found in tick vectors. Ecological studies and the characterization of agents of TBZ are therefore of major importance for Public Health. In this study we collected host-seeking ticks and material from rodents and passerine birds in an area in Tuscany were cases of Lyme Borreliosis were previously reported. DNA was extracted from samples using a commercial kit. To verify the correct extraction of each sample, we amplified a 450bp region in the 16s rDNA gene of ticks. The detection of *B.burgdorferi* s.l. was carried out by Touch Down Polymerase Chain Reaction (TD-PCR). Primers were specific for the intergenic spacer region included between genes codifying for 5s and 23s rRNA subunits (amplicon's length: 220 bp). The presence of *Rickettsia* spp. was investigated by using a specific PCR which amplifies a 450 bp fragment of the OmpA gene. *B.burgdorferi* s.l. was found in 22.1% of host seeking *I.ricinus* (n=167) and in one out of 12 *Rhipicephalus* spp.. 84% of *B.burgdorferi* s.l. strains belonged to *B.lusitaniae*. Spotted Fever Group Rickettsiae were found in 38.5% (strain IrITA2 and IrITA3) of host seeking *I.ricinus* (n=26) and in 50.0% (strain Bar29) of host seeking *Rhipicephalus* spp. (n=12). *B. afzelii* was found in an ear biopsy from an *Apodemus* mouse, whereas 11 *I.ricinus* larvae collected from the same mouse were infected by *B. garinii.* Recently, *B.lusitaniae* was isolated from the skin of a Lyme disease patient in Portugal. The possible pathogenic power and the high prevalence of this genospecie lead our study to investigate principally the *reservoirs* animals. To identify the *B.lusitaniae* hosts we are using a method based on the analysis of the blood meal remnant in the tick gut.

4. AQUATIC HABITAT CONNECTIVITY AND THE DISPERSAL POTENTIAL OF *BULINUS NASUTUS* IN COASTAL KENYA.

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Human infection with the trematode *Schistosoma haematobium* remains a major cause of disease along the southern coast of Kenya where suitable habitats (e.g., ponds and temporary water pools) for *Bulinus nasutus* snails, intermediate hosts of the parasite, are abundant. Fine resolution satellite imagery and field data from aquatic habitats were used to elucidate the potential dispersal pathways of *B. nasutus* snails, and then identify probable locations of snail aestivation. In Msambweni, water bodies undergo vast intra- and inter-annual fluctuations in size and depth, and may even dry up for months. Until now, the ponds in the area have been studied as if they were unconnected. However, recent field data indicate that many of these ponds are in fact connected at least during and following sufficient rains. Thus, even if all the *Bulinus* snails are eliminated from a pond, repeated reintroductions of *B. nasutus* are likely to result in reinfestation. Determining the spatial patterns of connectivity between aquatic habitats allows for identification of potential dispersal pathways of *B. nasutus* snails, and needs to be considered prior to the implementation of control measures targeting *B. nasutus* snail populations.

5. GROWTH KINETICS OF EHRLICHIA CANIS IN 030F CELL-LINE

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Ehrlichia canis (*E. canis*), the etiologic agent of canine ehrlichiosis, is a small pleomorphic obligatory intracellular gram-negative bacterium. The organism replicates in membrane-bound vacuoles in the cytoplasm of eukaryotic cells, chiefly canine monocytes. *Ehrlichia canis* is tick-transmitted and causes a subclinical or clinical disease called characterized by fever, anorexia, hematological abnormalities, lymphadenopathy and elevated liver enzyme

activity. The organism was first described in the 1930s, but a systematic investigation on the pathogenesis of the associated disease has been largely hindered by lack of a reliable quantitative assay. Using state-of-the art molecular biological tools, quantitative real time PCR (QRT-PCR), a reliable assay suitable for quantification of *E. canis* propagating in an *in vitro* culture system has been developed. The assay was validated against a conventional manual enumeration method. A positive correlation was demonstrated between scaled *E. canis* DNA concentration and the *E. canis* morulae counts. The assay made it possible for the first time to delineate the growth kinetics of *E. canis* in the canine myeloma 030F cell-line. It was demonstrated that 030F cells support the survival and proliferation of *E. canis* to a level of 90-100% infectivity. A prepatent (lag) period of 7 days, and a log phase of 7-17 days, respectively, were established. A stationary phase started at day 17 and persisted until the experiment was terminated.

6. EVALUATION OF STREPMYCIN-RIFAMPICIN TREATMENT IN SPRAGUE-DAWLEY RATS INFECTED WITH *BRUCELLA ABORTUS*

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Thirty(30) Sprang- Dawley rats were inoculated with 1.0×10^9 colony-forming units *B. abortus*, and randomized for treatment with rifampin administrated orally(Group A), and streptomycin administered intramuscularly (Group B) over 13 weeks starting at 7 days post infection (PI). A placebo recipient (Goup C) was inoculated with sterile saline without antibiotics. All animals were monitored by the Rose Bengal test (RBT), tube agglutination test (TAT) and AMOS-PCR to evaluate the efficiency of the combined treatment to B. abortus infection. The antibody titers in Groups A, B and C were 1:800 as measured by RBT at the first week PI. The antibody titer in Group A decreased to 1:400,by the 13th week PI (not understand). The control remained high for 13 weeks PI, but the antibody response in Group B was negative from the 5th week to the 13th week PI. antibody response using the TAT in all groups had similar profile to that observed using the RBT. AMOS-PCR there was evidence of relapse of *B. abortus* in group A in liver and spleen specimens at the 13th week PI(not understand). *B. abortus* was detected in Group C in liver and spleen specimens from the 7th to 13th week PI by AMOS-PCR. However AMOS-PCR could not detect any organism in Group B from the first week PI until the end of the study.

This study demonstrated that administration of a combination of rifampin and streptomycin is more efficacious than administration of rifampin alone. A significant reduction in antibody titer was observed when a combination of 15mg/kg/day of rifampin *per os* and 15mg/kg/day streptomycin i/m was used in comparison with the control group antibody.

7. EFFECT OF BACTERIAL TOXINS ON ADIPOGENESIS IN 3T3-L1 CELLS

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Pasteurella multocida Toxin (PMT) is a mitogen that exerts its effects through activation of the G_q-dependent signaling pathways, including phospholipase C (PLC) and inositol phosphate. PMT has been shown to stimulate Rho activation, characterized by subsequent stress fiber formation and focal adhesion assembly dependent on Rho activity. Dermonecrotic Toxin (DNT) from *Bordetella bronchiseptica* and Cytotoxic Necrotizing Factors 1 and 2 (CNF1, CNF2) from *Escherichia coli* also act on Rho. DNT does this through transglutamination or deamination, whereas CNF1 and CNF2 have been shown to cause deamination of Rho, as well as rac and cdc42. Thus, DNT, CNF1, and CNF2 directly activating Rho proteins, while PMT appears to do so indirectly. DNT, CNF1, and CNF2, however, do not activate G_q-PLC signaling pathways. There is a direct relationship between adipogenesis and $G_q\alpha$ regulation. PMT has been shown to block adipogenesis and 3T3-L1 adipocyte differentiation, inducing morphological changes and transient proliferation in pre-adipocytes. We tested PMT, DNT, CNF1, CNF2, as well as Pertussis Toxin (PT) from *Bordetella pertussis* and Cholera Toxin (CT) from *Vibrio cholerae* to determine what their effects on adipogenesis. PT has been shown to ADP-ribosylate G_i-alpha subunits, increasing cAMP levels, and increase the amount of free G_q-alpha by sequestering G-beta/gamma subunits in general. CT ADP-ribosylates G_s-alpha subunits, increasing cAMP levels. We found that PMT and DNT completely blocked 3T3-L1 adipocyte differentiation at similar concentrations, confirmed through CEBP-alpha, PPAR-gamma, and Pref-1 RNA expression levels. CNF1, CNF2, and PT were much less efficient at inhibiting the process, showing only partial blocking at the highest concentrations used. CT treatment enhanced adipogenesis. Similarly, PMT and DNT inhibited further lipid accumulation when administered at various timepoints during the process. Collectively, these findings show that 3T3-L1 cell adipogenesis is more directly dependent on PMT and DNT effector signaling pathways when compared to the effector signaling pathways of the other bacterial toxins studied.

8. COMMON POND INVERTEBRATES CONSUME *RIBEIROIA ONDATRA* CERCARIAE

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Since the mid-1990s, amphibian populations have been declining at an unnatural rate. Coinciding with this decline is an increase in the frequency of frog limb deformities, with a 50% or higher deformity rate occurring in many populations. Although these deformities may be attributed to factors such as UV radiation and toxicity, lab research and field studies have pointed to infection of tadpoles by trematode cercariae, especially of the species *Ribeiroia ondatrae*, as their most likely cause.

This study explored the roles other pond organisms may play in helping or hindering cercariae infection of tadpoles. Several pond species were tested for cercariophagic activity; organisms were placed in wells of multi-well culture plates with discreet numbers of cercariae. The numbers of swimming cercariae were recorded over time and compared with numbers in control wells that contained no predators. Of the species tested, hydra, damselfly larvae and copepods consumed significant amounts of cercariae in feeding trials. Hydra were also observed paralyzing cercariae on contact, whether or not they consumed the cercariae, greatly decreasing the numbers of swimming cercariae in the wells. Preference studies, which tested the cercariophagic activity of hydra and damselflies in the presence of other foods, indicated some change in the feeding efficiency of predators on cercariae.

9. FARNESYL PYROPHOSPHATE / GERANYLGERANYL PYROPHOSPHATE SYNTHASE OF *TOXOPLASMA GONDII*: THE DRUG TARGET FOR BISPHOSPHONATES.

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Short chain prenyl diphosphate synthases mainly contain farnesyl pyrophosphate synthase and geranylgeranyl diphospate synthase and play important roles in the isoprenoid pathway. They provide important intermediates for biosynthesis of sterols, ubiquinones, dolichols, heme a, and prenylated proteins.

Bisphosphonates have been used clinically to treat and prevent osteoporosis, Paget's disease, hypercalcemia caused by malignancy, tumor metastases in bone, and other ailments. Recently they have also been found to inhibit the growth of T. gondii both in vitro and in vivo. Over the past several years, several groups have narrowed the site of action of the bisphosphonates to the short chain prenyl diphosphate synthase.

We have cloned and sequenced the gene of a *T. gondii* short chain prenyl diphosphate synthase, farnesyl diphosphate / geranylgeranyl diphosphate synthase (TgFGGPS). TgFGPPS were expressed in the baculovirus expression system with a C-terminal his-tag. The recombinant protein was purified by using a nickel column and biochemically characterized. Reverse phase thin layer chromatography (TLC), indicated that the main products of this enzyme are fpp and ggpp indicating that it is a bifunctional enzyme.

60 bisphosphonates were screened by *T. gondii* growth inhibition test *in vitro*. Three of the best inhibitors were found to be good inhibitors of the recombinant enzyme. The inhibition order for the parasite and the enzyme is the same, strongly indicating that TgFGGPS is the molecular target for those active bisphophonates.

In addition, we have constructed a mutant of *T. gondii* over-expressing TgFGPPS and showed that these cells are more resistant to growth inhibition by bisphosphonates. This result further verified the target role of TgFGGPS.

10. INTERACTION OF *PASTEURELLA MULTOCIDA* TOXIN (PMT) WITH G_q

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Pasteurella multocida toxin (PMT) is an intracellularly acting protein toxin produced by the gram-negative coccobacillus *Pasteurella multocida*. PMT exerts its effects by activating phospholipase C and mitogenic signaling pathways via the heterotrimeric G_q protein. Experimental evidence has led to the hypothesis that PMT is acting upon the G□$_q$ subunit of the heterotrimeric G_q protein. My research involves determining whether or not PMT is directly interacting with the G□$_q$ subunit, and if so what part of PMT interacts with the □ subunit. The BacterioMatch® II Two-Hybrid System is being used to determine whether PMT binds directly to G_q and if so to further determine which parts of the proteins are responsible for these interactions. A library screening approach is also being used to identify any other intracellular targets of PMT. Initial library screens have identified several signaling proteins as potential intracellular targets of the N-terminus or the C-terminus of PMT. In addition, probing a ProtoArray™ Human Protein Microarray with full-length PMT identified a number of other signaling proteins as potential intracellular targets. Results obtained from the two-hybrid

assay and protoarray experiments will be verified by co-elution of the proteins via column chromatography, co-immunoprecipitation, fluorescence-based protein-protein interaction techniques, and cross-linking studies.

11. EVALUATING THE POTENTIAL IMPACT OF VAGINAL MICROBICIDES TO REDUCE THE RISK OF ACQUIRING HIV IN FEMALE SEX WORKERS

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[1]Department of Mathematics and Department fo Veterinary Pathobiology, University of Illinois at Urbana-Champaign
[2] Department of Biomathematics and UCLA AIDS Institute, University of California, Los Angeles

Vaginal microbicides are chemical compounds which can be applied topically to prevent or reduce the transmission of HIV. The following questions are addressed: Would the introduction of vaginal microbicides substantially reduce the risk of female sex workers (FSWs) acquiring HIV? Which factor would it be most important to maximize, microbicide efficacy or microbicide use? What level of microbicide efficacy and use would be necessary to counterbalance a possible reduction in condom use? We developed risk equations and performed Monte Carlo simulations to model an FSW's daily risk of HIV acquisition currently, and after, microbicide introduction. We used uncertainty and sensitivity analyses, and tornado plots for two ranges of microbicide efficacy (30-50%) and (50-80%). Risk was estimated for FSWs whose clients sometimes (10-50%) use condoms, and those whose clients never use condoms. We estimated an analytical threshold for which reducing condom use increases risk. We determined that microbicides could substantially reduce FSWsâ€™ risk of acquiring HIV; absolute decrease in risk would be greatest in high-prevalence regions. Public health impact of microbicides will depend upon useage and efficacy. Even if the microbicides that become available are only low-to-moderately effective, the probability that risk in FSWs will increase (due to replacing condoms with microbicides) is low.

CENTER FOR ZOONOSES RESEARCH AT A GLANCE

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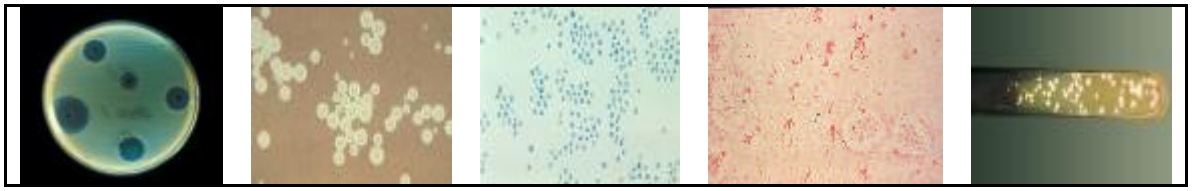

CZR LEADERSHIP

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 in 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

VII Conference on New and Re-Emerging Infectious Diseases (April 15-16, 2004; now planning for VIII Conference, April 21-22, 2005)
Veterinary Student Education in Infectious Diseases (Summer Training Program, 2004; preparing for 2005)
GIS Workshop (May 2004)
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)
NCRR Training Grant for Veterinary Students in Animal-Oriented, Hypothesis-Based Research (NIH, Sept. 20, 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
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OUR SPONSORS

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 Environmental Council's mission is to help build the University's capacity for leadership in environmental discovery, learning, and public engagement. The Council stimulates and promotes interdisciplinary discovery and learning in an effort to prepare students for the world that awaits them, to address the challenges associated with sustainability, and to improve public policies through engagement with citizens and public officials.

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.

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.

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.

One of only twenty-eight veterinary schools in the United States, Illinois has earned a reputation for excellence. Not only does the College graduate superbly educated veterinarians, but it is fertile ground for innovative research, comprehensive animal health care, and diagnostic services.

The College of Veterinary Medicine is located on forty acres at the south edge of the campus of the University of Illinois at Urbana-Champaign. State-of-the-art physical facilities, including the Veterinary Teaching Hospital for small and large animals and the Veterinary Medicine Basic Sciences Building, provide a solid foundation for activities in all aspects of veterinary medicine.

The hospital's annual patient load provides abundant teaching opportunities for students: 12,000 small animal patients, more than 2,000 horses, more than 1,000 cows, sheep, pigs, and other farm animals; and about 2,000 farm visits made by the ambulatory unit. Clinical specialists in a dozen fields draw upon the latest equipment in veterinary medicine, including radiation therapy for animals with cancer; a 24-hour intensive care unit; and diagnostic imaging via nuclear medicine, ultrasound, radiology, computed tomography (CT), and magnetic resonance imaging (MRI).

More than 100 full-time faculty members work in three departments: Veterinary Biosciences, Veterinary Clinical Medicine, and Veterinary Pathobiology. Faculty research interests are quite varied in biomedical and clinical sciences. The College has research strengths in the areas of reproductive biology, environmental toxicology, infectious disease, clinical imaging, oncology, and food safety. Research activity provides employment and learning for students and increases interactions between faculty and students.

In addition to nearly 400 Doctor of Veterinary Medicine (D.V.M.) students, the College has more than 100 graduate students, postdoctoral research associates, residents, and interns. They study such areas as anesthesiology, cell/molecular biology, immunology, internal medicine, microbiology, parasitology, pathology, pharmacology, physiology, surgery, and toxicology.