

**SEVENTH ANNUAL CONFERENCE ON NEW AND
RE-EMERGING INFECTIOUS DISEASES**

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CENTER FOR ZOOZOSES RESEARCH
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APRIL 15-16, 2004

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NEW AND RE-EMERGING INFECTIOUS DISEASES

We are very proud to present the Seventh Annual Conference on New and Re-Emerging Infectious Diseases. Our keynote speaker, Dr. Virginia L. Miller, of the Department of Molecular Microbiology, Washington University, St. Louis, will speak on factors involved in invasion of host cells by *Yersinia enterocolitica*, an enteric pathogen that causes several diseases in humans. Dr. Andre Dhont will discuss his efforts to understand the dynamics of *Mycoplasma gallisepticum* infection in house finches as a model of a new emerging infectious disease. Dr. Elizabeth S. Didier will report advances made in the chemotherapy against microsporidia, important opportunistic pathogens in patients with AIDS. Dr. Joseph Heitman will discuss efforts to understand the signaling cascades that govern virulence of the human fungal pathogen *Cryptococcus neoformans*, another opportunistic pathogen of immunocompromised patients. Dr. James D. Bangs will describe his work on differentiation and surface coat exchange in trypanosomes that cause African sleeping sickness. Dr. Samuel L. Stanley will show his studies on the response of human colonic xenografts to infection with either *Shigella flexneri* or *Entamoeba histolytica*. Dr. Olaf Schneewind will present his results on new strategies to combat *Staphylococcus aureus* infections through disruption of enzymes involved in their cell wall biogenesis. Dr. James Maguire will talk about what happened in China during the SARS epidemic last year. Finally, the Poster Session will give students and faculty at the UIUC and other regional research institutions an opportunity to showcase their research in new and re-emerging infectious diseases. Conference organizers hope that the Conference will stimulate interdisciplinary initiatives to address them and draw much needed attention to the problem of infectious disease.

Acknowledgments

The Conference Committee wishes to thank all that have helped to make this meeting possible. We especially want to acknowledge Dr. Herbert Whiteley, Dean, College of Veterinary Medicine, for his support, and Debra Domal and Judy Mewes for their invaluable organizational help. We would like to thank the Office of Public Engagement of the College of Veterinary Medicine for organizing the space we needed. The financial support of our main sponsors, the Veterinary Medical Research Fund, the College of Veterinary Medicine, the Department of Veterinary Pathobiology, the Great Lakes Regional Center for Excellence in Biodefense and Emerging Infectious Diseases, and Invitrogen Corporation greatly contributed to the Conference's success.

CONFERENCE SCHEDULE

Thursday April 15

Rooms 314A/B Illini Union, 1401 W. Green Street, Urbana, IL 61801

5:00 p.m.: HERBERT WHITELEY, Dean, College of Veterinary Medicine, UIUC;
Welcome

5:15-p.m.: VIRGINIA L MILLER, Department of Molecular Microbiology, Washington
University School of Medicine
"Invasin, RovA and the Peyer's Patch in *Yersinia
enterocolitica* infection"

6:15-7:15 p.m.: Reception

4:30-6:00 p.m.: Registration (Illini Union)

Friday April 16

Holiday Inn, 1001 W. Kilarney, Urbana, IL 61801

7:30-8:15 a.m. Registration/Check-in/Poster Setup

8:00-8:15 a.m. Conference Introduction and Welcome

8:15-9:00 a.m. ANDRE A. DHONDT, Department of Ecology and Evolutionary Biology,
Cornell University, NY.
"*Mycoplasma gallisepticum* in house finches: the dynamics
of an emerging pathogen in an introduced host"

9:00-9:45 a.m. ELIZABETH SCHMIDT DIDIER, Division of Microbiology and
Immunology, Tulane National Primate Research Center,
Covington, LA.
"Microsporidiosis: an emerging and opportunistic
infection in humans and animals"

9:45-10:15 a.m. Coffee break

10:15-11:00 a.m. **JOSEPH HEITMAN**, Departments of Molecular Genetics and Microbiology, Pharmacology and Cancer Biology, and Medicine, Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC

“Sex and the evolution of virulence in *Cryptococcus neoformans*”

11:00-11:45 p.m. **JAMES D BANGS**, Department of Medical Microbiology and Immunology, University of Wisconsin Medical School, Madison, WI

"Differentiation and surface coat exchange In African trypanosomes"

11:45-2:30 p.m. Lunch break and poster viewing

2:30-3:15 p.m. **SAMUEL STANLEY**, Departments of Medicine and Molecular Microbiology, Washington University School of Medicine, St. Louis, MO

“New insights into enteric infections from mouse-human chimeras”

3:15-4:00 p.m. **OLAF SCHNEEWIND** Committee on Microbiology, University of Chicago, Chicago, IL

"Strategies to combat *Staphylococcus aureus* infection"

4:00-4:45 p.m. **JAMES MAGUIRE** Division of Parasitic Diseases, National Center for Infectious Diseases, Center for Disease Control and Prevention, NIAID/NIH, Rocky Mountain Laboratories, Atlanta, GA

“SARS: what really happened in China?”

5:00-6:00 p.m. Reception & Poster Viewing

PRESENTATION ABSTRACTS

RovA, INVASIN AND THE PEYER'S PATCH IN *Yersinia enterocolitica* INFECTION

Virginia L. Miller (Keynote Speaker)

Department of Molecular Microbiology, Washington University, School of Medicine, St. Louis, MO

Yersinia enterocolitica is a Gram-negative enteric human pathogen that causes several diseases; the most common manifestation is enterocolitis. As with many enteric pathogens, tissue invasion is a critical first step in the pathogenesis of *Y. enterocolitica* infections. Invasin, encoded by the *inv* gene, is the primary factor required for efficient translocation of the bacteria across the intestinal epithelium. In the laboratory *inv* is maximally expressed at 26°C or at 37°C, pH5.5 in early stationary phase. We have identified both positive and negative regulators of *inv* expression. A small histone-like protein, YmoA, is required for repression of *inv* expression at 37°C. A member of the MarR/SlyA family of transcriptional regulators, RovA, is required for expression of *inv* under all conditions in the laboratory and during infection. In a mouse model of infection an *inv* mutant has a wild-type LD₅₀ even though the kinetics of infection is changed. In contrast, the *rovA* mutant has altered kinetics, as well as an increase in the LD₅₀. One of the notable features of infection with the *rovA* mutant compared to infection with the wild type strain or the *inv* mutant is the lack of inflammation. These data suggest that RovA regulates other virulence factors in addition to *inv*. We are currently screening for RovA regulated genes using a variety of methods. In addition host genes responsive to the *Yersinia* infection are being identified and characterized.

***Mycoplasma gallisepticum* IN HOUSE FINCHES: THE DYNAMICS OF AN EMERGING PATHOGEN IN AN INTRODUCED HOST**

André A. Dhondt

Laboratory of Ornithology, Department of Ecology and Evolutionary Biology, Cornell University, NY

In early 1994 a novel strain of the poultry pathogen *Mycoplasma gallisepticum* appeared around Washington DC causing severe conjunctivitis in House Finches. The new disease spread rapidly in the eastern (introduced) part of the finch's range, but has now also reached the western (native) range. *M. gallisepticum* also causes disease in several other finch species, but these seem to be spill-over infections. The study system is interesting in that the house-finch strain of *M. gallisepticum* is most likely a novel strain (it causes only mild clinical signs in poultry) that emerged recently and invaded a new host that itself was introduced in 1940 in eastern North America. House Finches increased by about 6 orders of magnitude in 60 years. They are a mobile, patchily distributed, seasonally

breeding social species. As the bacterium cannot survive outside a host for more than a few days it is assumed that transmission occurs primarily through direct contact and/or through fomites, less through vertical transmission. Disease prevalence shows strong seasonal fluctuations, whose amplitude and cycle, though, varies geographically. To understand and model the dynamics of *M. gallisepticum* in House Finches we combine data from three lines of investigation: volunteers to describe disease prevalence at large geographic scales; intense capture-mark-recapture studies in local populations to determine the effects of disease on survival and behavior; and controlled experimental infections in captivity to understand factors influencing the course of the disease in individuals in controlled conditions. I will present recent advances in our understanding of the disease dynamics using results from all three lines of investigation. The project is funded by NSF (DEB) and carried out in collaboration with colleagues at Cornell University, Princeton University, UW at Madison and Emory University.

MICROSPORIDIOSIS: AN EMERGING AND OPPORTUNISTIC INFECTION IN HUMANS AND ANIMALS

Elizabeth Schmidt Didier

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Microsporidia have emerged as causes of opportunistic infections in AIDS patients, organ transplant recipients, children, travelers, contact lens wearers, and the elderly. These organisms are small single-celled, obligate intracellular parasites that were considered early eukaryotic protozoa but were recently reclassified with the fungi. The two most commonly-identified species that infect humans, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*, have been associated with diarrhea and systemic disease. Species of microsporidia infecting humans have been identified in water sources as well as in farm, wild, and domestic animals, raising concerns for waterborne and zoonotic transmission. Current therapies for microsporidiosis are variably effective. Albendazole, a benzimidazole that inhibits microtubule assembly, is effective against several microsporidia including the *Encephalitozoon* species, but is less effective against *E. bieneusi*. Fumagillin, an antibiotic and antiangiogenic compound produced by *Aspergillus fumigatus*, is more broadly effective against *Encephalitozoon* spp. and *Enterocytozoon bieneusi* but is toxic when administered systemically in mammals. Gene target studies have focused on methionine aminopeptidase 2 (MetAP2) for characterizing the mechanism of action and for identifying more effective, less toxic fumagillin-related drugs. MetAP2 genes from *Encephalitozoon* species were cloned, sequenced, and found to relate more closely with those of eukaryotes than those of the eubacteria. During attempts to clone the MetAP2 gene in the human microsporidian, *Vittaforma corneae*, a topoisomerase IV gene was identified. This was of phylogenetic interest because microsporidia are eukaryotes, but until now, topoisomerase IV had only been identified in prokaryotes. Furthermore, fluoroquinolones target topoisomerase IV and based on early *in vitro* and *in vivo* murine studies, may prove to be promising antimicrosporidial drugs.

***Cryptococcus neoformans* AS A MODEL FUNGAL PATHOGEN**

Joseph Heitman

Departments of Molecular Genetics and Microbiology, Pharmacology and Cancer Biology, and Medicine, Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC

We study the human fungal pathogen *Cryptococcus neoformans*, which causes life-threatening infections of the central nervous system, most commonly in immunocompromised hosts. This organism is a basidiomycete that is divergent from other common human fungal pathogens. *C. neoformans* is an excellent model pathogen. The organism has a defined sexual cycle involving haploid α and **a** cells, genes can be readily disrupted following transformation and homologous recombination, and animal virulence models are well established. \Genome sequencing projects are in progress for three related but divergent varieties, which are all pathogenic in humans and have unique environmental and virulence attributes. Our studies focus on signaling cascades that govern virulence, and an unusual mating type locus linked to differentiation and virulence. We have defined signaling cascades that control virulence and mating, and cloned and sequenced the **a** and α alleles of the mating-type locus from divergent strains. The MAT locus spans >100 kb and contains more than 20 genes, several of which function in differentiation and virulence. The MAT alleles are composed of divergent sets of the same genes that evolved by extensive remodeling from a common ancestral DNA region. We have defined the sexual cycles for the most common pathogenic variety, recapitulated the sexual cycle for the divergent *gattii* variety that infects immunocompetent hosts with implications for an unusual fertile isolate causing an outbreak on Vancouver Island, and discovered a unique population of serotype A strains undergoing active recombination in sub-Saharan Africa. Our findings highlight the potential of sexual recombination in evolution and divergence of human fungal pathogens.

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DIFFERENTIATION AND SURFACE COAT EXCHANGE IN AFRICAN TRYPANOSOMES

James D. Bangs and Amy E. Gruszynski*

Department of Medical Microbiology and Immunology and Department of Biomolecular Chemistry, University of Wisconsin Medical School, Madison, WI

African trypanosomes regulate the composition of their cell surface in a stage-specific manner. The mammalian bloodstream form (BSF) has a coat composed of a single abundant variant surface glycoprotein (VSG). There are hundreds of VSG genes but only one is expressed in any single cell, and by altering the expression of VSG genes the parasite avoids the host immune response (antigenic variation). The procyclic insect form (PCF) has a surface coat composed of an invariant protein, procyclin, which is protease resistant thereby providing protection in the hydrolytic environment of the tsetse fly midgut. Both VSG and procyclin are glycosylphosphatidylinositol (GPI)-anchored, but their GPI anchors differ in a stage-specific manner; the VSG anchor is sensitive to phosphatidylinositol-specific-phospholipase C (PI-PLC) whereas the procyclin anchor is resistant. In the mammalian host replicating long slender BSF transform in a density-dependent manner into non-replicating short stumpy BSF that are pre-adapted for transmission to the *tsetse*; when taken up in a bloodmeal these differentiate into replicating PCF. Using enriched short stumps this process can be mimicked in vitro by temperature shift from 37°C to 27°C plus *cis*-aconitate. Differentiation is synchronous over ~12 hours after which PCF log phase growth begins. The most striking event in differentiation is the remodeling of the parasite cell surface by which VSG is replaced with procyclin. The old VSG coat is shed by both endoproteolysis and GPI-hydrolysis. However, the active protease has not been identified, and in addition the role of endogenous GPI-PLC has been discounted for two reasons. First, endogenous GPI-PLC is located on the cytosolic face of internal membranes and is therefore topologically sequestered from VSG, and second, GPI-PLC nulls can complete differentiation. We previously demonstrated that transgenic PCF have a surface metalloprotease activity that releases recombinant VSG by endoproteolysis. To determine if this protease is involved in VSG release during differentiation we have exploited the in vitro assay with short stumpy BSF. We find that in intact cells VSG release occurs initially (0-2 hrs) by GPI hydrolysis and later (2-4 hrs) by endoproteolysis; removal is complete by 8-12 hrs. Endoproteolysis is blocked by zinc chelation and by peptidomimetic collagenase inhibitors indicating that the differentiation-specific protease is also a zinc metalloprotease. Recently a major surface metalloprotease family has been identified by the trypanosome genome project. Northern analysis indicates

that MSP-B, is expressed at low levels in BSF and is upregulated in PCF. We hypothesize that MSP-B is specifically upregulated during differentiation to remove VSG, and then is maintained in PCF for as yet unknown purpose(s). With collaboration (J. Donelson, U. Iowa), we have used RNAi to silence MSP-B in transgenic PCF expressing VSG. MSP-B ablation has no effect on cell growth but markedly reduces endoproteolytic VSG release indicating it to be the major surface protease of replicating PCF. A similar RNAi approach is under development to test whether MSP-B is also the differentiation-specific protease, but consistent with our hypothesis, actinomycin D treatment does block both proteolytic release and MSP-B upregulation during differentiation. Our finding that VSG release occurs before upregulation of proteolysis suggests that GPI-hydrolysis is 'primed' in the starting population prior to induction of differentiation. Indeed, surface biotinylation with rigorous controls for plasma membrane integrity reveals that GPI-PLC is on the external surface of short stumpy parasites, and thus that there are no topological constraints on its action during differentiation. Clearly then, the role GPI hydrolysis in differentiation cannot be discounted. Endogenous GPI-PLC is known to be reversibly thioacylated and we hypothesize that acylation regulates access to the cell surface during differentiation. We are currently testing this model by complementation of GPI-PLC null cell lines with wildtype GPI-PLC and GPI-PLC acylation mutants.

NEW INSIGHTS INTO ENTERIC INFECTIONS FROM MOUSE-HUMAN CHIMERAS

Samuel L. Stanley Jr.

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The clinical presentations of bacillary dysentery caused by shigella, and amebic dysentery caused by the protozoan parasite *Entamoeba histolytica*, can be indistinguishable, with both organisms causing colonic mucosal damage and ulceration. However, the two organisms are quite distinct, and have very different pathogenic mechanisms. This raises the fundamental question of whether the similar clinical manifestations reflect a stereotypic response of the human gut to mucosal injury, or whether there are differences at the molecular level in the host response to individual gut pathogens. To characterize the human colonic response to each pathogen at the molecular level, we measured the differential transcription of nearly 40,000 human genes in sections of human colonic xenografts obtained 4 and 24 hours following infection with *S. flexneri* or *E. histolytica*. Our results indicate that much of the human colonic response to these two pathogens is stereotypic, with increased expression of genes activated in cells undergoing stress and/or hypoxic responses, genes encoding cytokines, chemokines, and mediators that are involved in immune and inflammatory responses, and genes encoding proteins involved in responses to tissue injury and in tissue repair. The responses to ameba and *Shigella* were not identical however, and we found unique elements in each response that may provide new insights into the distinct pathogenic mechanisms of *E. histolytica* and *S. flexneri*.

STRATEGIES TO COMBAT *Staphylococcus. aureus* INFECTIONS

Olaf Schneewind

Committee on Microbiology, University of Chicago, Chicago, IL

The cell wall of Gram-positive bacteria is composed of a peptidoglycan macromolecule that serves multiple functions. In addition to providing protection against osmotic lysis, the peptidoglycan functions as a cytoskeletal element for the anchoring of surface proteins. Proteins can be immobilized to the cell wall of Gram-positive bacteria through covalent and non-covalent linkages. A large number of surface proteins are covalently anchored to the cell wall peptidoglycan by a mechanism requiring a C-terminal sorting signal with an LPXTG motif. Sortases, membrane anchored transpeptidases, cleave surface proteins at the LPXTG motif and catalyzes the formation of an amide linkage between the C-terminal end of the polypeptide chain and the cell wall crossbridge of peptidoglycan. The first sortase identified in Gram-positive bacteria was found in *S. aureus* and is known as sortase A. Sortase A (*srtA*) is responsible for the cell-wall anchoring of at least 20 proteins involved in various processes including nutrient acquisition, ligand binding, and immune system evasion. Three proteins of the Isd system, iron regulated surface determinants, are anchored to the cell wall by sortase A (IsdA, IsdB, and IsdH). Analysis of the *S. aureus* genome for *srtA* homologs revealed a second sortase gene, *srtB*. *srtB*, encoding for sortase B, is located in the transcriptional unit *isdCDEFsrtBisdG*, and functions as the transpeptidase in anchoring the heme binding protein IsdC to the cell wall envelope. The cell wall anchor structure of sortase A and B products appears to be distinct, suggesting unique substrate requirements for polypeptide and peptidoglycan components. Because sortase enzymes and iron uptake strategies of *S. aureus* are required for the establishment of animal infections, inhibitors that disrupt the activity of sortase enzymes may be useful therapeutics.

SARS: WHAT REALLY HAPPENED IN CHINA

James Maguire

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The first case of SARS occurred in November 2002 in Guangdong Province, P.R. China. By the time of the last case of the outbreak in July 2003, there had been 8,098 cases and 774 deaths in 30 countries and regions; global economic losses were an estimated \$11 billion. This was the first pandemic of the 21st century, but undoubtedly not the last. Although the source of the SARS coronavirus remains unknown, there are lessons to be learned from this experience that may be applicable to future emergences of infectious diseases. The epidemic in China and the Chinese Government's response to the epidemic deserve special attention, not only because this was the site of the first case, but also the greatest numbers of cases (5,327) and deaths (349). China's handling of the epidemic was sharply criticized by the international community, but a balanced view of events is warranted in order to take best advantage of the lessons learned. This session will focus on China's role in the detection of the epidemic and identification of the SARS coronavirus, its response to the epidemic, and the role of the international community in bringing the epidemic to an end.

POSTER ABSTRACTS

1. SPATIAL ANALYSIS OF EQUINE WEST NILE VIRUS CASE RATES BY COUNTY IN ILLINOIS IN 2002 AND COMPARISON WITH LAND COVER DATA

Amy Jo Wolf

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West Nile Virus (WNV) is a flavivirus historically found in Africa, West Asia and the Middle East (www.cdc.gov/ncidod/dvbid/westnile/qa/overview.htm) with outbreaks also documented in Europe, South Africa and Israel. In 1999, WNV was detected in New York City and by 2001 the virus had reached Illinois. The subsequent outbreaks involved humans, equids and both mammalian and avian wildlife. Approximately 1200 equids tested positive for the virus in 2002 (www.idph.state.il.us). Developing a reliable means of identifying equine outbreak locations and the expected severity of outbreaks before disease occurs could potentially decrease loss of life, both human and non-human, from the virus. The purpose of the study was to both describe the spatial pattern of equine WNV and to determine if vegetation data can be used as a predictor for equine WNV incidence. Areas of intensive agriculture have higher rates of equine WNV, while upland areas, that is, areas of closed-canopy deciduous forest, and areas of grasses and other agriculture have lower rates of equine WNV. Although the correlations seen were statistically significant, only the regression coefficient for uplands was found to be significant in a linear regression analysis. The negative correlations may be due to the fact that the habitat in open grassland and forests are not ideal for vector breeding and survival. One future hypothesis to be tested is to consider whether the horses in areas with more intensive agriculture have relatively less pasture land, thus putting them in closer contact with barns and other peridomestic structures where the mosquito vectors may find more places to breed. Further research will need to be completed in these areas to elucidate the processes that are responsible for the results seen.

2. IDENTIFICATION OF IN VIVO TARGETS OF P. AERUGINOSA EXOS ADP-RIBOSYLTRANSFERASE

Anthony W. Maresso and Joseph T. Barbieri

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Pseudomonas aeruginosa is a gram negative bacterium which causes life-threatening infections in immunocompromised and cystic fibrosis patients. Pathogenesis stems from a number of virulence factors, including four cytotoxins: ExoS, ExoT, ExoU, and ExoY. These cytotoxins are delivered directly into host cells by a specialized apparatus termed the type-III secretion system. ExoS is a bifunctional enzyme. The N terminus consists of a Rho GTPase Activating Protein (GAP) activity which inactivates the Ras-like GTPases Rho, Rac, and Cdc42 in vitro and in vivo, and a C terminal ADP-ribosyltransferase domain which covalently attaches an ADP-ribose moiety onto target substrates such as Ras, Ral, Rap, and ExoS itself. The ADP-r domain is cytotoxic to mammalian cells. Recently, amino acids 51-72 (termed the Membrane Localization Domain or MLD) have been implicated in the localization of ExoS to the perinuclear ER-region of mammalian cells. Infection with ExoS *f*'MLD inefficiently ribosylates Ras yet is cytotoxic, prompting an investigation into ExoS-mediated cytotoxicity. One and two-dimensional SDS-PAGE analysis of cell lysates infected with either ExoS or ExoS *f*'MLD revealed different target profiles. Examining substrates common to both ExoS and ExoS *f*'MLD, as well as substrates modified at the earliest time points during infection, allowed identification of proteins which potentially mediate cytotoxicity. Using this analysis, approximately ten proteins were identified. The identification of high affinity targets will help elucidate the mechanisms behind ExoS-mediated cell death, allowing substrate-based ExoS-inhibitor design.

3. CHARACTERIZATION OF CANINE MYELOMA CELL-LINE, 030F AS A COMPETENT HOST FOR *Ehrlichia canis* PROPAGATION: SEROLOGICAL AND BIOCHEMICAL CHARACTERISTICS

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¹University of Illinois, Urbana, IL

²North Carolina State University, College of Veterinary Medicine, Raleigh, NC

Ehrlichia canis (*E. canis*) is a small gram-negative obligate intracellular bacterium. The organism replicates in membrane-bound vacuoles in the cytoplasm of monocytes. The agent is tick-transmitted and causes a clinical or subclinical disease characterized by fever, anorexia, hematological abnormalities, lymphadenopathy, and elevation of liver enzyme activity. A major landmark in ehrlichiosis research was the development of the *in vitro* method for the propagation *E. canis* using canine monocytes by Nyindo et al.¹ The myeloma cells of canine monocyte lineage has since then replaced the primary canine monocyte cultures for propagation of *E. canis*. This development has facilitated our understanding of parasite-host cellular interactions in the pathogenesis of ehrlichiosis. There is need for a complete delineation of various pathways of the parasite and host responses that would enhance the development of therapeutic and vaccination intervention strategies. These *in vitro* systems offer the greatest research potential outside the natural host.

The present report presents data on a new (030F) cell-line that has been shown to be remarkably susceptible to *E. canis* (up to 100% susceptibility). The cell-line was shown to express CD4, CD18 and CD45 markers as demonstrated by Flowcytometric analysis and confocal microscopy. The cell-line also has been optimized for the measurements of various inhibitors of cellular function (EGTA, MDC, Neomycin, Verapamil, BAPTA-AM, Genistein, 2-APB, SKF-96365, Thapsigargin, U-73122 and Cytochalasin D). These data now lay a foundation for a systematic analysis of the pathogenesis pathways of *E. canis* and form a potential model for the study of other intracellular parasites that can be cultivated in 030F cell-line or its various clonal derivatives.

4. EXPERIMENTAL INFECTION WITH *Brucella abortus* BIOTYPE 1, *Brucella abortus* STRAIN RB51, *Brucella suis*, *Brucella melitensis*, and *Brucella canis* IN KOREAN MONGREL DOGS

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²Biosafety Research Institute, Chonbuk National University, Jeonju 561-756, Korea

This study was carried out to investigate immunological responses and bacteriological infection status in mongrels experimentally inoculated with *Brucella abortus* (*B. abortus*) biotype 1 and *Brucella canis* (*B. canis*) isolated from Korean animals, *Brucella abortus* strain RB51 (SRB51), *Brucella suis* biotype 1 (*B. suis*) and *Brucella melitensis* biotype 1 (*B. melitensis*). The six groups of dogs (each group consisting of three dogs) were orally inoculated with 5×10^9 CFU (colony-forming units) of *B. abortus* biotype 1, *B. canis*, SRB51, *B. suis*, *B. melitensis* and with sterile pyrogen-free PBS, pH 7.4, respectively. The animals were monitored at regular intervals to the 12th week post-inoculation (PI) by standard tube agglutination test (STAT), plate agglutination test (PAT), rose bengal test (RBT), enzyme-linked immunosorbent assay (ELISA) and 2-mercaptoethanol rapid slide agglutination test (2ME-RSAT). By ELISA, using cytoplasmic fractions of *Brucella* as antigen, antibodies against *B. abortus* biotype 1 and *B. suis* as well as *B. canis* were detectable. However, by STAT and RBT, the only detectable antibodies were against *B. abortus* biotype 1 and *B. suis*. Furthermore, by PAT, antibodies against only *B. abortus* biotype 1 could be detected. Antibodies to SRB51 and *B. melitensis* were not detectable by the tests used in this study. *B. canis* was isolated beginning with the first week PI, while the others were not isolated. These data show that ELISA, using cytoplasmic fractions of *Brucella* as antigen, has good potential as a screening test for canine brucellosis.

5. VACCINATION WITH *Brucella abortus* STRAIN RB51 PROTECTS KOREAN MONGREL DOGS AGAINST VIRULENT *Brucella abortus* BIOTYPE 1, and *Brucella canis* isolats FROM KOREAN ANIMALS

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This study was carried out to test the hypothesis that *Brucella abortus* strain RB51 (SRB51) protects Korean mongrel dogs from *Brucella abortus* (*B. abortus*) biotype 1 and *Brucella canis* (*B. canis*) isolates from Korea. Two groups of dogs (each group consisting of three dogs) were intramuscular inoculated with 1.0×10^9 CFU (colony-forming units) of SRB51, respectively. At the twelfth week post-vaccination, each group of dogs was orally challenged with 5.0×10^9 CFU (colony-forming units) of *B. abortus* biotype 1 or *B. canis*, respectively. The animals were monitored at regular intervals for eighth week post-challenge (PC) by standard tube agglutination test (STAT), plate agglutination test (PAT), rose bengal test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) using *B. abortus* strain 1119-3 as antigen, 2-mercaptoethanol rapid slide agglutination test (2ME-RSAT) using the whole of *B. canis* M-, and 2-mercaptoethanol tube agglutination test (2ME-TAT) using whole *B. canis* RM6/66 antigen. Antisera to *B. abortus* biotype 1 or *B. canis* were not detectable by all the tests used this study. The first week PC until the end of the experiment, *B. abortus* biotype 1 or *B. canis* were never isolated from the blood. At the 8th week PC, the microorganisms were not isolated from liver, spleen, kidney, lymph nodes, and reproductive organs of the dogs inoculated with the bacteria. The data suggest that SRB51 is a good candidate as an effective vaccine against canine brucellosis caused by *B. abortus* biotype 1 or *B. canis* especially among Korean indigenous dogs.

6. SUSCEPTIBILITY TO ANTIMICROBIAL AGENTS OF *Listeria monocytogenes* ISOLATED FROM LIVESTOCK PRODUCTS AND THE IMMEDIATE ENVIRONMENT

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This study was carried out to investigate the antibiotic susceptibilities of *Listeria monocytogenes*. A total of 70 strains were isolated from poultry meats, pork, hamburger, animal feces, dry cattle food, and knives at a pork producing plant in Seoul, Korea and Kyonggi province during the period from 1998 to 2003. Serotyping of *L. monocytogenes* isolates was performed according to the manufacturer's instruction. Minimum inhibitory concentrations (MICs) were determined by the microdilution method according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS). All the isolates were tested against 20 antimicrobial agents as follows; Ampicillin (Am), Amikacin (An), Cephalothin (Cf), Chloramphenicol (C), Ciprofloxacin (Cip), Erythromycin (E), Gentamicin (Gm), Imipenem (Ipm), Kanamycin (K), Minocycline (Mi), Neomycin (N), Norfloxacin (Nor), Ofloxacin (Ofx), Penicillin (P), Streptomycin (S), Tetracycline (Te), Tobramycin (Nn), Trimethoprim (Tmp), Trimethoprim/Sulfamethoxazole (Sxt), Vancomycin (Va). The serotypes of 70 isolates were serotype 1/2a (20%), serotype 1/2b (17.1%) and serotype 1/2c (62.9%). Of the *L. monocytogenes* isolates, 65.7% were resistant to Te (MIC, 64 to 128 $\mu\text{g}/\text{mL}$), 57.1% to Mi (8 to 32 $\mu\text{g}/\text{mL}$), 11.4% to Nor (16 $\mu\text{g}/\text{mL}$), 5.7% to Cip (4 $\mu\text{g}/\text{mL}$), 2.8% to N (4 $\mu\text{g}/\text{mL}$), 1.4% to C (16 $\mu\text{g}/\text{mL}$) and 1.4% to Cf (32 $\mu\text{g}/\text{mL}$). However, all isolates were 100% sensitivity to antibiotics such as Am, An, E, Gm, Ipm, K, Nn, P, Sxt, Tmp, and Va. The multiple resistance patterns of the isolates were observed in Te-Mi-Nor-Cip (1.4%), Te-Mi-Nor (7.1%), Te-Mi-Cip (2.9%), Te-Mi-N (1.4%), and Te-Mi (42.9%). The results of this study indicate that many *L. monocytogenes* isolates are resistant to antimicrobial agents including Te and Mi, and further. There is potential risk that these organisms may gradually acquire multiple antimicrobial resistant properties.

7. A QUANTITATIVE REAL-TIME PCR ASSAY FOR THE DETECTION OF PATHOGENIC *Leptospira* spp.

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Leptospirosis continues to be an important zoonosis of worldwide concern. Primarily caused by serovars of *Leptospira interrogans*, leptospirosis, if left untreated or misdiagnosed, can progress to hepatic or renal failure. Leptospirosis is transmitted via contact with water contaminated by the urine of carrier animals that persistently shed the organism. Although leptospirosis typically exhibits a low mortality and morbidity in this country, current research suggests that there may be a possibility for a re-emergence of the disease.⁴ For example, an increasing incidence in leptospirosis cases resulting from infections by novel serovars of *Leptospira interrogans*, other than historically significant serovars *canicola* and *icterohemorrhagiae*, has been observed.² Further, the encroachment of suburban developments and retention ponds into wildlife habitats could presumably create an environment predisposed to the spread of leptospires. A rapid, sensitive method for the detection of pathogenic leptospires in urine, tissue and environmental samples would be valuable in obtaining accurate diagnoses and aid in tracking emerging trends. To this end, we adapted a TaqMan® (quantitative) real-time polymerase chain reaction (PCR) assay for use in the SmartCycler®, by Cepheid. The assay is based on the *rrs* gene (16S rRNA) alignments published in GenBank and has a detection limit of 25 cells and does not cross-react with common urinary pathogens or non-pathogenic leptospires. Sample inhibition was of no consequence in kidney or liver specimens; however, a slight inhibition was seen with urine specimens resulting in a detection limit of 45 cells. A blinded test performed on 22 randomly spiked negative urine specimens, subsequently subjected to PCR, yielded a sensitivity and specificity of 93% and 100% respectively.

8. ENHANCED INNATE IMMUNITY TO SYSTEMIC *Yersinia pseudotuberculosis* INFECTION IN B CELL-DEFICIENT MICE

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Studies of pathogenic *Yersinia* spp. over the last several decades have identified many virulence factors and provided important insights into the mechanisms of bacterial pathogenesis. However, very little is understood of the complex interplay between *Yersinia* and the host immune system. We challenged wild type mice and mice that completely lack T cells ($TCR^{-/-}$), B cells ($BCR^{-/-}$), or both ($RAG-1^{-/-}$) with *Y. pseudotuberculosis* to study the mechanisms of immunity to *Yersinia* infection and of host-pathogen interactions. Both wild type and $TCR^{-/-}$ mice are highly susceptible to blood-born *Yersinia*, while $BCR^{-/-}$ and $RAG-1^{-/-}$ animals are relatively resistant. The innate immune system

appears to be more active in the absence of B cells, as both BCR^{-/-} and RAG-1^{-/-} mice exhibit increased phagocytic killing and decreased bacterial burdens at a very early stage of *Yersinia* infection. This enhanced innate immunity is inhibited by the introduction of *Yersinia*-nonspecific B cells via a hen egg lysozyme (HEL)-specific immunoglobulin transgene, Ig^{HEL}. These observations suggest that *Yersinia* or a *Yersinia*-secreted product can interact with B cells irrespective of their antigen receptor specificity and induce the production of inhibitory factor(s) to suppress the host innate immune response. Consistent with this interpretation, we found that wild type mice produce significantly more IL-10 than BCR^{-/-} animals after *Yersinia* challenge. Furthermore, we found that recombinant *Yersinia* LcrV protein elicits IL-10 production in purified B cells *in vitro*. These results suggest a possible mechanism by which pathogenic bacteria exploit B cells to subvert the host innate immunity.

9. The ROLE OF B CELLS IN PRIMARY AND MEMORY T-CELL RESPONSE TO *Yersinia* INFECTION

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Previous studies demonstrate T-cells are essential for the clearance of *Yersinia* infection in mice. However, the role of B cells in host defense against *Yersinia* is not well established. We used B cell-deficient (BCR^{-/-}) mice to investigate the role of B cells in *Y. pseudotuberculosis* pathogenesis. We found wild type (wt) mice that survived a low-dose *Yersinia* infection are resistant to re-challenge with a much higher dose that is lethal to naive mice. However, BCR^{-/-} mice that survived primary infection are only slightly more resistant than naive BCR^{-/-} mice to lethal re-challenge. The protective immunity observed in wt mice during re-challenge could be the result of both remaining antibody from the primary immune response and T-cell memory. The lack of robust immunological memory in BCR^{-/-} mice suggests that B cells are involved in the generation of effector T-cells during primary infection or the maintenance of memory T-cells, or both. In experiments designed to examine the role of B cells in primary T-cell response, BCR^{-/-} CD4^{-/-} mice (B and CD4⁺ T-cell deficient) were found to be no more susceptible to *Yersinia* than BCR^{-/-} mice alone in primary infection, suggesting lack of CD4⁺ T-cell activation in BCR^{-/-} mice. Moreover, we find that Ig^{HEL} BCR^{-/-} mice (with hen egg-white lysozyme specific B cells) are more resistant to *Yersinia* in primary infection than wt and BCR^{-/-} mice, and they exhibit robust immunological memory in a re-challenge experiment. The enhanced resistance and immunological memory of Ig^{HEL} BCR^{-/-} mice occurs in the absence of significant levels of anti-*Yersinia* antibody. BCR^{-/-} mice immunized less than twice with heat-killed *Yersinia* do not exhibit a memory response when challenged with live *Yersinia*. These data suggest T-cell memory may be maintained in the absence of B cells and antibody production may not be the critical role of B cells in *Yersinia* infection; rather the critical role of B cells may be

T-cell activation through a yet unidentified process. In brief, our investigation is starting to define a role for B cells in regulating the primary and memory T-cell response during *Yersinia* infection.

10. A FUNCTIONAL AQUAPORIN CO-LOCALIZES WITH A VACUOLAR PROTON PYROPHOSPHATASE TO ACIDOCALCISOMES OF *Trypanosoma cruzi*

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Water channels or aquaporins (AQPs) are intrinsic membrane proteins that allow water or small specific solutes to pass unhindered, but block the passage of ions to prevent dissipation of the transmembrane potential. Many of these channels are present in intracellular vacuoles. Since acidocalcisomes, acidic organelles containing calcium, pyrophosphate, polyphosphates, and other elements, have been postulated to be involved in osmoregulation in trypanosomatids we decided to study aquaporins in *T. cruzi*. We have cloned an aquaporin gene from *T. cruzi* (*TcAQP*) that encodes a protein of 231 amino acids, which is highly hydrophobic. The protein has six putative transmembrane domains and two signature motifs NPA that are involved in the formation of an aqueous channel spanning the bilayer. *TcAQP* was resistant to endo H treatment and sensitive to PNGase F, suggesting that the protein was modified in the Golgi apparatus. The water transport properties of *TcAQP* were analyzed by expressing the gene in *Xenopus* oocytes and performing oocyte swelling assays. *TcAQP*-expressing oocytes swelled under hyposmotic conditions indicating water permeability, which was abolished after preincubating oocytes with AQP inhibitors HgCl_2 and AgNO_3 . To investigate the localization of *TcAQP* we generated fusion proteins containing the green fluorescent protein (GFP) at the C-terminal of *TcAQP*. Immunofluorescence microscopy of *T. cruzi* epimastigotes expressing these proteins showed co-localization in acidocalcisomes of GFP-*TcAQP* with the V-H⁺-PPase detected with monoclonal antibodies prepared against a conserved epitope in the *T. cruzi* enzyme. In addition, there was an anterior localization of *TcAQP* in a vacuole, close to the flagellar pocket, that was distinct from the acidocalcisomes. These results were confirmed by immunocytochemical evidence. *TcAQP*-GFP containing acidocalcisomes was translocated when epimastigotes of *T. cruzi* were subjected to hyposmotic stress. Taking together the data argue that aquaporin present in acidocalcisomes plays an important role in osmoregulation in *T. cruzi*.

11. THE EXOPOLYPHOSPHATASE FROM *Trypanosoma cruzi* HAS HIGHER AFFINITY FOR SHORT-CHAIN POLYPHOSPHATE

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We report the cloning, overexpression, purification, and characterization of the *Trypanosoma cruzi* exopolyphosphatase (*TcPPX*). The product of this gene (*TcPPX*), the first related to polyphosphate (polyP) metabolism isolated from this parasite, has 383 amino acids and a molecular mass of 43.1 kDa. *TcPPX* differs from other exopolyphosphatases previously investigated, but has similarities to the *Leishmania major* exopolyphosphatase (Rodrigues et al., *J. Biol. Chem.* 277, 50899-50906, 2002). Heterologous expression of *TcPPX* in *Escherichia coli* produced a functional enzyme that was similar to the yeast exopolyphosphatase with respect to its Mg²⁺ requirement, optimum pH, and sensitivity to cations, amino acids, and heparin, but, that in contrast to the yeast enzyme and other exopolyphosphatases investigated before, it acts on polyP of short-chain lengths with higher rates and affinity. *TcPPX* does not hydrolyze pyrophosphate, ATP, or *p*-nitrophenylphosphate. The unique characteristics of *TcPPX* and *T. cruzi* polyP metabolism may facilitate the development of novel trypanocidal agents.

12. A LIPID-MODIFIED PHOSPHOINOSITIDE-SPECIFIC PHOSPHOLIPASE C (*TcPI-PLC*) IS INVOLVED IN DIFFERENTIATION OF TRYPOMASTIGOTES TO AMASTIGOTES OF *Trypanosoma cruzi* *

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The phosphoinositide-specific phospholipase C (PI-PLC) is an important component of the inositol phosphate/diacylglycerol signaling pathway. A newly discovered *Trypanosoma cruzi* PI-PLC (*TcPI-PLC*) is lipid modified in its *N*-terminus, located to its plasma membrane, and believed to play a role in differentiation of the parasite because its expression increases during the differentiation of trypomastigote to amastigote stages. To determine if *TcPI-PLC* is involved in this differentiation step, antisense inhibition using phosphorothioate-modified oligonucleotides, or overexpression of the gene were performed. Antisense oligonucleotide-treated parasites showed a reduced rate of differentiation in comparison to controls, as well as accumulation of intermediate forms. Overexpression of *TcPI-PLC* led to a faster differentiation rate. In contrast,

overexpression of a mutant *TcPI-PLC* that lacked the lipid modification at its *N*-terminus did not affect the differentiation rate. Therefore, *TcPI-PLC* is involved, when expressed in the plasma membrane, in the differentiation of trypomastigotes to amastigotes, an essential step for the intracellular replication of these parasites.

13. A P-TYPE H⁺-ATPase ACTIVITY IS PRESENT IN THE ENDOCYTIC PATHWAY OF *Trypanosoma cruzi* EPIMASTIGOTES

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Mammalian cells control their intracellular pH (pH_i) through a variety of plasma membrane transporters including a Na⁺/H⁺ antiport, a Cl⁻/HCO₃⁻ exchanger that may or may not be sodium dependent, and ATP-dependent proton pumps of the vacuolar type (V-H⁺-ATPases). These proton pumps are also involved in vesicular trafficking and acidification of endosomal and lysosomal compartments. P type proton pumps (P-type H⁺-ATPases) are not present in mammalian cells but are important for pH_i and membrane potential regulation in plants and fungal cells. Previous studies carried out in our laboratory have shown that pH_i homeostasis in *T. cruzi* epimastigotes is not dependent on Na⁺, K⁺ or HCO₃⁻, requires ATP and is affected by inhibitors of the P-type H⁺-ATPase, suggesting a major role for this pump in this developmental stage. We have also obtained biochemical evidence indicating the presence of P-type H⁺-ATPase, not only in the plasma membrane, but also in intracellular compartments in the three developmental stages of *T. cruzi*. In a recent study we have identified 2 isoforms of this enzyme arranged in tandem in the parasite genome which we named *TcHA1* and *TcHA2*. Here we used an anti-serum against a sequence common to both isoforms to show that *T. cruzi* P-type H⁺-ATPases are in fact localized not only in the plasma membrane of epimastigotes, but also in intracellular structures belonging to the parasite endocytic pathway. Immunofluorescence analysis showed that the anti-serum recognized intracellular compartments known as the reservosomes. These organelles have pre-lysosomal features and are the last compartment of the epimastigote endocytic pathway. Labeling of reservosomes by uptake of gold-conjugated bovine serum albumin (BSA) followed by immunoelectron microscopy added more evidence to the presence of this enzyme in this compartment. We were also able to detect an ATP-dependent proton uptake activity in subcellular fractions enriched in reservosomes. This activity was resistant to high concentrations of bafilomycin A₁, but highly sensitive to vanadate demonstrating the presence of a P-type proton pump in these structures. Using parasites transfected with tagged forms of *TcHA1* or *TcHA2* we were able to localize both

isoforms in the reservosomes while only TcHA1 was in the plasma membrane. Together, these results suggest the presence of a P-type H⁺-ATPase in the endocytic pathway of *T. cruzi*.

14. LIPID COMPOSITION OF ACIDOCALCISOMES OF *Trypanosoma cruzi*

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Acidocalcisomes are acidic calcium-storage organelles characterized by their high electron density and high content of pyrophosphate, polyphosphate, calcium and other cations. We report here the lipid composition of *T. cruzi* acidocalcisomes. The acidocalcisome fraction was obtained according to Scott et al. *J. Biol. Chem.* 275, 24215-24221, 2000, with some modifications to minimize membrane contamination, which was less than 10% as shown by three different membrane markers. An equal amount in protein of acidocalcisome and microsomal fraction was used to evaluate lipid enrichment. The phospholipids phosphatidylcholine and phosphatidylinositol were around 70% enriched in the acidocalcisome fraction as observed by thin layer chromatography. The structure of these lipids is being confirmed by ESI/MS/MS. The amount of glycoinositolphospholipids (GIPLs) was quantified by measuring inositol. GIPLs were hydrolyzed and free inositol was quantified by High Performance Anion Exchange Chromatography (HPAEC-PAD) in MA-1 columns with 80 mM NaOH. The GIPLs amount in the acidocalcisome fraction was 30% of the microsomal amount, and 20% of it could not be justified by contamination. An antibody against GIPLs containing galactofuranose (*galf*) failed to react with the acidocalcisome fraction ruling out the presence of *galf* in these glycolipids. We are comparing the carbohydrate structure of GIPLs from membrane and acidocalcisome fractions by hydrolysis and neutral sugar analysis by HPAEC-PAD.

15. IDENTIFICATION OF A POTENTIAL DRUG TARGET FOR BISPHOSPHONATES IN *Toxoplasma gondii*

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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 be effective as anti-protozoal agents. The existing treatment for toxoplasmosis, a protozoal disease caused by *Toxoplasma gondii*, is not adequate. We selected bisphosphonates as candidate drugs to inhibit *T. gondii*, because they were found to inhibit *T. gondii* growth *in vitro* and *in vivo* and also because they are already FDA-approved for human use. Our interest focuses on optimizing the structure of these compounds, and characterizing their molecular target. We have measured and calculated the IC₅₀s of 95 bisphosphonates against *T. gondii* *in vitro*, and QSAR studies are being performed. Some of them, showed promising inhibition of *T. gondii* growth *in vitro* and the most potent, compound 22, has an IC₅₀ of 0.28 ± 0.02 μM. The potential target of bisphosphonates in *T. gondii* is the farnesyl pyrophosphate synthase (FPPS), an enzyme that has been found to be the molecular target of bisphosphonates in several other organisms. We have cloned and sequenced the putative gene of the *T. gondii* farnesyl pyrophosphate synthase (*TgFPPS*) which encodes a protein of 605 amino acids. The seven conserved amino acid motifs known to be characteristic of other FPPSs are also found in the *TgFPPS*. Southern blot analysis indicated that the *TgFPPS* gene exists as a single copy in the genome of *T. gondii*. Western blot analysis with affinity-purified antibody against *TgFPPS* showed that this enzyme is expressed in *T. gondii* tachyzoites with an apparent molecular weight of 61 kD. *T. gondii* cells over-expressing *TgFPPS* (*TgFO*) were more resistant to bisphosphonates suggesting that *TgFPPS* is the target. A typical mitochondrial localization pattern is observed by immunofluorescence of *TgFO* using either anti-FLAG or anti-*TgFPPS* antibody. Further experiments are needed to confirm the localization of *TgFPPS*. Purification and biochemical characterization of *TgFPPS* is underway.

16. CHARACTERIZATION OF THE VACUOLAR PROTON PYROPHOSPHATASE (*TgVP1*) IN *Toxoplasma gondii* BY TARGETED GENE DISRUPTION

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The addition of PP_i promoted the acidification of a subcellular compartment in cell homogenates of *Toxoplasma gondii* tachyzoites, implying the presence of a proton-translocating pyrophosphatase (*TgVP1*). The proton gradient was collapsed by addition of the K^+/H^+ antiporter nigericin, and was also inhibited by addition of the PP_i analog aminomethylenediphosphonate (AMDP) (Rodrigues et al., *Biochem. J.* 349, 737-745, 2000). To investigate the physiological role of this *TgVP1* further, we have used homologous recombination to produce a knock out mutant in an RH dhxgprt strain (RHwt2) a clonal derivative of the RH strain (RHwt1). The targeting construct *pTgVP1/HXGPRT* was linearized with *Sca I* and electroporated into RHwt2, using mycophenolic acid and xanthine to select for stably transfected parasites. Genomic DNA from 64 clones was analyzed by PCR, using *TgVP1*-specific or *HXGPRT*-specific primers. Seven clones might have replaced the wild-type locus via a double crossover event. Two clones, 4C7 and 1C12 were confirmed to contain a disruption of the *TgVP1* gene by Southern blot, RT-PCR, Western blot, and immunofluorescence microscopy analysis. These two clones were characterized in regard to their acidocalcisome content and growth rate.

17. PRESENCE OF A SODIUM/HYDROGEN EXCHANGER IN *Toxoplasma gondii* AND ITS INVOLVEMENT IN CALCIUM REGULATION

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Sodium/Hydrogen Exchangers (NHEs) are ubiquitous membrane proteins involved in the exchange of H^+ for Na^+ . Although NHE have been described in *Leishmania donovani* (Vercesi et al., 2000), *Trypanosoma brucei* (Vercesi and Docampo, 1996) and *Plasmodium* (Bosia et al., 1993), no NHE has been previously described in *Toxoplasma* parasites. Micromolar concentrations of the NHE inhibitor amiloride reduced proton efflux by half in a parental strain (RH Δ *hpt*) of *Toxoplasma* tachyzoites. However, in a *Toxoplasma* strain that has the NHE gene disrupted (RH Δ *nhe1*), amiloride did not have any effect in the proton efflux. The resistance to the reduction of proton efflux exhibited by

RH Δ *nhe1* is specific for the NHE inhibitor since the V-H⁺-ATPase inhibitor, bafilomycin A₁, affected proton efflux to the same level in both the parental strain and the RH Δ *nhe1* parasites. Studies in NHEs of other intracellular parasites suggested a relationship between NHE and calcium regulation and therefore we measured intracellular calcium concentrations ([Ca²⁺]_i) in the parental and NHE knockout strains. Knockout NHE strain was less able to regulate their [Ca²⁺]_i and exhibited an elevated [Ca²⁺]_i as compared to the wild type strain. These results indicate that the disruption of NHE1 results in a deficient ability to regulate [Ca²⁺]_i levels and in accumulation of Ca²⁺ in the cytosol of the parasites. In addition, we studied the possible role of NHE in pH regulation. Intracellular pH was similar in the parental strain and NHE KO parasites under steady-state conditions suggesting that under normal conditions the knockout parasites compensate for the lack of NHE.

18. INTRAMUSCULAR SARCOCYSTS IN A DOG

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This poster presents evidence to extend the known range of intermediate hosts of *Sarcocystis neurona*. This organism is best known for causing a common neurological disease of horses, equine protozoal myelitis. Cysts in skeletal muscle (sarcocysts) of *S. neurona* were recently discovered to occur in striped skunks (*Mephitis mephitis*) and domestic cats. Horses appear to be aberrant intermediate hosts because their muscles are not infected. Opossums (*Didelphis virginiana* and *D. albiventris*) are definitive hosts, which shed sporocysts in feces after consuming infected muscle. In the present investigation, a pet Labrador Retriever was euthanized due to a history of polymyositis and progressive muscular atrophy. Histopathology revealed microscopic protozoal cysts within striated myocytes. Electron microscopy revealed villus protrusions on cyst walls with a character suggestive of *S. neurona* sarcocysts. BLAST analysis of PCR amplified cyst DNA using 18s rRNA primers demonstrated 100% homology with *S. neurona*. A part of the 18s sequence analyzed also aligned completely with *S. falcatula*, a species very similar to *S. neurona* and differentiated only by molecular analysis. Presently, we are in the process of cloning DNA amplified using primers described by Tanhauser et al to confirm the species. Previously, there have been few reports of sarcocysts in dogs, and the organisms have not been speciated precisely. Although, it is unknown if the dog in the present investigation acquired infection from ingestion of another intermediate host, or from ingestion of sporocysts, the domestic dog appears to be an intermediate host of *Sarcocystis neurona*.

19. CHARACTERIZATION OF A MYST-FAMILY HISTONE ACETYLTRANSFERASE IN *Toxoplasma Gondii*

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The protozoan *Toxoplasma gondii* is an obligate intracellular parasite that is a member of the phylum Apicomplexa. *T. gondii* causes congenital birth defects and has emerged recently as a serious opportunistic infection in immunocompromised individuals such as AIDS patients and transplant recipients. Other members of this phylum, such as *Plasmodium* (malaria) and *Eimeria* (coccidiosis), also cause significant medical and economic concerns. While pyrimethamine and sulfa drugs are effective at controlling acute *T. gondii* infection, they produce toxic side effects and fail to rid the body of the encysted form of the parasite, which is the primary source of reactivated toxoplasmosis during immune impairment. In hopes of delineating the transcriptional regulatory events modulating parasite cyst development, we have been examining parasite histone acetyltransferases (HATs). HATs covalently modify nucleosomal histones and generally enhance the rate of gene transcription. We have previously cloned members of the GCN5 HAT family (see related abstract by Bhatti and Sullivan), and here we present our cloning of a MYST-family HAT from *T. gondii* (TgMYST-A). The TgMYST-A gene contains an open reading frame of 1236 nt with 5' and 3' untranslated regions of ~1.5 and ~700 nt respectively. The genomic locus is present as a single copy in the genome and contains 10 exons. The predicted protein consists of 411 amino acids (47.6 kD) and contains a chromodomain and an atypical (C2HC) zinc finger in addition to the MYST HAT domain. We have expressed and purified a recombinant version of TgMYST-A in *E. coli* that exhibits preferentially histone H4 activity. A bioinformatics survey reveals that *T. gondii* possesses at least one additional MYST HAT and several candidate homologues of proteins shown to associate with MYST complexes in other systems. Chromatin immunoprecipitation experiments using anti-acetylated H4 have been employed to access the importance of this modification in *T. gondii*.

20. TgSRCAP: A CHROMATIN REMODELING HOMOLOGUE AND POTENTIAL DRUG TARGET AGAINST *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular protozoan parasite belonging to phylum Apicomplexa. The phylum also harbors pathogens such as *Plasmodium* (malaria), *Eimeria*, and *Cryptosporidium*, all of which are a major

concern in medical and veterinary fields. *Toxoplasma* is notorious for causing congenital toxoplasmosis, an acute infection of the developing fetus resulting in perinatal morbidity and mortality. Toxoplasmosis is generally not considered a major concern in immunocompetent individuals, but it is a life-threatening opportunistic infection of immunosuppressed individuals. In these cases, infection is chronic because the rapidly growing tachyzoites continually re-emerge from slow-growing bradyzoites (cyst form). The current drug therapy, besides having severe side effects, is effective only against tachyzoites. Understanding pathways involved in differentiation of tachyzoites to bradyzoites is critical for developing novel drugs. We have previously cloned TgSRCAP (Snf2-Related CBP Activator Protein), an ATP-dependent chromatin remodeler, and shown that it specifically enhances CREB-mediated transcription like its human homologue. TgSRCAP message levels increase during *in vitro* differentiation, giving us the impetus to examine its role in parasite physiology. Attempts to “knock out” the TgSRCAP gene by homologous recombination failed, implying that its function may be essential for parasite survival and hence may be a suitable new drug target. In an attempt to elucidate the role of TgSRCAP, we sought to identify interacting proteins by employing the yeast two-hybrid system using the lengthy “spacer” region between ATPase domains IV-V as bait (in humans, the corresponding domain binds CBP). Here we describe the results of candidate proteins that may associate with this region of TgSRCAP, which have been confirmed by co-immunoprecipitation *in vitro*. To complement the yeast two-hybrid screen, we are performing GST “pull-downs” of *Toxoplasma* lysates using GST-fused to the equivalent domain of TgSRCAP.

21. NUCLEAR TRAFFICKING OF A HISTONE ACETYLASE IN *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular parasite in phylum Apicomplexa. *T. gondii* threatens neonates and immunocompromised (AIDS) patients, and is recognized as a model for the study of other Apicomplexan pathogens such as those causing malaria or coccidiosis. Virtually nothing is known about how nuclear proteins reach their destination in these parasites. Study of parasite nuclear trafficking promises to reveal insight into how this system evolved in early eukaryotic cells and may lead to novel antiprotozoals to treat disease. Our lab has previously cloned a histone acetyltransferase (HAT) homologue in *T. gondii* that is a member of the GCN5 family (TgGCN5). TgGCN5 contains unusual features warranting further investigation, most notably a lengthy N-terminal extension of 820 amino acids that bears no resemblance to any known protein sequence or functional motif. We have determined that at least one role for the unique N-terminal extension is nuclear

localization, and subsequently used TgGCN5 as a model to explore nuclear trafficking in *T. gondii*. To that end, we have mapped a six amino acid motif that represents a necessary and sufficient nuclear localization signal (NLS) in these parasites. It is the first demonstrated NLS delineated in the phylum Apicomplexa. Searches of *T. gondii* and *Plasmodium* (malaria) EST databases have revealed this particular motif is rare. We have also cloned *T. gondii* importin-alpha (TgIIMA), the main transport receptor in the importin/karyopherin nuclear import pathway, and evaluated if it interacts with the NLS we elucidated for TgGCN5. TgIIMA possesses conserved features including an importin-beta binding domain and several super helical armadillo repeats. Interestingly, TgIIMA more closely resembles plant importin-alpha proteins rather than metazoan, suggesting it may be a useful target for drug design. Bioinformatic searches of the *T. gondii* database suggest two other components of the importin/karyopherin nuclear import pathway are conserved in the parasite: importin-beta and the Ran G-protein. Further characterization of these putative homologues and the identification of more NLSs will facilitate our understanding of the importin/karyopherin nuclear trafficking pathway in Apicomplexa.

22. MICROARRAY ANALYSIS OF *Candida albicans* GENE EXPRESSION DURING BIOFILM FORMATION

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Many variables can affect which genes are differentially expressed between planktonic and biofilm-grown *Candida albicans* cells. For example, *C. albicans* strains vary in their ability to form biofilms, and growth surface influences architecture of the mature biofilm. Since different stages of biofilm development are marked by specific morphologic characteristics, differential gene expression data must also be interpreted within this context. In this work, we sought to identify genes that were differentially expressed in biofilms irrespective of the *C. albicans* strain or growth surface. The experimental design included two different biofilm models (denture and catheter), and two *C. albicans* strains were grown in each model (GDH2346 and SC5314 on denture acrylic; M61 and SC5314 on silicone elastomer disks). Planktonic and biofilm specimens were collected at three time points during biofilm development (6, 12 and 48 hr). Total RNA was extracted from two independent replicates of each specimen and microarray targets were prepared. A 4-block (one for each strain and surface combination), closed-loop design (with inter-nodal connections and dye swap) was used for hybridizations. The microarrays consisted of PCR products representing 6392 *C. albicans* ORFs. Controls included *Arabidopsis* and mammalian DNA fragments, *C. albicans* genes of known expression profiles, and

buffer blanks. All sequences were printed in duplicate on each array. Data were analyzed using a mixed model, and a least square means method was used for comparing specific treatments. As expected, strain-specific differences in gene expression were found in the data. The nature of these differences was influenced by growth surface and stage of biofilm development. Because SC5314 was used in both the denture and catheter models, we were able to identify genes that were differentially expressed on the two growth surfaces. Data analysis also revealed a set of genes that were up-regulated consistently in planktonic cells as compared to their expression level in biofilm specimens. Genes up-regulated in biofilm samples compared to planktonic cells varied with developmental stage, consistent with the changing biofilm morphology at the three time points sampled. Data analysis also revealed a set of genes that were up-regulated in biofilm samples irrespective of strain or surface. Genes from this candidate list will be disrupted to identify those that are required for wild-type biofilm formation.

23. CASE STUDIES IN FUNGAL MICROARRAY DESIGN AND DATA ANALYSIS APPROACHES

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Genome-wide analysis of *Candida albicans* and other fungal organisms is becoming commonplace. There are a large number of experimental and analytical design issues that must be considered to maximize the utility of such efforts. We have addressed a number of these issues and present results from several case studies in both *C. albicans* and *Saccharomyces cerevisiae*. We have assessed the importance of replication in a four-treatment case study and compared the results obtained using both a closed-loop hybridization design and reference design. We present clear evidence of the importance of replication and will discuss general considerations in terms of pair-wise hybridization design issues. In addition, we have addressed the importance of dye swap in either hybridization design. Finally, we compare clustering results using different distance metrics (Euclidean, Manhattan, Sup, Correlation Coefficient) and clustering algorithms (K-means, SOMs, K-medoids, Hierarchical) and highlight the importance of including the variance estimate in such approaches. Overall, the results and comparisons presented are intended to stimulate discussion about various experimental and analytical design issues in this rapidly evolving area of investigation.

24. COMPARISON OF *Candida albicans* ALS GENE EXPRESSION IN HUMAN CLINICAL SPECIMENS AND MODELS OF VAGINAL CANDIDIASIS

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The ALS (agglutinin-like sequence) gene family of *Candida albicans* encodes cell wall glycoproteins, some of which are involved in adhesion to host surfaces. This study used RT-PCR to examine expression of the eight ALS genes in human clinical vaginal specimens and compared the results to gene expression observed in vaginal candidiasis models. Analysis of RNA isolated from vaginal fluid collected from women who were symptomatic or asymptomatic for vaginal candidiasis showed expression of all ALS genes with *ALS1*, *ALS2*, *ALS3* and *ALS9* observed most frequently. Analysis of total RNA and poly(A)-selected RNA extracted from the same clinical specimen indicated that, in general, expression of more genes was detected in the poly(A) sample. Exceptions to this conclusion were *ALS6* and *ALS7*, which were more difficult to detect in poly(A) RNA compared to total RNA. In the clinical specimens, *ALS4* and *ALS5* were detected least frequently overall, but tended to be associated with samples collected from women who were asymptomatic for vaginal candidiasis and not pregnant. Two *C. albicans* strains isolated from the clinical specimens and laboratory strain 3153A were inoculated into models of vaginal candidiasis. Similar to the results from analysis of the human clinical specimens, *ALS1*, *ALS2*, *ALS3* and *ALS9* were detected most frequently in a murine model of vaginal candidiasis. Expression of *ALS4*, *ALS5*, *ALS6*, and *ALS7* was observed, but much less commonly than for the other genes. Comparable results were derived for a reconstituted human vaginal epithelium (RHVE) model inoculated with the same three *C. albicans* strains. In the RHVE model, gene expression results were very similar for the different strains. All ALS gene transcripts were detected across the set of samples, but detection of *ALS4*, *ALS6* and *ALS7* expression was the most sporadic. Although the *ALS4* message was detected readily in *C. albicans* cells used to inoculate the model, its presence diminished or disappeared within the first 24 h of incubation and reappeared as time progressed. Results from these studies demonstrate similarities between ALS gene expression trends in human vaginal specimens and those observed in vaginal candidiasis models.

24b. CELLULAR AND MOLECULAR BIOLOGY OF *Candida albicans* ESTROGEN RESPONSE

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Candida albicans is the most common etiological agent of vaginal candidiasis. In some reports, there is a positive association between elevated host estrogen levels and the incidence of vaginal candidiasis. Elevated estrogen levels may affect host and/or fungal cells. The goal of this work was to examine the effect of 17-beta-estradiol on *C. albicans* cells when added at physiological (10^{-10} M) or supraphysiological concentrations (10^{-7} and 10^{-5} M). Three different *C. albicans* strains and two growth media (synthetic complete and phenol-red-free RPMI 1640) were used. An estrogen-free control was also included for each combination of strain and growth medium. Culture biomass and cellular morphology were examined at four different time points (2, 4, 6 and 8 h in synthetic complete medium and 1, 2, 3 and 5 h in RPMI 1640). Culture biomass did not differ in the presence of any of the concentrations of estrogen. However, at early time points, certain strain and growth medium combinations showed an increase in the percentage of cells forming germ tubes that was directly related to the concentration of estrogen added to the culture. In all cultures examined, germ tube length was increased by the addition of estrogen in a concentration-dependent manner. The availability of genomic microarrays provides a powerful tool for evaluating the effect of estrogen on the *C. albicans* transcriptome. Analysis of the effects of estrogen on *C. albicans* cells focused on the early events by harvesting cells 10 min following estrogen exposure. For microarray analysis, *C. albicans* SC5314 was grown in either synthetic complete or phenol-red-free RPMI 1640 medium in the absence or presence (10^{-5} M or 10^{-10} M) of 17-beta-estradiol. Microarray data analysis identified genes that were up-regulated at either estrogen concentration as well as genes that responded to a specific estrogen concentration. Some genes were differentially expressed by estrogen exposure in both synthetic complete and RPMI 1640 medium while expression of others was only altered in response to estrogen exposure in one growth medium. Further molecular analysis of these genes will define the mechanisms by which their transcription is increased in the presence of estrogen and provide a better understanding of the effects of estrogen exposure on *C. albicans*.

25. USE OF GREEN FLUORESCENT PROTEIN TO MONITOR ALS GENE EXPRESSION IN CULTURED *Candida albicans* CELLS

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We constructed a set of reporter strains in which production of yeast-enhanced green fluorescent protein (GFP) was controlled by ALS gene promoters. For each ALS gene, GFP was integrated into the locus encoding the small ALS allele in the SC5314 background. Yeast forms of the reporter strains were cultured in various media and GFP production was monitored by flow cytometry. Hourly time points were taken across the growth curve for each strain. Some PALS-GFP reporter strains showed an association between GFP production and growth stage of yeast forms. For example, GFP production by yeast forms of the PALS1- and PALS2-GFP reporter strains increased within the first hour after transfer of cells to fresh growth medium and was undetectable as cells exited logarithmic growth. Fluorescence of the PALS4-GFP reporter strain was strong during late stages of growth and decreased during log phase. All of the PALS-GFP reporter strains were inoculated into hyphal induction media and fluorescence followed by flow cytometry at 15 min intervals. Large increases in fluorescence were measured for the PALS1- and PALS3-GFP reporter strains during germ tube formation. The PALS2- and PALS4-GFP reporter strains also showed increased fluorescence during germ tube formation. Increased expression of *ALS6* was detected in germ tubes, although only a small fraction of the cell population produced GFP. This population effect was confirmed by two-photon laser scanning confocal microscopy of hyphal cells. The overall conclusions from flow cytometry and confocal microscopy analysis of the reporter strain collection are consistent with previous Northern blot and RT-PCR analysis of ALS gene expression. This set of PALS-GFP reporter strains provides a powerful tool to define ALS gene expression patterns under various culture conditions. Understanding gene expression patterns is important for identification of meaningful growth conditions for Als protein functional assays and for defining growth conditions useful for microarray analysis of mutant strains.

26. USE OF GFP REPORTER CONSTRUCTS TO DETECT *Candida albicans* ALS GENE EXPRESSION IN MODEL CATHETER BIOFILMS AND DISSEMINATED MURINE CANDIDIASIS

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Yeast-enhanced green fluorescent protein (GFP) was placed under control of each of the different ALS gene promoters in *Candida albicans*. In each reporter strain, GFP was integrated into the small ALS allele in the SC5314 background. The PALS-GFP reporter strains were grown in model catheter biofilms on silicone elastomer to define the pattern of ALS gene expression under these growth conditions. Fluorescence was detected by two-photon laser scanning confocal microscopy for all biofilms grown from PALS-GFP reporter strains, suggesting transcription from each ALS locus occurred in catheter biofilms. Fluorescence from the PALS1-, PALS2-, PALS3- and PALS4-GFP strains was visible early in biofilm formation, while GFP production driven by other ALS promoters became detectable at the later time points in biofilm development. Fluorescence intensity differed for the various reporter strains with the PALS3-GFP strain producing one of the most intense signals and the PALS6-GFP strain producing one of the weakest signals. The set of PALS-GFP reporter strains was also evaluated in the murine model of disseminated candidiasis by inoculating each strain into the tail vein of BALB/cByJ mice. GFP production was monitored immunohistochemically using an anti-GFP antiserum. Tissues were collected from mice 12, 24 and 48 hr following inoculation. PALS1- and PALS3-driven GFP production was easily detected in kidney and heart lesions at each time point with the anti-GFP serum, but the other reporter strains did not produce a detectable signal using this method. Expression of more ALS genes was detected using ALS gene-specific RT-PCR analysis of RNA extracted from homogenized *C. albicans*-infected kidneys at each time point. RT-PCR analysis using GFP-specific primers also revealed expression of more ALS genes than were detected immunohistochemically. Use of the PALS-GFP reporter strains and the various methodologies to assess ALS gene expression provided insight about the sensitivity of each method and the relative level of expression of each ALS gene in different model systems.

27. *Candida albicans* Als3p IS REQUIRED FOR WILD-TYPE BIOFILM FORMATION ON SILICONE ELASTOMER SURFACES

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Candida albicans ALS3 is expressed preferentially in germ tubes and hyphae; Als3p functions in adhesion to endothelial and epithelial surfaces. A PALS3-GFP reporter strain was used to demonstrate expression of ALS3 in model catheter biofilms grown on silicone elastomer. Analysis of the biofilm by two-photon laser scanning confocal microscopy showed fluorescence within hyphal elements throughout the biofilm structure. Als3p was required for wild-type biofilm formation because a biofilm grown with an *als3/als3* strain was structurally disorganized and extremely weakened. Dry weight measurements for the *als3/als3* biofilms were significantly less than those grown with wild-type strains. Complementation of the *als3/als3* mutant with a wild-type copy of the large ALS3 allele from strain SC5314 stabilized the biofilm structure and produced dry weight measurements equivalent to the wild-type strain. Because of its predicted structural resemblance to the adhesive domain of *Saccharomyces cerevisiae* alpha-agglutinin, the Als protein N-terminal domain has been suggested to mediate adhesion. We constructed a *C. albicans* strain that produced a cell-surface-localized protein fusion between the N-terminal domain of Als3p and the C-terminal (non-adhesive) domain of alpha-agglutinin. This strain did not produce native Als3p. The fusion protein restored adhesion of *als3/als3* *C. albicans* strain to buccal epithelial cells demonstrating the adhesive capacity of the Als3p N-terminal domain. However, growth of this strain on a silicone elastomer disk did not restore wild-type biofilm production as measured microscopically and by dry weight analysis. Because Als3p length is known to affect functional properties of the protein, lack of complete restoration of wild-type biofilm growth may be attributable to the relatively shorter fusion protein size. *C. albicans* strains lacking Efg1p cannot form wild-type biofilms. Since ALS3 is a downstream effector of *EFG1*, we grew a *C. albicans* strain that constitutively overproduces Als3p in the catheter biofilm model. Analysis of these biofilms showed that overproduction of Als3p was not sufficient to rescue the *efg1/efg1* mutant strain and suggested that multiple Efg1-regulated factors are required for wild-type biofilm growth.

28. FUNCTIONAL COMPARISONS BETWEEN THE *Candida albicans* ALS PROTEINS

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The *Candida albicans* ALS (agglutinin-like sequence) gene family encodes large, cell-surface glycoproteins that are involved in several cellular functions including adherence to host surfaces. Our research goals include determining the function of each Als protein and also understanding the role of gene families in *C. albicans* pathogenesis. We constructed a set of *C. albicans* strains, each mutant in one of the ALS genes. Each ALS open reading frame was excised precisely from the *C. albicans* genome in order to avoid creation of partial ALS sequences that may produce biologically active protein fragments. Since ALS allelic size can greatly affect conclusions from functional assays, multiple replacement strains were constructed, each with a different ALS allele. Growth rates of each set of mutant/replacement strains were matched using strain CA112 as a control. The *als/als* mutant strains were assayed for defects in filamentation; some exhibited growth-medium-specific filamentation defects. The set of *als/als* mutant strains was used to assess functional differences in adhesion among the Als proteins. Even some of the most closely related Als proteins showed different adhesion specificities. For example, Als3p contributed to endothelial cell and buccal epithelial cell adhesion, but not to adhesion to fibronectin. In contrast, *C. albicans* strains lacking Als1p showed reduced adherence to endothelial cells, but no change in adhesion to buccal epithelial cells or fibronectin. These differences were apparent despite the fact that the amino acid sequences of Als1p and Als3p are 84% identical within the N-terminal domain. These adhesion assays were conducted using germ tubes grown in RPMI 1640 medium, where expression of *ALS1* and *ALS3* greatly increases. Analysis of GFP reporter strains for *ALS2* and *ALS4* showed increased expression of these genes in RPMI 1640, providing growth conditions that could be used to evaluate Als2p and Als4p for their contribution to adhesion. Results from initial experimentation with the reconstituted human epithelium (RHE) model of oral candidiasis closely reflected those obtained with other in vitro adhesion assays. Analysis of the entire set of *als/als* mutant strains in this model highlighted the variability with which Als proteins contribute to adhesion and subsequent epithelial cell destruction. These data, together with information from other experimental approaches, have provided a more complete picture of the roles of the Als proteins in *C. albicans* biology and pathogenesis.

29. MAPPING THE SPREAD OF EMERGENT WILDLIFE DISEASE: THE IMPORTANCE OF THE DENOMINATOR

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Emergent wildlife diseases, particularly those that are zoonotic, can achieve a very high public profile and attract considerable media attention. Public discussion and media coverage tend to focus on the spatial distribution of diagnosed cases, with the underlying distribution of the susceptible and/or tested population receiving minimal attention. Recognition of disease emergence is usually associated with a progressive increase in surveillance activity over time, so qualitative assessment of genuine spread of infection is frequently confounded by the increased probability of detecting existing infection as survey effort rises. The obvious solution to this problem is to provide the public and policy-makers with maps of disease prevalence rather than case distribution, however unbiased data on the denominator can be challenging to obtain. Furthermore, maps of prevalence require at least a cursory understanding of how to aggregate, or smooth, spatial data. These issues are illustrated using an analysis of changes in the spatial distribution of bovine tuberculosis infection among free-ranging white-tailed deer in Michigan's northeastern Lower Peninsula over the period 1995 – 2000. Although tuberculosis is widely perceived to have spread rapidly through the deer population during this period, there is no statistical evidence whatsoever to support that interpretation.

30. DIURNAL PATTERNS OF ACTIVITY OF *Ixodes scapularis*, THE TICK VECTOR OF LYME DISEASE.

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As part of a long-term ongoing study of the epidemiology of Lyme disease and the ecology of its tick vector in Illinois and surrounding states, tick bionomics were studied in one infested site in central Illinois. Transmission of Lyme disease is the result of encounters between questing ticks and susceptible vertebrates, thus examining diurnal patterns of tick activity as a function of time of day, temperature, and relative humidity may help determine times of high and low encounter probability.

Ixodes scapularis larvae were collected during a three day period from 14 July to 17 July 2003 using drag-sampling techniques at Natural Land State Park in Putnam County, IL. Three individual grids were established at the park and were dragged during four separate times of the day. Relationships between *I. scapularis* larval activity and temperature, relative humidity, and time of day were examined.

The number of larvae on individual drags ranged from 0-14 with most drags containing between zero and three larvae. Of the three grids, grid one had significantly ($P<0.05$) more larvae per drag. This difference may be related to vegetation, slope or other land cover features. There was no significant relationship found between the numbers of larvae collected and temperature, relative humidity, and time of day.

Based on extensive collections in the past, we predicted that tick activity would be lowest during the hottest part of the day, due to increased water loss and susceptibility to desiccation. We did not find such a relationship in our study. Because our collection dates were cooler than normal, we will repeat our study under more extreme conditions to verify the lack of association of time of day and tick activity.

31. SPATIAL PATTERNS OF URINARY SCHISTOSOMIASIS INFECTION BY AGE AND SEX FOR A HIGHLY ENDEMIC AREA IN COASTAL KENYA

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Urinary schistosomiasis, *Schistosoma haematobium*, is a significant disease burden on the southern coast of Kenya, where it is highly endemic. Within the 25km² study area of Msambweni Division, Kwale District, demographic, parasitological, and household location data for in five villages were obtained and integrated with biotic, abiotic, and location data for local water sources in a geographic information system. The prevalence of infection was found to be similar between males (47 percent) and females (45 percent) ($p>0.05$), but varied by age group ($p<0.0001$). Infection intensity was also comparable between the sexes ($p<0.0001$). Local spatial statistics were applied to detect household clusters of infection levels broken down by age and sex, and examine how infection levels vary with distance from contaminated ponds water sources. High infection levels were found to cluster around a water source known to have had high prevalence of snails shedding human cercariae. When comparing age groups regardless of sex, clusters were found to start at different distances near such water sources. Of particular interest is the focal clustering pattern of infection intensity detected for children ages 6-13 vs. younger and older children, a pattern that is in agreement with changes in water contact behavior and immunity by age. This pattern may reflect that children under 6 who live close to Nimbodze Pond have more contacts with infected water and develop immunity earlier, while children who live farther away do not have considerable water contact with infected water until they are older.

SPONSORS

CENTER FOR ZONOOSES RESEARCH

The Center for Zoonoses Research (CZR) was established by the Board of Trustees of the University of Illinois on January 20, 1960. The Center is composed of outstanding scientists from 15 departments and units at the University of Illinois at Urbana-Champaign. The goals of the CZR are:

- Promote collaborative work among the College of Veterinary Medicine and other UIUC faculty as well as faculty from other institutions worldwide in an integrated dynamic program.
- Develop a 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.
- Promote the dissemination of information concerning zoonoses research through the organization of conferences and seminars, publication of brochures, proceedings of meetings and reports of research activities, and press releases.
- Promote the establishment of training grants to attract top graduate students, post-doctoral trainees and visiting scientists.
- Promote collaborative efforts and service to the Illinois Departments of Public Health and Agriculture.
- Promote the interest and awareness from UIUC faculty and administration about ongoing research on infectious diseases and food safety
- Become a recognized research and training center by international organizations.

COLLEGE OF VETERINARY MEDICINE

The College of Veterinary Medicine was founded fifty five years ago, in 1949. Since 1970 it has occupied a 56,500 square-foot companion-animal hospital and clinic, a 71,700 square-foot food-animal hospital, and a 158,000 square-foot basic sciences building. The College operates the Laboratories of Veterinary Diagnostic Medicine, that receives some 12,000 samples yearly. Operational units within the diagnostic laboratory include virology, immunology, parasitology, clinical pathology, toxicology, microbiology, and pathology. Further access to veterinary medical accessions is provided through the veterinary teaching hospital.

A variety of support facilities is available in the College. The College has its own photography, histology and library facilities and houses the Center for Microscopic Imaging. The College of Veterinary Medicine Library has 700 specialty serial titles and 32,000 reference books and is part of the University of Illinois library system. The Biomedical Communications Center is available to aid in planning and producing communication materials in art, photography, and television to support research and education. The 12,000 square-foot laboratory animal care facility is designed to meet the needs of research in both noninfectious and infectious diseases. Other animal care facilities, including a 75-acre farm, a fully-equipped companion- and food-animal hospital, and isolation and conditioning quarters, are readily available. The Office of Continuing Education and Public Service offers a variety of consultative services to companion- and food-animal practitioners and the general public. It provides a medium for the dissemination of research findings through regular continuing education conferences and publications.

The College also maintains its own Gopher and World-Wide Web server (<http://www.cvm.uiuc.edu/>). These information services provide a two-way street for College faculty, students and staff to access information anywhere in the world via Internet, and for people outside of the College to learn about College resources and programs.

DEPARTMENT OF VETERINARY PATHOBIOLOGY

The Department of Veterinary Pathobiology plays a central role in the University of Illinois's 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 (<http://www.cvm.uiuc.edu/vp/>).

The majority of departmental research addresses two critical global issues: infectious diseases and environmental toxicology. Both are relevant to human and animal health and have a significant impact on global economic trade. There is extensive collaboration across basic science and clinical departments within the College, across campus, and nationally and internationally. Faculty participates in campus-wide interdisciplinary programs, including those in nutritional sciences, neurosciences, cell and molecular biology, genetics, and environmental toxicology. Support for research comes from the National Institutes of Health, the Centers for Disease Control and Prevention, the U.S. Department of Agriculture, the Illinois Department of Agriculture, the American Heart Association, the World Health Organization, and many other sources.

Departmental research in infectious diseases is coordinated through the Center for Zoonosis Research, which addresses diseases of local, national, and global importance. Major research areas include food safety (diseases due to *E. coli*, *Salmonella* sp., and *Toxoplasma gondii*), vector-borne diseases (malaria, trypanosomiasis, leishmaniasis, Lyme disease, rickettsial diseases, and arbovirus), AIDS-related diseases (cryptosporidiosis, and candidiasis), and animal diseases of economic importance (including those due to *E. coli*, *Salmonella* sp., pseudorabies, rotavirus, porcine reproductive and respiratory syndrome [PRRS] virus, and parasites).

The department has a strong graduate program that attracts students with a B.S., M.S., or D.V.M. degree. 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. Residency programs in pathology and continuing education courses, such as molecular biology, are also offered. Undergraduate teaching is increasing through courses taught collaboratively with other departments and through research opportunities within the department. Computer technologies are used to enhance instruction and develop distance learning, which is critical to education today and in the future.