Deans’ Forum on
INFECTIOUS DISEASES

September 28-29, 2008
Virginia Polytechnic Institute and State University

www.infectiousdiseases.cod.vt.edu
In *The Entry of Animals into Noah's Ark*, Jan Brueghel the Elder depicts Noah shepherding animals of every type towards the safety of the ark in the far distance. The painting illustrates the interconnectedness of animals, humans, and the environment. Just as their existence is reliant upon many inter-dependencies, so too can their demise be propagated through the incubation, transmission and pathogenesis of infectious diseases.
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A transmission electron micrograph of a number of Lassa virus virions adjacent to some cell debris.
Letter From the Deans

Dear Participant,

Welcome to the Deans’ Forum on Infectious Diseases, the fourth in a series of forums created in support of the University Strategic Plan. The goal of this forum and those of its predecessors is to highlight the research of our faculty, staff and students as well as encourage new collaborations all across the university.

As you all know, infectious diseases pose a serious risk to all levels of society. Virginia Tech has a vast and significant array of research underway to fight emerging and re-emerging diseases and improve life around the world.

We hope you will use this forum as an opportunity to showcase your research while meeting, exchanging ideas, and creating synergies with others who share common interests.

This book contains over 100 abstracts submitted by faculty, staff, students, and guests of the university. In the back you will find contact information for the primary author of every abstract. Please continue to use this as a resource even after the conclusion of this forum’s proceedings to further foster inter-college partnerships in learning, discovery and engagement.

Thank you for your participation. We hope you enjoy the forum.

Sincerely,

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Virginia-Maryland Regional College of Veterinary Medicine

Lay Nam Chang
Dean
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Deans’ Forum on Infectious Diseases
presented by the Deans of Virginia Tech

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The Council of Deans recognizes the efforts of the individuals behind the creation and implementation of this forum. Their contributions and efforts are greatly appreciated.

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Deans’ Forum on
INFECTIONOUS DISEASES

Host-Pathogen Interaction
An important aspect of systems biology is the elucidation of the protein-protein interactions (PPIs) that control important biological processes within a cell and between organisms. In particular, at the cellular and molecular level, interactions between a pathogen and its host play a vital role in initiating infection and a successful pathogenesis. Despite recent successes in the advancement of the systems biology of model organisms to understand complex diseases, the analysis of infectious diseases at the systems-level has not received as much attention. Since pathogen-related disease is responsible for millions of deaths and billions of dollars in damage to crops and livestock, understanding the mechanisms employed by pathogens to infect their hosts is critical in the development of new and effective therapeutic strategies. The research presented here is one of the first computational approaches to studying host-pathogen PPI networks. There are two main aims. First, we discuss analytical tools for studying host-pathogen networks to identify common pathways perturbed and manipulated by pathogens. We present the first global comparison of the host-pathogen PPI networks of 190 different pathogens and their interactions with human proteins. We also present the construction and analysis of three highly infectious human-bacterial PPI networks: *Bacillus anthracis*, *Francisella tularensis*, and *Yersinia pestis*. The second aim of the research is the development of predictive models for identifying PPIs between host and pathogen proteins. We present two methods: (i) a domain-based approach that uses frequency of domain-pairs in intra-species PPIs, and (ii) a supervised machine learning method that is trained on known inter-species PPIs.
Suppression of LPS-induced IFNg and Nitric Oxide in Splenic Lymphocytes by Select Estrogen-regulated miRNA: A Novel Mechanism of Immune Modulation

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MicroRNAs (miRNA), recently identified, non-coding, small RNAs, are emerging as key regulators in homeostasis of the immune system. Therefore, aberrant expression of miRNA may be linked to immune dysfunction, such as in chronic inflammation and autoimmunity. In this study, we investigated the potential role of miRNA in estrogen-mediated regulation of innate immune responses, as indicated by upregulation of LPS-induced IFNg, inducible nitric oxide synthase (iNOS), and nitric oxide in splenic lymphocytes from estrogen-treated mice. We found that miR-146a, a negative regulator of Toll-like receptor (TLR) signaling, was decreased in freshly-isolated splenic lymphocytes from estrogen-treated mice compared to placebo controls. Increasing the activity of miR-146a significantly inhibited LPS-induced IFNg and iNOS expression in mouse splenic lymphocytes. Further, miRNA microarray and real-time RT-PCR analysis revealed that estrogen selectively upregulates/downregulates the expression of miRNA in mouse splenic lymphocytes. miR-223, which is highly upregulated by estrogen, regulates LPS-induced IFNg, but not iNOS or nitric oxide in splenic lymphocytes. Inhibition of miR-223 activity decreased LPS-induced IFNg in splenic lymphocytes from estrogen-treated mice. Our data are the first to demonstrate the selective regulation of miRNA expression in immune cells by estrogen and are indicative of an important role of miRNA in estrogen-mediated immune regulation.

![Figure 1. LPS-induced IFNγ and nitric oxide in mouse splenic lymphocytes is promoted by estrogen.](image.png)
Figure 2. LPS-induced IFNγ, iNOS and nitric oxide in mouse splenic lymphocytes is regulated by the estrogen-regulated miRNA, miR-146a.

Figure 3. miRNA expression profile in mouse splenic lymphocytes is altered by in vivo estrogen treatment.
Genomic Sequences of Mesogenic Strains of Pigeon Paramyxovirus-1 Isolated in the United States

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Pigeon paramyxovirus type 1 (PPMV-1) is an antigenic variant of Newcastle disease virus (NDV) or avian paramyxovirus type-1 (APMV-1), belonging the Avulavirus genus in the Paramyxovirinae subfamily. PPMV-1 strains have been frequently isolated from pigeons in the U.S. The potential risk of dissemination from the pigeon population into commercial poultry as documented in Britain and Austria raise the need for a better characterization of these viruses. To develop a reverse genetics system and to understand the role of these viruses in the causation of outbreaks in domestic chickens, we have initiated genetic and pathotypic characterization studies of three viruses (PPMV-1/New York/1984, PPMV-1/ Maryland/1984, PPMV-1/Texas/1998) isolated from pigeons in the United States. All three viruses were mesogenic in nature based on standard characterization tests. The genome of all three viruses consisted of six non-overlapping genes in the order 3'-NP-P-M-F-HN-L-5', coding for the nucleocapsid (NP), phospho- (P), matrix (M), fusion (F), hemagglutinin-neuraminidase (HN), and large polymerase (L) proteins, respectively. The gene junctions contained highly conserved gene start and transcription termination sequences with variable intergenic sequences as seen in Newcastle disease virus and other paramyxoviruses. The cleavage motif of the F protein conformed to the consensus motif with multibasic amino acids (112RRQKRF117) for furin cleavage, found in velogenic NDV strains. The 15REAKNTWR22 pattern within the intracellular domain of HN proteins, specific for APMV-1 strains, was found conserved in all three viruses. The deduced amino acid sequences of the F, HN and L proteins of these three viruses were used to infer phylogenetic relationships with other Paramyxoviridae. All three viruses clustered with PPMV-1 strains in the genus Avulavirus.
An Anomalous Type IV Secretion System in *Rickettsia* is Evolutionarily Conserved

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**Background:** Bacterial type IV secretion systems (T4SSs) comprise a diverse family of transporters that function in conjugation, competence, DNA release, and effector molecule (DNA and/or protein) translocation. Thirteen genome sequences from *Rickettsia*, obligate intracellular symbionts/pathogens of a wide range of eukaryotes, have revealed a T4SS similar (yet reductive) to the Agrobacterium archetype (vir). However, the *Rickettsia* T4SS has not yet been functionally characterized for its role in symbiosis/virulence, and none of its prospective substrates have been identified.

**Results:** Superimposition of structural and functional information from other T4SSs over previously identified *Rickettsia* Vir-like genes (*vir*B3, *vir*B4, *vir*B6, *vir*B8-*vir*B11, and *vir*D4) suggests that the *Rickettsia* T4SS is a functional transporter. *vir*B4, *vir*B8 and *vir*B9 are duplicated, yet only one copy of each gene adheres to the conserved features of the T4SS. An extraordinarily duplicated *Vir*B6 gene encodes five highly variable proteins constrained in hydrophobicity, all of which are conserved in a region essential for substrate transfer to *Vir*B8. Bioinformatic analysis revealed the presence of *vir*B1, *vir*B2 and *vir*B7; thus, the *Rickettsia* T4SS is only devoid of *vir*B5 relative to the *Agrobacterium* archetype. Phylogeny estimation suggests all 18 *Vir*-like components have been vertically inherited, despite the rearrangement of these genes into an archipelago of five islets. Similarities of *Rickettsia* VirB7/VirB9 to ComB7/ComB9 proteins of γ-proteobacteria, as well as overall phylogenetic affinities to the *Legionella* lvh T4SS, imply the *Rickettsiales* ancestor acquired a *vir*-like locus early in its evolution from distantly-related bacteria, probably while residing in a protozoan host. Modern modifications of these systems likely reflect diversification with various eukaryotic host cells.

**Conclusion:** We present the rvh (*Rickettsiales* vir homolog) T4SS, an evolutionary conserved transporter with an unknown role in rickettsial biology. This work lays the foundation for future laboratory characterization of this system, and also targets the *Legionella* lvh T4SS as a suitable genetic model.
Porcine DC-SIGN: Molecular Cloning, Gene Structure, Tissue Distribution and Binding Characteristics

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DC-SIGN, a human C-type lectin, is involved in the transmission of many enveloped viruses. Here we report the cloning and characterization of the cDNA and gene encoding porcine DC-SIGN (pDC-SIGN). The full-length pDC-SIGN cDNA encodes a type II transmembrane protein of 240 amino acids. Phylogenetic analysis revealed that pDC-SIGN, together with bovine, canis and equine DC-SIGN, are more closely-related to mouse SIGNR7 and SIGNR8 than to human DC-SIGN. pDC-SIGN has the same gene structure as bovine, canis DC-SIGN and mouse SIGNR8 with eight exons. pDC-SIGN mRNA expression was detected in pig spleen, thymus, lymph node, lung, bone marrow and muscles. pDC-SIGN protein was found to express on the surface of monocyte-derived macrophages and dendritic cells, and alveolar macrophages but not monocytes or peripheral blood lymphocytes. A BHK cell line stably expressing pDC-SIGN binds to human ICAM-3 and ICAM-2 immunoadhesins in a calcium-dependent manner, although there was no apparent augmentation of endocytosis ability. The results will help better understand the biological role(s) of DC-SIGN family in innate immunity during the evolutionary process.
Host Derived Inflammatory Phospholipids Regulate rahU (PA0122) Gene, Protein and Biofilm in Pseudomonas aeruginosa

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1-Palmitoyl-2-arachidonoyl-sn-phosphatidylcholine (PAPC) is a naturally occurring phospholipid containing polyunsaturated arachidonic acid (AA) and lysophosphatidylcholine (lysoPC), which are integral parts of mammalian cell membranes and oxidized-low-density lipoprotein (Ox-LDL). These lipids have been reported to modulate immune cells and inflammation in humans. We have recently identified Pseudomonas aeruginosa PA0122 as an Ox-LDL- and lysoPC-binding protein whose function remained elusive. Functional genomic analysis of rahU using host-derived phospholipids showed: (a) rahU not only binds to lysoPC but augments its own synthesis in the presence of lysoPC in wild-type P. aeruginosa (rahU+). (b) PA0122 promoter activity was increased by lysoPC and inhibited by PAPC, Ox-PAPC and AA, the latter effect being reversible by free lysoPC [or lysoPC released from PAPC] (c) A constructed P. aeruginosa rahU mutant (rahU-) exhibited spontaneous increase in biofilm formation as compared to rahU+. (d) Phospholipids (PAPC and AA (but not lysoPC)) significantly augmented biofilm formation in rahU+ cells, but not in rahU- cells. In summary this study shows for the first time that host derived phospholipids signal P. aeruginosa gene and protein expression as well as regulate biofilm formation via rahU. We now refer to the PA0122 protein as rahU, derived from a Sanskrit word ‘Rahu’ for “the seizer”. This unique, multifunctional gene/protein seizes various environmental conditions to modulate biofilm formation and inflammation.
rahU: A Functionally Pleotropic Protein in *Pseudomonas aeruginosa* Modulates Innate Immunity and Inflammation

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In the accompanying report, we show the regulation of expression of *rahU* gene in *Pseudomonas aeruginosa* by human inflammatory phospholipids and its role in biofilm formation. In the present report, we demonstrate two additional functions of *rahU* in host macrophages and T cells. *rahU* was cloned into pET 28b expression plasmid, and the expressed protein was affinity purified, detoxified and identified by western blotting. Incubation with native-endotoxin free *rahU* at 1.0, 2.0 and 10 µg/ml inhibited the accumulation of nitric oxide (NO) in murine macrophage (RAW 246.7) cells in the presence of LPS by 10 percent to 50 percent. The IC50 of *rahU* was similar to 50 µM of Prednisone and 100 µM of NMMA. Moreover, immunoadsorption or proteinase K treatment of *rahU* resulted in loss of this NO production inhibitory activity. Monocyte Chemotactic Protein-2 (CCL2)-induced recruitment of T cells was also inhibited significantly with 30 µg/ml of recombinant *rahU* but not proteinase K treated *rahU*. Similar observations were made when other chemo-attractants (which represent physiological concentrations of phospholipids) such as supernatants procured from apoptotic T cells were used in the chemotactic assay. In summary, *rahU* interferes with macrophage activation and NO production-associated with innate immunity. *rahU* interferes with, and inhibits, at least two chemotactic signals involved in inflammation, infection and recruitment of macrophages and T cells during host–bacteria interactions. In conclusion, these two reports identify previously unknown pleitropic functions of a single bacterial derived protein *rahU*: which acts as an “innate guru”, modulating “opportunistic behavior” synonymous with *Pseudomonas*. Thus *rahU* underscores itself as a candidate target for therapeutics and biomarkers.
Synergistic Interplay between Macrophages, Alveolar Type II Cells and Neutrophils in Viral Predisposition to Secondary Bacterial Infections

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Mild and self-limiting viral or bacterial infections can become fatal when they occur concomitantly or as secondary infections. One of the reasons for disease exacerbation in polymicrobial infections is attributed to alterations in innate immune responses. Our laboratory has previously described a mouse infection model of synergistic disease exacerbation of respiratory viral and bacterial agents, in which a significant overexpression of specific cytokines and chemokines was observed in mice challenged with *Streptococcus pneumoniae* following infection with influenza A virus (IAV). Here, we have focused our studies on the role of the alveolar macrophage and its interplay with the respiratory epithelium as well as neutrophils in respiratory disease exacerbation. Preliminary results using primary mouse bone marrow-derived macrophages (BMDM), and the alveolar macrophage cell line (MH-S), infected with IAV followed by superinfection with *S. pneumoniae* or *Staphylococcus aureus* revealed distinct mRNA expression profiles compared to singly exposed macrophages. IAV induced gene expression of pro-inflammatory cytokines such as IL12, iNOS, and TNF-α, whereas upon superinfection with *S. pneumoniae*, BMDM gene expression of M2 markers IL-10 and Arg-1 was upregulated by more than 600 fold and 17 fold, respectively. Bacterial superinfection of BMDM cells also produced high levels of chemokines such as KC, MIP2 and MCP compared to singly or sham-infected cells. Synergistic infection of MH-S cells revealed similar patterns of gene expression profiles. Interestingly, treatment of BMDM and MH-S cells with LPS or IFN-γ, prior to infection with *S. pneumoniae* or *S. aureus* showed a different expression pattern compared to superinfection of virus infected cells, suggesting that viral infection alters macrophage polarization. We are currently evaluating the synergistic interplay between differentially activated macrophages with the respiratory epithelium (alveolar type II cells) and neutrophils. A co-culture infection model of epithelial cells, macrophages and neutrophils would provide valuable information toward better understanding and combating of polymicrobial infections.
Despite the increasing interest in *Ochrobactrum anthropi* as an emerging nosocomial pathogen resistant to most commonly used antimicrobials, relatively little is known about pathogenesis and factors contributing to its virulence. Also, many aspects of interaction between *Ochrobactrum* spp. and their hosts remain unclear. The ability to monitor *O. anthropi* infection in the host will facilitate our understanding of the pathogenic mechanisms and will lead to better choices of antimicrobial or additional therapeutic strategies. We have demonstrated the ability to stably express three reporter genes (green fluorescence protein GFP, red fluorescence protein RFP and luciferase Lux) and track the infection in a J774A.1 murine macrophage cell line as well as in the BALB/c mouse. Our results suggest that these reporter genes should improve genetic studies in *O. anthropi*, particularly those aimed at understanding pathogenesis, virulence factors, and host interaction.
Enhanced Lymphocyte Proliferation in Response to Monocyte Derived Dendritic Cells from Cows with Prior *Staphylococcus aureus* Mastitis

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*Staphylococcus aureus* is a versatile pathogen causing a variety of diseases in both humans and animals. It has been reported that infected individuals are not protected from subsequent infection because of defective immunological memory. Even though several *S. aureus* vaccines have been developed, none offer specific and persistent protection. As an alternative approach, dendritic cells (DC) are being used as biological adjuvants in vaccines to enhance T cell memory. The objective of this study was to evaluate the ability of monocyte derived DC to induce antigen specific lymphocyte proliferation in response to *S. aureus*. Peripheral blood monocytes (CD14+) were isolated from cows previously diagnosed with *S. aureus* mastitis (infected, n=5). Control cows (n=5) were chosen based on lack of previous record of *S. aureus* mastitis. For DC differentiation, CD14+ cells were cultured for seven days in RPMI based medium supplemented with recombinant bovine granulocyte-monocyte colony stimulating factor and recombinant bovine interleukin-4. Differentiated DC had a greater intensity of expression of CD205, MHC II, CD11c and low expression of CD14 as assessed by flowcytometry. To measure lymphocyte proliferation, 105 DC were loaded with *S. aureus* isolates from infected cows at a low multiplicity of infection (104 CFU). Autologous lymphocytes were added after overnight incubation. There was a significant increase in lymphocyte proliferative response in infected cows compared to control animals, indicative of a memory response to *S. aureus*. Understanding the DC-T cell interactions in response to *S. aureus* might be valuable for the design of a successful vaccine.
The United States contributes roughly half of the world’s $14 billion soybean industry. A major problem for producers is infectious diseases which annually cause over a 10 percent yield loss in the U.S. Our Plant Genomics laboratory focuses on genomic approaches to investigate soybean disease resistance and develop tools for breeders to use when integrating resistance genes into high yielding soybean varieties. *Phytophthora* root and stem rot and Soybean Mosaic Virus (SMV) are two diseases that can cause substantial yield losses annually. Our current research focuses on creating soybean lines with durable disease resistance and dissecting gene expression patterns during *Phytophthora* infections. Pathogen populations are continually evolving to overcome single gene-mediated resistance in widely grown commercial soybean cultivars. Therefore, single gene resistance has been shown to be effective for eight to 15 years, placing pressure on breeders to develop new commercial cultivars quickly. One potential solution is to incorporate multiple resistance genes for a given disease into one cultivar. The “pyramided” resistance genes create lines which are resistant to multiple strains or races of a given pathogen. Another solution is to integrate quantitative (multigenic) resistance into agronomically superior cultivars. Quantitative resistance confers resistance to all strains of a pathogen and cannot be overcome. To facilitate efficient gene pyramiding and quantitative resistance incorporation into soybean cultivars, DNA molecular markers are needed to allow breeders to artificially select for resistance, avoiding costly disease assays. When a plant comes under attack by a pathogen there is a genome-wide response with changes in gene expression. Microarrays allow researchers to measure the expression levels for thousands of genes simultaneously, providing a snapshot or profile of genes which are expressed during the initial invasion and defense response. These expression profiles allow for identification of potentially important defense-related genes which are specifically regulated during pathogen attacks.
Over Expression of a *Brucella suis* Urease Gene into *Brucella melitensis* for Complementation of Urease Activity

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Brucellosis, especially caused by *Brucella melitensis*, remains the most common zoonotic disease worldwide with more than 500,000 cases reported annually. The bacterial pathogen is also classified by the CDC as a category (B) pathogen that has the potential for development as a bio-weapon. Although eight genomes of *Brucella* have been sequenced, little information is available regarding urease genes and their regulation and activity in *Brucella* spp. It has been proposed that urease activity is responsible for providing the microenvironment necessary for the *Brucella* to survive passage through the acidity of gastric mucosa. *Brucella suis* has been shown to have the highest urease activity of the three main *Brucella* spp. (*B. abortus*, *B. melitensis*, and *B. suis*), which could be attributed to a nine base pair insert in the *ureE* gene (metallo chaperone that helps maintain the confirmation of the multisubunit urease enzyme) of *B. melitensis* and *B. abortus*. In this study, we cloned the *ureE* gene from *B. suis* strain 1330 into a *Brucella* expression vector (pNSGroE) in order to compliment urease activity of *B. melitensis* strain 16M. The over expression of *B. suis* ureE in *B. melitensis* caused a slight increase in urease activity when compared to wild type *B. melitensis*. However, the urease activity was not increased to the same extent as that of *B. suis*. This would indicate that the absence of the nine base pair insert in the *ureE* gene of *B. suis* is not solely responsible for the increased urease activity of *B. suis* when compared to *B. melitensis*. 
Computational Approach for Predicting Broad-spectrum Host Response Targets

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During the last few years, significant progress has been made toward developing counter-measures for some of the most infectious pathogens. However, it is clear that the “one-bug one-drug” approach is difficult to extend to all categories of pathogen due to the high cost associated with the drug discovery process. Furthermore, the range of infectious disease is too vast to dwell on this approach. NIAID has proposed adopting a broad-spectrum drug discovery strategy, an approach aimed at developing counter-measures that are effective against a variety of pathogens and toxins. The realization of this vision requires computational approaches that are capable of identifying drug targets that promise to be effective against multiple pathogens. We propose to develop a computational approach for predicting broad-spectrum host response targets for infectious diseases by integrating three biological datasets: i) gene expression data on human and mouse response to infectious pathogens, ii) molecular interaction datasets that capture known cellular wiring diagram of the hosts, and iii) manually curated interaction pathways. Our goal is to identify pathways that are commonly perturbed in multiple infectious diseases and to prioritize host genes in such pathways for validation as drug targets. The hypothesis underlying this approach is that such pathways (i.e. those perturbed by multiple pathogens) will contain many candidates for broad-spectrum host targets. Another important aspect of the proposed approach is that targeting the host genes, proteins and pathways (as opposed to the pathogen’s enzymes) is believed to have a potential in overcoming the capacity of pathogens to mutate and become resistant to drugs.
A Porcine Epithelial Cell Model for Study of Innate Immune Responses to Rotavirus Infection and Probiotic Bacteria Colonization

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Rotaviruses replicate in epithelial cells of the small intestine and cause dehydrating diarrhea in infants, young children and young animals. Innate responses to rotavirus infection and commensal bacteria colonization of the intestinal epithelial cells may contribute significantly to the resistance or the pathogenesis of rotavirus infection; however, there is little information on innate immune responses to rotavirus infection in the intestinal epithelial cells. In this study, we evaluated a porcine small intestinal epithelial cell line (IPEC-J2) as an in vitro model of porcine or human rotavirus infection and commensal lactobacilli colonization. MA104 cell-culture-adapted porcine rotavirus OSU strain (OSU PRV) and human rotavirus Wa strain (Wa HRV) and probiotic Lactobacillus acidophilus (LA) and L. rhamnosus GG (LGG) were used to inoculate the IPEC-J2 cells. The OSU PRV was able to infect and replicate in the IPEC-J2 cells to a much greater extent than the Wa HRV. The innate cytokine IL-6, IL-8, IL-15 and TNF-α, production by IPEC-J2 cells after OSU PRV or Wa HRV infection or LA and LGG colonization were measured in the culture supernatants by ELISAs. The concentrations of IL-8 induced by the OSU PRV were significantly higher than that of the Wa HRV. LA plus LGG induced significantly lower IL-6 levels, but significantly higher IL-15 levels than the OSU PRV. Our results suggest that the IPEC-J2 cell line is a useful model for studying interactions among rotavirus, probiotic bacteria and host small intestinal epithelial cells.
Characterization of Seasonal Diversity of the Spinach (*Spinacea oleracea*) Phylosphere Microbial Community Using Culture and Non-Culture Dependent Techniques

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A number of recent food borne pathogen outbreaks are associated with leafy greens. It is important to identify the native microbial communities of phyllosphere surfaces of leafy greens in order to begin to elucidate the mechanisms by which food borne pathogens are able to persist in edible leaves. It is likely that the influence of the microflora could have a role in the establishment and survival of pathogens. The purpose of this work was to determine changes in spinach leaves microbial diversity and its relation with the time of harvesting and the spinach cultivar. Three spinach cultivars (Monza, Menorca, Unipack) were organically grown during fall 2007 at the Virginia Tech Experimental Research Farm. Spinach leaves (length<10 cm) were harvested during the first week of October, November, and December respectively. Aerobic plate counts were performed using minimal media and potato dextrose agar for a period of 16 days after harvest. Total microbial DNA, without enrichment, was isolated for each harvest and for each cultivar. 16S rRNA genes for the community were amplified using universal primers and fragments analyzed through denaturant gradient gel electrophoresis (DGGE). Dendrograms were constructed to study the differences among the three harvest periods and cultivars. Total counts and colonial morphology of culturable epiphytic microbial population showed significant differences (p>0.05) amongst the three cultivars and the three different harvest periods. Microbial counts were influenced by the surface of leaves, finding higher microbial counts in the Unipack (savoy) leaves than in the Monza (flat) leaves. DGGE patterns showed that samples with higher microbial counts also correspond to samples with higher diversity however no differences among the three cultivar DGGE patterns were found within each harvest period. We conclude that cultivar and time of harvest influence the microbial community. These seasonal and cultivar differences could impact the survival of bacteria antagonistic to food borne pathogens and in consequence may favor the persistence of these microorganisms.
Immunohistochemical Identification of Dendritic Cells in the Bovine Mammary Gland

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Dendritic cells (DC) are a heterogeneous population of professional antigen presenting cells and are potent stimulators of T-cells. DCs play an integral role in immunity, and essentially bridge the gap between the innate and adaptive immune system. DCs can be differentiated morphologically by their long dendrites and by relatively high expression of antigen capture and presentation proteins, including MHC-II, CD80, CD86, CD205, and others. Immature DCs can be found in a wide variety of tissue, where they serve as sentries to pathogens. Upon stimulation by foreign particles, such as viral or bacterial components, DCs expand their expression of proteins associated with lymphocyte stimulation. They then migrate to secondary lymphoid tissues where they present antigens and stimulate lymphocytes. Using immunohistochemistry, we have identified DCs in the epidermis and dermis of bovine teat tissue, by using antibody markers for MHC-II and CD205. Future work will attempt to characterize DCs in other parts of the mammary gland, and identify any spatial relationships that exist between immune cells and structural tissue. This information will help us to better understand the immune response in the mammary gland, which may aid in the design of therapeutic treatments.
Identification and Relative Abundance of Small RNAs in Chikungunya Virus Infected Mosquitoes

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RNA interference (RNAi) is a mechanism triggered by dsRNA resulting in a sequence specific silencing of gene expression. In cells, dsRNA is recognized and cleaved into 21-28 nucleotide small dsRNA products. Small RNAs found in somatic cells include microRNAs (miRNAs) and small interfering RNAs (siRNAs). miRNAs are derived from the cleavage of endogenous dsRNA and regulate host gene expression. siRNAs are typically derived from both endogenous and exogenous sources of dsRNAs, and in invertebrates function as the sequence-specific mediators of an antiviral RNAi pathway. While there is evidence to support a functional antiviral RNAi response in mosquitoes, it remains unclear if siRNAs affect the ability of mosquitoes to vector human diseases caused by arthropod-borne viruses (arboviruses). To begin to address this question we used molecular cloning and high-throughput sequencing to identify virus derived siRNAs (viRNAs) in infected mosquitoes. In brief, mosquitoes were injected with an alphavirus. After an incubation period, total RNA was size fractionated and small RNAs (18-30nts) were isolated. RNA adapters of known sequence were ligated to the 5’ and 3’ ends of the small RNAs. This was followed by RT-PCR amplification. Reverse transcribed and amplified products were then sequenced on an Illumina genome analyzer. Our results show both the relative abundance and strand-specific genome-wide distribution of viRNAs in relation to the chikungunya virus (CHIKV) genome. This information provides novel insights into the origin and processing of viRNAs in mosquitoes infected with CHIKV.
Comparison of Virulence Factors among Klebsiella Isolates with Varying Prevalence

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Klebsiella spp are environmental, gram negative, coliform bacteria, and are the third most common cause of community acquired and nosocomial infections in humans that result in high mortality rates. As with infections found in humans, Klebsiella spp account for high mortality rates in dairy cows leading to significant financial loss. Recently, common Klebsiella strains responsible for mastitis infections in dairy cows have been identified in multiple animals at the same time on the same farm which is unusual for a pathogen considered not contagious. In this study, Klebsiella spp isolates from multiple and single infected dairy cows were evaluated for their ability to evade neutrophil killing, the primary immune defense in the bovine mammary gland. Additionally, each isolate of Klebsiella was evaluated for capsule and biofilm production. Bacteria isolates found in multiple animals showed evasion rates, capsule sizes, and biofilm production different from isolates found in single animals. Our results suggest that isolates found in single animals are better able to evade neutrophil killing and have larger capsules than isolates found in multiple animals. Our future research will focus on differential expression of adhesion factors that allow bacterial adherence to mammary epithelial cells. Our current results and future studies will explain the increased rate of single Klebsiella isolates causing infections in multiple animals. The determination of Klebsiella’s evasion and adhesion factors will identify potential routes for prevention and treatment of this infection.
Staphylococcus aureus Suppresses Immune Response of Bovine Mammary Epithelial Cells during Intracellular Infection

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Staphylococcus aureus is a major cause of nosocomial and community acquired infections as well as chronic and subclinical infections of the mammary gland. Antibiotic resistant S. aureus strains are a present concern for treatment; therefore, understanding the mechanisms of infection and host-pathogen interactions may convey new targets for therapy. This gram positive bacterium has developed a number of virulence factors that contribute to its versatility in infection and allow for adherence to the epithelial cells in the mammary gland. In addition to adherence, S. aureus can invade and cause intracellular infections in bovine mammary epithelial cells (bMEC). Intracellular S. aureus may manipulate the immune response to secure a chronic infection. Our goal is to determine the inflammatory and coagulation protein expression of bMEC during a S. aureus intracellular infection. Epithelial cells were infected with live S. aureus, Escherichia coli, or stimulated with S. aureus supernatant. RNA was collected for protein expression throughout several hours post-infection. Gene expression profiles of bMEC infected with intracellular S. aureus hardly fluctuated throughout time and remained close to baseline, comparable to unstimulated bMEC. Gene expression profiles of bMEC stimulated with S. aureus supernatant increased as compared with unstimulated cells, but was lower than bMEC treated with E. coli. Our results suggest that S. aureus suppresses cellular responses defenses by causing intracellular infection and thereby avoiding immune detection.
Influenza A viruses (IAV) are generally host specific and they rarely establish stable lineages in another species. Although whole viruses may rarely transmit, gene segments can reassort and viruses cross the species barrier. However, swine influenza A viruses (SIV) have established stable genetic lineages in turkeys in the United States in recent years. Despite their uniform ability to bind to oligosaccharide-containing sialic acids, IAVs show differences in receptor specificity. The preferential binding to α-2,3 (avian) or α-2,6 (swine and human)-linked oligosaccharides can be altered by changes in HA-specific amino acids that influence both host specificity and cell tropism. To identify the minimal amino acid changes involved in receptor specificity and efficient transmission of SIV to turkeys, we have sequentially passaged five recent triple reassortant H3N2 SIV isolates from Minnesota in nine to 11 day-old specific-pathogen-free turkey embryos. Complete nucleotide sequencing of the PB1, HA and NA genes before and after each passage was undertaken to identify the host-adapted changes in the antigenic sites and host specific amino acids. We showed that virus replication in turkey embryos leads to mutations in the HA gene of SIVs that are similar to those found in triple reassortant turkey H3N2 viruses. Glycan-HA interactions in the respiratory epithelial cells of swine and turkeys was analyzed. Homology modeling of HA1 proteins indicated a switch in the glycan topology from the open umbrella structure to the cone form after passaging the viruses in turkey embryos. Our results suggest that turkey embryos could serve as an alternative model to study virus evolution and interspecies transmission of SIVs.
Effect of Different Growth Conditions on Gene Expression of *Brucella suis*

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*Brucella suis* is a gram negative, facultative intracellular bacterium that primarily infects swine but can also infect humans causing severe clinical manifestations and thereby classified as a Category B bioterrorism agent by the U.S. Centers for Disease Control and Prevention. Expression profiling of the genome at both pre-infection and post-infection stages would help us in identifying virulence factors or critical steps in the infection. As a baseline study, analysis of RNA from wild type *B. suis* strain 1330 grown in enriched (control) and minimal media (mimicking host environment) was performed using a custom designed Affymetrics microarray containing 4770 *B. suis* probe sets. A total of 432 genes were differentially regulated (P<0.05 and at least two fold) between the two treatments with 280 genes up-regulated and 152 genes down-regulated. The major group of genes (33 genes) that were down-regulated are transporters (ABC-, sugar- and others). *B. suis* is known for its pronounced urease activity compared to other species of *Brucella*. A urease mutant of *B. suis* was constructed and its differential gene expression also was studied under enriched and minimal media growth conditions. In case of urease mutant, additional genes were differentially regulated when compared to wild type strain that could play a role in infection. Overall, in the urease mutant, 375 genes were differentially regulated (at least two fold, p<0.05) whereas 236 genes were down-regulated, while only 139 were up-regulated. Thus a mutation in urease has pleiotrophic effects on gene expression and precludes direct assessment of whether the urease is directly responsible for virulence.
Intracellular Drug Targeting of Mycobacterium tuberculosis

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Currently, about one third of the world's population is infected with Mycobacterium tuberculosis (Mtb). Each year approximately eight million people develop active tuberculosis and about three million die from the disease worldwide. In the United States a resurgence of Mtb cases has occurred in recent years and is attributed to emigration from countries with high Mtb prevalence, the HIV epidemic, outdated control strategies and poor patient compliance. In 1993, the World Health Organization declared Mtb a global public health emergency. The ability of Mtb to evade destruction inside the macrophage is the key to its virulence. Developing newer drug strategies that target the intracellular environment are needed to improve patient compliance, decrease the development of resistant bacteria and lessen the toxic side effects of the drugs. The goal of this project is to explore if different nanoparticles loaded with various antibiotics versus the conventional therapeutic regimen will effectively enter and eliminate M. tuberculosis in the macrophage without toxic side effects. In-vitro cell culture studies using the J774A.1 murine macrophage cell lines were done to estimate the efficiency of delivery and efficacy of these nanoparticles. The results and the comparative efficacy of different nanoparticles will be discussed.
Francisella tularensis Infection in IRAK1-deficient Macrophages: Role of TLR-2 Signaling

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Francisella tularensis is a gram-negative coccobacillus, the etiological agent of tularemia, and a category A biowarfare agent. F. tularensis is atypical in that it survives and grows inside host MØ by escaping into the cytoplasm before phagosome-lyzosome fusion, its LPS is non-toxic, and it inhibits TLR-4 and IL-1 signaling. Interluken-1 Receptor Associated Kinase 1 (IRAK1) plays a critical role in regulation of the host inflammatory response via TLR-4, TLR2, and IL-1 signaling pathways. Lipopolysaccharide (LPS) activates IRAK1 in macrophages (MØ) and IRAK1-deficient mice can survive lethal LPS challenge. The role of IRAK1 in protection of mice against F. tularensis was investigated. Bone marrow derived macrophages (BMDMs) were collected from IRAK1-deficient and wild-type (WT) mice, and were co-incubated with live F. tularensis live vaccine strain (LVS) for plate counts, labeled with colloidal gold for transmission electron microscopy or with LVS containing green fluorescent protein (GFP) for flow cytometry. We evaluated three criteria for quantification of an in vitro response: rate of phagocytosis, number of bacteria phagocitized, and percent lyzosome present in infected BMDMs. At 15, 30, and 60 minute post infection, IRAK1-/- BMDMs were found to phagocitize bacteria at a faster rate than WT cells. IRAK1-/- BMDMs and peritoneal macrophages contained twice as many bacteria as macrophages from WT cells. In addition, the percent of lysosome in WT cells was greater than in IRAK1-deficient MØ showing a decreased inflammatory response to the pathogen regardless of the rate. Since F. tularensis does not signal through TLR4, these results were likely from the effects of TLR2 signaling.
Mechanism of EsaR Regulation by Acylated Homoserine Lactone

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In *Pantoea stewartii subsp. stewartii*, two regulatory proteins play a key role in the bacterial cell-cell communication system known as quorum sensing. These proteins EsaI, an acylated homoserine lactone synthase, and EsaR, a response regulator, control capsular polysaccharide production and virulence in the plant pathogen. The mechanism by which these regulatory proteins confer this regulation is opposite of that found in most quorum sensing networks studied to date. Previous studies have demonstrated that EsaR is stable and functions as a repressor under low concentration of N-(3-oxo-hexanoyl)-L-homoserine lactone (AHL). It is only in high concentrations that the AHL appears to bind and cause some conformational shift in the protein leading to derepression of the system. This study focuses on the mechanism by which AHL regulates EsaR. Through the use of pulse-chase experiments and *in vitro* protease digestion, insights into the proteolytic regulation of EsaR within the cell are being gained. Experiments are also being performed with gel filtration chromatography to examine the multimeric state of the protein in both the presence and absence of AHL. Taken together these results will help reveal the mode of AHL regulation of EsaR leading to control of the quorum sensing system of *P. stewartii*. 
Innate Immune Interactions of Murine Bone Marrow Derived Dendritic Cells to Heat Killed, Irradiated and Live *Brucella abortus* Rough and Smooth Strains

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*Brucella* are Gram-negative facultative intracellular bacteria, causing abortion and infertility in livestock and chronic illness in humans. Transmission occurs through inhalation and ingestion from infected animals. The Centers for Disease Control and Prevention (CDC) categorizes it as a class B pathogen. There are no human vaccines available. Strain RB51 and strain 2308 are the live attenuated rough and virulent smooth strains of *Brucella abortus*, respectively. Protection to Brucellosis is conferred by a strong innate response and resultant CD4+ Th1 and CD8+ Tc1 response. Dendritic cells (DCs) are the key mediators bridging innate immunity to adaptive immunity. Recent results show that in contrast to smooth strain 2308, rough vaccine strain induces phenotypic and functional maturation of DCs. However, specific mechanisms underlying this immune phenomenon are not fully understood. In this study, we used heat killed and irradiated rough (strain RB51) and smooth (strain 2308) strains of *B. abortus* to compare their differential effects on DC maturation and activation. The phenotypic maturation of infected BMDCs at MOIs 1:10 and 1:100 were characterized by analyzing the surface expression of MHC class II and co-stimulatory markers (CD40 and CD86). The results show that both heat killed and irradiated rough strains stimulate dose dependent increased expression of DC-MHC class II compared to corresponding smooth strains. BMDCs infected with heat killed RB51 also show dose dependent increases in DC, CD40 and CD86 expression compared to heat killed 2308 strain. The results from this experiment will enhance the knowledge base towards developing a successful human Brucellosis vaccine.
Individual Based Model of HIV Spread and Evolution within the Body–What Drives Strain Emergence?

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Genetic recombination plays a critical role in HIV divergence and evolution, which has negative consequences in terms of immunological escape and treatment resistance. One of the greatest determinants of recombination occurrence and the consequent product is the infection of a single CD4+ T-cell with multiple HIV viral genomes (superinfection). Research indicates that this process is mediated by the CD4 receptor density on the surface of the target cell as well as the cell’s proximity to other superinfected T-cells and antigen-presenting cells in the body. We are investigating the application of high resolution individual-based modeling techniques to simulate HIV superinfection of CD4+ T-cells and the subsequent recombination rate of bodily viral population as a function of immune cell-cell contacts in the body. Our approach is unique in that it seeks to represent each cell as individual agents, includes organ and tissue locations distinguished by cell presence and interactions permitted, detailed representation of viral transmission points, as well as a model of viral genetic evolution upon each cell-cell contact and infection. I will present a preliminary model of interactions among CD4+ T-cells, free virions, macrophages, dendritic cells, and follicular dendritic cells as they move among tissue, organs, and lymphnodes. Later phases will include parameters specific to different organs and tissues as well as effects of HIV on cell behavior and movement. The simulation will be used to test hypothesis pertaining to the aspects of our physiology that affect HIV recombination rates, e.g. how does immune activation/suppression affect the superinfection and subsequent recombination rate of an HIV population? What role do tissue reservoirs play in viral genomic diversification throughout infection? How do these effects compare to the effect of heterogeneity of the initial infecting population? The main purpose of the presentation is to gather relevant critiques particularly pertaining to biological assumptions of this preliminary model.
Histophilus somni Biofilm Formation in Cardiopulmonary Tissue of the Bovine Host following Respiratory Challenge

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Biofilms form in a variety of host sites following infection with many bacterial species. However, the study of biofilms in a host is hindered due to the lack of protocols for experimentally studying biofilms in vivo. Histophilus somni is an agent of respiratory and systemic diseases in bovines, and readily forms biofilms in vitro. In the present study the capability of H. somni to form biofilms in cardiopulmonary tissue following experimental respiratory infection in the bovine host was examined by light microscopy, transmission electron microscopy, immunoelectron microscopy of ultrathin cryosections, scanning electron microscopy of freeze-fractured samples, and fluorescent in situ hybridization. Biofilms were evident and most prominent in the myocardium, and were associated with a large amount of amorphous extracellular material. Furthermore, Pasteurella multocida was often cultured with H. somni from heart and lung samples. This investigation demonstrated that H. somni is capable of forming a biofilm in its natural host, and that such a biofilm may be capable of harboring other bovine respiratory disease pathogens.

Fig. 1. Cardiac and lung tissue visualized by light microscopy. (A-B), H&E-stained sections of cardiac tissue with aggregates of bacteria. (C), lung tissue with infiltrations of neutrophils and macrophages. Magnification: (A-B), 20X; (C), 100X.
Fig. 2. TEM of cardiac and lung tissue. Top panel: (A-B), cardiac tissue containing a large number of bacteria dispersed within an extracellular substance (matrix). Scale bars: (A), 10 μm; (B), 1 μm. Bottom panel: (C-D), lung tissue with neutrophils and macrophages (C), and small bacterial clusters surrounded by amorphous material (D). Scale bars: (C), 10 μm; (D), 1 μm.

Fig. 3. IEM of ultrathin cryosections of cardiac tissue. (A), IEM images of cardiac tissue containing bacteria surrounded by an electron-lucent halo; (B-C), immunolabeling with gold particles around bacterial cells and on the matrix between the cells. Scale bars: (A) 0.5 μm; (B-C) 0.2 μm.

Fig. 4. SEM of freeze-fractured samples of cardiac and lung tissue. Top panel: (A), SEM images of normal cardiac tissue; (B-C), infected cardiac tissue containing coccus-shaped bacteria surrounded in a network of matrix. Scale bars: (A), 2 μm with magnification 20,000X; (B-C), 5 μm with magnification 10,000X. Bottom panel: (D), normal lung tissue; (E-F), infected lung tissue containing few coccobacilli within an extracellular matrix. Scale bars: (E), 1 μm with magnification 30,000X; (F), 0.5 μm with magnification 60,000X.

Fig. 5. FISH of bacterial biofilms on cardiac and lung tissue using a specific oligonucleotide 16S rRNA probe for *H. somni* labeled with Cy3. Top panel: (A), normal cardiac tissue; (B-C), infected cardiac tissue with microcolonies of bacteria within the biofilm matrix. The bacteria are stained red and the surrounding matrix is a diffuse light red (autofluorescent). Scale bars: (A), 20 μm; (B-C), 10 μm. Bottom panel: (D), normal lung tissue; (E-F), infected lung tissue with individual bacteria stained red. Scale bars: (D-F), 20 μm.
Using a Plant–Bacterial Pathogen Interaction Model to Study Host Range and Virulence Evolution of Infectious Bacteria

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*Pseudomonas syringae* is a wide-spread plant pathogen species that causes damage to global crops. *P. syringae* is also the most intensively studied model bacterial plant pathogen. *P. syringae* utilizes the Type III Secretion System (T3SS), which is very similar to that employed by bacterial animal pathogens, such as *E. coli*. To investigate host range and virulence evolution of this species, we have selected a group of closely related isolates that have distinct host range differences. The selected group of isolates includes the completely sequenced *Arabidopsis thaliana* and tomato pathogen PtoDC3000 while other members of this group, such as PtoT1 and PtoJL1065, are not pathogens of *A. thaliana*. Genome sequencing of T1 has revealed diversity in some genomic regions, which are believed to be the primary contributors to the observed virulence and host range differences between isolates. We are currently in the process of comparing the defense responses of *A. thaliana* to DC3000 and T1-like isolates. We found evidence that suggests that unknown pathogen associated molecular patterns of T1 are eliciting basal defense mechanisms. On the other hand, some of T1’s Type III effectors seem to suppress defense responses. These preliminary results confirmed the complexity of plant-microbe interactions and further demonstrated the potential threat of emerging novel pathogens through horizontal gene transfer and gene deletion. We believe our research using this system will contribute to the fundamental understanding of pathogen evolution and thus facilitate research in public health and infectious diseases of humans and animals.
Deans’ Forum on

INFECTIOUS DISEASES

Infectious Disease Ecology and Epidemiology
Disease Dynamics in Freshwater Trematode Systems

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Digenean trematodes are a diverse group of parasitic flatworms (Phylum Platyhelminthes, Class Trematoda, Subclass Digenea). They can infect all vertebrate classes, and are common parasites in freshwater systems containing aquatic snails, which serve as obligate first intermediate hosts. Most digenean trematodes infect three successive host species, although there is considerable variation in the life cycle. In an ecological sense, trematodes can be viewed as representative of a group of wildlife parasites and pathogens that have complex life-cycles involving multiple host or vector species that operate at very different spatial scales. Research in the Belden lab is focused both on understanding the factors that influence trematode dynamics in free-living wildlife populations, and also on using trematode systems as experimental models for elucidating some of the general underlying ecological mechanisms that operate in host-parasite systems. Examples of our research along both of these lines of inquiry will be presented.
Determining the Origins of Waterborne Pathogens in the Yangtze River, China

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In China, waterborne pathogens that cause diseases such as cholera and typhoid fever are endemic, are spread through contaminated water, and seasonal outbreaks occur every year. The goal of this new project is to deploy modern microbiological methods to trace pathogens found in the Yangtze River to the sources of the pollution. The Yangtze River, the third longest river in the world, flows some 5,800 km from Qinghai Province in west China to the East China Sea. The river passes through several large cities including Wuhan, Nanjing, and Shanghai, which combined include more than 35 million people. This project is sponsored by the Chinese Center for Disease Control in Nanjing. The first objective is to determine if the microbial pollution in the river is from human or non-human sources. Methods being introduced that are specific to human-origin pollution include the Enterococcus esp gene, Enterococcus speciation, the Bacteroides thetaiotamicron assay, the Bacteroides human-specific marker, human polyomavirus, and optical brighteners (compounds found in laundry detergents). The microbial methods are all PCR based and will involve quantitative and real-time as well as conventional PCR where appropriate. The initial field site for testing the methodology is the Guwui reservoir on the Huai River (a tributary of the Yangtze), where the sources of human pollution have already been identified. An epidemiological approach will be used to detect and identify fecal indicator bacteria (FIB) and specific bacterial pathogens, and then attempt to correlate these with the specific human-origin markers. To date intensive sampling has identified areas in the reservoir where large concentrations of both FIB and pathogens are present. The next step will be to test and evaluate the human-specific markers in these same areas to determine how well the markers can be used as an alternative to the other more conventional approaches.
Emergence of Leishmaniasis and Trypanosomiasis as Potential Zoonotic Diseases in the United States

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Visceral leishmaniasis and American trypanosomiasis (Chagas disease) are potentially fatal diseases in both dogs and humans. Dogs serve as reservoirs for both parasites. Leishmaniasis caused by Leishmania infantum has recently emerged in the foxhound population within the U.S. and in parts of Canada. Leishmania infections are usually spread to mammals by infected sand flies. In the U.S., epidemiological data do not support a role for sand fly transmission. Our experiments in pregnant dogs and mice revealed that vertical transmission occurred in dogs and mice and that direct transmission occurred in mice with a U.S. isolate of L. infantum. Prevalence studies in wild and domestic dogs indicate that L. infantum is almost exclusively restricted to foxhounds in the U.S. The World Health Organization estimates 16-18 million people are infected with Trypanosoma cruzi, the agent of Chagas disease, in Latin America with 100 million people at risk. A sylvatic cycle of T. cruzi that involves parasite transmission between triatomin bugs and wildlife reservoir hosts is well recognized in the U.S. In our survey of raccoons from an urban area of Virginia for antibodies to T. cruzi we found that 154 (33%) of the 464 samples were positive. In our survey of wild canids from South Carolina, two (8%) of 26 gray foxes were positive for T. cruzi. As emerging pathogens in the U.S., L. infantum and T. cruzi pose a public health risk. There is a need to monitor canine hosts for the presence of these zoonotic parasites.
*Encephalitozoon cuniculi* Antibodies in Dogs from Urban Areas of Brazil and Colombia: Do Dogs Represent a Potential Reservoir for Human Infections?

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*Encephalitozoon cuniculi* is an intracellular microsporidian parasite. Microsporidian parasites are found in many hosts including mammals, fish, and insects. There are three strains of *E. cuniculi* that occur in mammals. Strain III is associated with clinical disease in dogs but many dogs can be asymptomatic carriers and excrete spores in their urine. Several cases of human *E. cuniculi* infection caused by strain III have been observed in immunocompromised patients indicating that *E. cuniculi* should be considered a zoonosis. Additionally, fatal *E. cuniculi* strain III infections have been observed in monkeys further indicating it is a potential zoonosis. *E. cuniculi* can cause fatal disease in maternally infected and young dogs. Clinical signs in these dogs included blindness, encephalitis, retarded growth rate and nephritis. The present study used the indirect fluorescent antibody test (IFAT, cut-off 1:5) and the direct agglutination test (DAT, cut-off 1:50) to examine the prevalence of antibodies to *E. cuniculi* in dogs from Brazil and Columbia. Nine (14.3%) of 63 dogs from Brazil and 18 (35.3%) of the 51 dogs from Columbia were seropositive by IFAT. Using DAG 31 (27.4%) of 113 dogs from Brazil and 47 (18.5%) of 254 dogs from Columbia were seropositive. These results indicate that dogs from Brazil and Columbia are exposed to *E. cuniculi.*
Produce Food Safety Graduate Certificate Program at Virginia Tech

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Increasingly, fresh and minimally processed produce and juice products have been implicated as vehicles of food-borne illness over the last three decades. In response to growing social and scientific concerns regarding the safety of fresh and minimally processed produce, the Department of Food Science and Technology at Virginia Tech has created a multidisciplinary graduate educational and training certificate program emphasizing produce food safety. This program focuses on relevant social, agricultural, scientific and educational issues relating to the safety of fresh and fresh-cut produce. Virginia Tech has created this program to address the projected shortage of qualified candidates for food safety positions declared by the USDA. One of the primary goals of the Produce Food Safety Graduate Certificate program is to train future scientists with the knowledge and skills necessary to address the growing food safety concerns relating to fresh produce in the professional worlds of academia, government or food industry. This program seeks to couple traditional classroom instruction and research with exposure to industrial perspectives and regulatory issues to provide the next generation of scientists the skill set necessary to impact the world of produce food safety. It is our goal to provide students experiential learning opportunities in diverse fields including: media relations, lobbying, regulatory activities, agribusiness, quality assurance and control, and cooperative extension. With the growing number of outbreaks in recent years attributed to produce items, it is essential to have the man-power throughout the food safety framework (industry, government, university) to address these issues.
Geospatial Information Technologies and Remote Sensing for Infectious Disease Research

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Geospatial information technologies provide powerful tools for managing spatial data and analyses for plant, animal, and human health and infectious disease research. A variety of geospatial applications will be demonstrated with primary examples at the landscape scale for two complex disease systems in Virginia: Lyme disease and Barley Yellow Dwarf disease. Geospatial information can be used for numerous applications, including ecological (reservoir, vector, and host mapping), characterization of biotic and abiotic factors (population modeling, soil, temperature, precipitation, ET, wind, terrain, etc.), remote sensing products (land cover, vegetation indexes, land surface temperature, photography), and for mapping demographic, social, and economic indicators. Geospatial technologies may be used for investigating patterns of transmission through diffusion, transport and network models, and can also serve as a spatial framework for a surveillance system that integrates multiple sources of health data and observations for spatio-temporal analysis, simulation, and visualization.
West Nile, Eastern Equine Encephalitis, and St. Louis Encephalitis Viruses in Crested Caracaras in Florida

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Numerous infectious diseases that threaten human populations and/or domestic animals are transmitted by arthropod vectors and amplified within avian hosts. West Nile virus (WNV), Eastern equine encephalitis virus (EEEV), and St. Louis encephalitis virus (SLEV) are such three pathogens that circulate in south Florida where agricultural and equine industries are of high economic importance. These viruses have been found in numerous raptor species which can be both competent amplifying hosts, and highly susceptible to disease. In Florida, the crested caracara (Caracara cheriway) is a non-migratory threatened raptor that breeds almost exclusively in agricultural areas. We captured caracaras throughout the species’ range in Florida, and tested sera for anti-arbovirus antibodies by plaque reduction neutralization test. In 2007 and 2008, we sampled 80 individuals from four age classes (adults, subadults, juveniles, and fledglings). All birds sampled were in apparent good health, were flight-capable, and were foraging normally when captured. The seroprevalence rate for WNV was 8.8 percent, and was 2.9 percent for both SLEV and EEEV. Two caracaras had anti-flavivirus (either WNV or SLEV) antibodies that we could not differentiate. Only adults of ≥ three years of age were antibody positive for any of the three viruses, and adults were significantly more likely to be infected than younger birds. We found infected individuals throughout the species’ range. The susceptibility of caracaras to adverse effects of WNV, SLEV, or EEEV infection is unknown. However, we found that some individuals survive infection and remain productive.
Evaluation of the Mood Stabilizing Agent Valproic Acid as a Preventative for Toxoplasmosis in Mice and Activity against Tissue Cysts in Mice

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Toxoplasma gondii is a common intracellular protozoal infection of humans worldwide. Severe disease can occur in immunocompromised individuals and unborn fetuses of non-immune pregnant women via transplacental transmission. More recently chronic infection is now associated with decreased mental functions, vision and hearing problems and some mental disorders such as schizophrenia. The mood stabilizing agent valproic acid has been shown to inhibit the development of \textit{T. gondii} in vitro at dosages that are normally achieved in the serum and cerebral spinal fluid of human patients. The present study was done to examine the \textit{in vivo} activity of valproic acid against acute and chronic toxoplasmosis in mice. Four experiments were done giving valproic acid in the drinking water or by interperitoneal injection. The concentration of valproic acid in drinking water was 1.5 mg/ml and 3.0 mg/ml (Experiments 1 and 2). Valproic acid was injected interperitoneally at 400 mg/kg/day and 600 mg/kg/day (Experiment 3). A high oral dose was used for treating chronic toxoplasmosis at 6.6 mg/ml (Experiment 4). Results indicate valproic acid was not effective in preventing acute toxoplasmosis in mice. Mice treated with valproic acid did not significantly (P >0.05) live longer than the controls in Experiments 1-3. The numbers of tissue cysts in the brains of mice in Experiment 4 were not decreased in treated mice. Our results indicate valproic acid was ineffective when used as a prophylactic or cystocidal drug.
Influence of Mercury Toxicity on Hemoparasite Prevalence in 
*Tachycineta bicolor*

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The link between ecotoxicology and infectious disease has not been widely explored in the literature and this study aims to partially fill that void. Before 1950, the South River in Virginia was contaminated with mercury. Recent work at the South River has shown that tree swallow (*Tachycineta bicolor*) females along the contaminated river had impaired reproductive success compared to those on uncontaminated reference tributaries (Brasso and Cristol 2008). Knowing that mercury contamination impacts their fitness leads to questions regarding its influence on health and disease susceptibility. The specific aims of this pilot study were to assess (a) cell-mediated immunity and (b) hemoparasite prevalence in tree swallows along the contaminated South River in comparison to those on nearby uncontaminated rivers in the same watershed. We hypothesized that tree swallows along the mercury contaminated sites would have a suppressed immune response and an increased prevalence of hemoparasites. Cell-mediated immunity was examined by the phytohaemagglutinin (PHA) skin test. Hemoparasite prevalence was determined by blood smear examination followed by confirmation with polymerase chain reaction. Based on the PHA skin test, cell-mediated immunity was significantly depressed in swallows on the contaminated sites compared to those on reference sites (p=0.006). While a difference was noted in the prevalence of *Plasmodium* across the sites, the results were not statistically significant. Further research is necessary to understand the relationship between mercury contamination and parasite abundance.
Oviposition Preferences of Three Container-Breeding Mosquitoes and the Possible Implications for La Crosse Virus Transmission

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The exotic container-breeding mosquitoes *Aedes albopictus* and *Aedes japonicus* have been collected in areas endemic to La Crosse virus (LACV) and its primary vector, *Aedes triseriatus*. Because both exotic species are potential vectors of LACV, it is important to understand their population dynamics as it may impact the epidemiology of the virus. A critical factor in the interaction among the container-breeding species is the selection of suitable oviposition sites. In laboratory experiments all three species were given a four choice test and allowed to oviposit in cages with cups containing preexisting eggs, first instar larvae, or larval rearing water (LRW) of the three species plus a control cup containing distilled water. *Ae. albopictus* showed no preference or avoidance in any of the tests. *Ae. japonicus* showed the strongest selection, avoiding cups containing *Ae. triseriatus* eggs or LRW. Field studies using ovitraps showed similar results to the caged experiments with preexisting eggs. Interaction among the vectors in the selection of oviposition sites could impact their comparative distribution and abundance. This, in turn, could have implications for LACV transmission.
The USDA approved vaccine *Brucella abortus* strain RB51 that was developed at Virginia Tech is a widely used vaccine for cattle against brucellosis caused by *B. abortus* (a bioterrorism agent). Our ultimate goal is to use vaccine RB51 as a platform for the delivery of protective antigens from other pathogens so that protection against multiple pathogens can be accomplished at the same time. Anthrax and plague have been the focus on much attention as a potential biological weapon for at least eight decades. It has been estimated that release of 100 kilograms of anthrax spores upwind of Washington DC would result in up to 3,000,000 deaths (U.S. Congress, 1993), cost to society of such a release is estimated at $26.2 billion per 100,000 persons exposed and spores can remain viable for thousands of years. Researchers at the VMRCVM are developing an improved RB51 vaccine that can protect not only against brucellosis, but also against tuberculosis, anthrax, plague, Rift Valley fever and neosporosis.
Sol–Gel Derived Silica Matrix as a Delivery System for Intracellular Pathogens

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The recently developed sol-gel technique offers new possibilities for embedding organic compounds within silica and for controlling their release from the host matrix into a surrounding medium. As yet, such sol-gel carrier systems for drug delivery are not widely known, despite their obvious advantages. The technology is simple and versatile, with the oxide matrix being cheap, inert and stable against light and heat without being hazardous to humans or the environment. We investigated a sol–gel derived silica matrix as a delivery system for the prolonged release of gentamicin for treatment of salmonella infection in mouse model. The particles sizes of our porous silica are in a broad range from 1.7 to 3.3 micron. The release of gentamicin from the inside hollow part of porous carrier can last comparatively a long time, leading to a delayed release of drug (90% of gentamicin released in five days). Administration of three doses of porous silica loaded with gentamicin reduced CFU of *S. thyphimurium* in livers of infected mice by 0.48 log compared to 0.13 log with free drug. The results showing the comparative efficacy of these new tools over the traditional drug therapy will be discussed.
Role of Chromosomal Inversions in Desiccation Resistance of Malaria Mosquitoes

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Adaptation to aridity allows malaria mosquitoes to occupy large regions and survive longer during dry seasons in tropics and subtropics. This adaptation significantly increases malaria transmission. The major malaria vectors in Asia, Anopheles stephensi, and Africa, Anopheles gambiae, adapt to aridity through multiple polymorphic inversions on chromosome 2—the genome rearrangements that result from flipping a chromosomal segment. Understanding the genetic and physiological mechanisms of how chromosomal inversions affect adaptation will allow manipulating with aridity-tolerant genes in wild mosquitoes and will assist in developing vector elimination strategies. The goal of this research was to determine possible association between polymorphic inversions and desiccation resistance in laboratory colonies. Indian wild type of A. stephensi (polymorphic for 2Rb inversion) and G3 strain of A. gambiae (polymorphic for 2Rbc and 2La inversions) were studied. Females were placed in scintillation vials that contained drierite and set in an incubator at 26C. Time to death represented the desiccation resistance of the individual. Each experiment was replicated three times. Cytogenetic analysis and polymerase chain reaction were used to karyotype the mosquitoes. The JMP software was used to perform the ChiSquare statistical test. A. stephensi mosquitoes with an inversion (2Rb/b) died at later times than their counterparts with a standard (2R+/+) or heterozygous (2Rb/+) arrangement of their chromosome, and thus were more resistant to water desiccation. Analysis of the A. gambiae G3 strain revealed no significant difference in resistance among the carriers of the inverted (2La/a), standard (2L+/+) and heterozygous (2La/+) chromosomal arrangements. When desiccation resistance in both species are compared, A. stephensi was more resistant to water desiccation then A. gambiae. The experiments demonstrated that 2Rb inversion is associated with desiccation resistance of A. stephensi females. Surprisingly, the study failed to demonstrate association of 2La inversion with desiccation resistance of A. gambiae females. A plausible explanation is that G3 strain of A. gambiae has lost the adaptive alleles during inbreeding in the absence of selection for aridity since its establishment in 1975.
Complete Nucleotide Sequence of Two Swine Parainfluenzaviruses Isolated from the United States

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Two novel paramyxoviruses 81-19252 (Texas-81) and 92-7783 (ISU-92) were isolated from the brain of pigs that experienced respiratory and central nervous system disease in the 1980s and 1990s from South and North Central United States. Antigenic analysis of these viruses indicated that they were closely related to human parainfluenzavirus (HPIV) types 1 and 3, and bovine parainfluenza virus (BPIV) type 3. To further characterize the viruses, we have initiated sequence analysis of the isolates. The complete genome of Texas-81 virus was 15456 nucleotides (nt) and ISU-92 was 15480 nt in length and consisted of six non-overlapping genes in the order 3'-N-P/C/V-M-F-HN-L-5'. The gene junctions contain highly conserved transcription start and stop signal sequences and trinucleotide (AAG) intergenic regions similar to those of other Paramyxoviridae. The initiating A residue of the successive N, P, M, F, HN, and L mRNAs of each virus was found at hexamer positions 2,1,1,1,1, and 2, respectively, similar to HPIV3 and BPIV3 and followed the "Rule of six." The complete genome of the two viruses had 92 percent identity with each other and the swine viruses shared 98.0 percent homology with BPIV3(SF or 910N) and 82.9 percent homology with HPIV3 (Washington). Individual genes shared 97.2 percent -100 percent homology with BPIV3 and 80.3 percent - 83.7 percent homology with HPIV3, while the predicted proteins shared 87.6 percent -100 percent homology with BPIV3 and 63.9 percent - 91.7 percent homology with HPIV3. Phylogenetic analysis based on the envelope glycoproteins placed these viruses into the Respirovirus genus of the Paramyxovirinae subfamily.
Oviposition Activity of La Crosse Virus Vector Mosquitoes in Southwestern Virginia

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Foci of La Crosse Virus (LACV), the primary cause of pediatric encephalitis in the U.S., have recently emerged in southwestern Virginia. Reasons for the rise in LACV activity may include the relatively recent invasions of the mosquito species *Aedes albopictus* and *Aedes japonicus*, which coexist in this area along with the primary LACV vector, *Aedes triseriatus*. The purpose of this study was to evaluate the oviposition activity of vector mosquitoes to determine their comparative abundance and distribution. Ovitrap surveys were conducted in 2005 and 2006 in three regions of southwest Virginia: one with previous reports of human LACV encephalitis and LACV positive mosquito isolates, another with LACV positive mosquito isolates, and another with no reported human LACV encephalitis cases and/or mosquito isolates. These study regions were predominately rural, suburban and urban areas respectively. Egg abundance and oviposition patterns of the three vectors, *Ae. triseriatus*, *Ae. albopictus*, and *Ae. japonicus* varied across the study regions. *Ae. triseriatus* was collected in the greatest abundance from all three study regions. *Ae. albopictus* was the second most abundant species collected, favoring urban environments in particular. *Ae. japonicus* demonstrated active range expansion, as it was collected at many more sites in 2006 than 2005. Because there is a correlation between the risk of human LACV infection in endemic areas and the density of breeding sites available to the vector, these data suggest that the levels of risk probably vary throughout these regions. Serological surveys of canines are ongoing and will be compared spatially with vector distribution and abundance.
Characterization of Native Spinach Microbiota that are Antagonistic to Growth of Foodborne Pathogens *Escherichia coli* O157:H7 and *Salmonella enterica* in vitro

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**Background:** Leafy greens, such as spinach, have been the object of several recent food borne pathogen outbreaks. The purpose of this project is to isolate epiphytic bacteria from spinach leaves that displays antagonism towards the pathogens *Escherichia coli* O157:H7 and *Salmonella enterica* in vitro.

**Methods:** Three spinach cultivars, Monza, Unipack and Menorca, were grown in the spring of 2008 using conventional agricultural methods. Additionally, the culture Monza was grown using organic agricultural practices. Microbial counts were performed on R2A, PDA and PCA media for 16 days at 25°C. The colonies that were isolated from the plates were tested for antagonism towards the pathogens *E.coli* O157:H7 and *S.enterica* Typhimurium by being transferred to plates with a lawn of the pathogen. The plates were incubated at room temperature and checked daily for antagonism, which was indicated by zones of inhibition surrounding the bacteria colony. The 16S rRNA gene from bacteria displaying antagonism towards one of the pathogens had was amplified and sequenced to determine the identity at the genera level.

**Results:** Forty seven isolates displayed antagonism towards *E.coli* O157:H7 or *S.enterica*. There was significant difference between both the cultivars and the organic and conventionally grown spinach in the microbial count and the number of antagonists that were present. Sequencing of the 16s rDNA revealed that the bacteria antagonistic towards *E.coli* O157:H7 belonged to the genus *Erwinia, Microbacterium, Brevundimonas, Pseudomonas, Stenotrophomonas, Acidovorax, Paenibacillus, Flavobacterium, Actinobacterium, Pseudoalteromonas* and *Bacillus*. The bacteria antagonistic towards *S.enterica* belonged to the genus *Stenotrophomonas* and *Erwinia*.

**Conclusions:** Bacteria were isolated from the spinach microflora displayed antagonism towards the pathogens *E.coli* O157:H7 or *S. enterica in vitro*. These bacteria could potentially have an impact on the survival of pathogens on spinach leaves that are in the field or have been minimally processed. Future research will focus on the mechanism of the interaction between the antagonist and the pathogen and if any of the microflora have a positive interaction with the pathogens.
Deans’ Forum on
INFECTIOUS DISEASES

Vector/Disease Prevention and Control
Indoor Residual Lining against Endophilic Malaria Vectors

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The renewed impetus to control or even eliminate malaria requires innovation. Indoor residual spraying is a vector control strategy that has reduced the burden of malaria in many regions. However, in sub-Saharan Africa with the most efficient vectors, this strategy has not produced the desired results. In rural areas where malaria is most prevalent, homes do not have adequate sprayable surfaces, and are therefore not suitable for residual spraying. In light of this, indoor residual lining (IRL) is proposed. IRL involves lining the interior of human dwellings with insecticide treated material in a fashion similar to decorative wallpapers. This will allow mosaic application of insecticides in localities where vectors are resistant to insecticides commonly used in public health campaigns. This strategy is expected to overcome most of the obstacles encountered in deploying indoor residual spraying and bed nets, and complement other malaria control interventions.
A Spatial Analysis of the 2008 Measles Outbreaks to Assess Possible Future Outbreak Locations in the U.S.

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A minor wave of measles outbreaks has occurred in the U.S. in 15 states and two cities resulting in the largest number of cases of this potentially life-threatening disease in a decade. So far in 2008, doctors have reported 132 measles cases, resulting from importations abroad and occurring almost exclusively in unvaccinated persons. As a result, there remains an ongoing risk for measles and other infectious, vaccine-preventable diseases among unvaccinated persons nationwide. Measles vaccination programs have been so successful since 1963 when the vaccine was first introduced, that parents no longer fear the disease but instead fear the potential adverse effects from the vaccine. The past decade has seen a marked increase in the number of parents refusing vaccines, and the vaccine which includes measles in particular, due to unsubstantiated beliefs that the vaccine causes autism. Despite every state requiring immunizations for public school entry, almost all grant exemptions on religious, medical, and even philosophical grounds. The number of parents requesting exemptions has increased in many states in the past several years. Thus, many health officials are not surprised that outbreaks are increasing as a result. This poster will reflect a spatial look at the percentage rates of immunization exemptions across the U.S. to see how they may relate to the current increase in measles cases. In addition, the data will be analyzed to predict future outbreak locations based on current exemption trends.
Sampling for Detection of Disease in High Impact Animal Disease Outbreaks

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Animal diseases like foot and mouth disease, classical swine fever, exotic Newcastle disease, and zoonotic diseases like avian influenza greatly impact animal welfare, economic well-being of producers and food security. Surveillance systems with accurate diagnostic tests and highly sensitive sampling strategies need to be in place to rapidly detect infection in an outbreak. Sampling in animal health surveillance is the selection of units (individual animals, pens, herds or flocks /premises) from a population to 'collect' specimens / data in order to determine presence or absence of a disease in that population. Sampling sensitivity, $se_{samp}$, is the ability to detect disease when present in the sampled population and is the product of multiple probabilities for each selected unit. This product includes the probability $p_H$ of presence of the disease agent in a herd/premises (herd prevalence), and the probability $p_A$ of presence of the agent in an individual animal (within herd prevalence), which multiplied provide the probability of 'finding' an infected animal in the infected population. Sampling sensitivity further includes the probability of determining that an infected animal is infected (diagnostic sensitivity), which is the product of the probability that the agent is present in the collected specimen ($p_S$) and the ability of the diagnostic test to detect the agent that is present in the specimen ($p_D$, analytical sensitivity). For $n$ units selected for specimen collection, $se_{samp} = 1 - (1 - p_Hxp_Axp_Sxp_D)^n$. It is essential in high impact disease outbreaks that sampling strategies be adjusted, in particular for $p_A$, such that $se_{samp}$ approach 1.
Infectious Disease and Mothers’ Milk: How to Save Babies with Cultural Studies

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Culture impacts medical thinking and scientific research outcomes. Nowhere is this more evident than in addressing mother-to-child transmission of HIV (MTCT) through breastfeeding. Effective scientific approaches to MTCT require understanding the cultural meanings of mothers’ bodies and critically assessing the influence of global political economy on their practices.

Public health has promoted breastfeeding as a partial solution to problems of infant health in resource-poor contexts and as a health enhancement in wealthier countries. HIV presents significant biological challenges to that paradigm. UNAIDS estimates that 300,000 infants die annually from HIV infections contracted through breastfeeding. Yet UNICEF believes that 1.5 million babies die annually because they are not breast fed.

Historically, breastfeeding advocates have portrayed human milk as preventive medicine. More recently, awareness of HIV transmission through breastfeeding and of contaminants in human milk has drawn attention to breastfeeding risks. So which perspective is correct? Will breast milk save the world’s children or kill them? Are breasts life-givers or toxic waste dumps? Is breast milk a medicine or a virus?

Infant formula continues to be the default alternative to breastfeeding in preventing MTCT, even though replacement feeding leads to higher rates of infant morbidity and mortality. Does the impulse to intervene between mother and baby result from good science or is it preferred because of (1) culturally powerful distrust of women’s bodies, (2) capitalistic emphasis on commodity solutions and the related influence of formula manufacturers, (3) modern trust in technology, or (4) all of the above? Answering these questions is a start to saving babies’ lives.
**Probing Mycothiol Biosynthesis as a Target for Antibiotic Development**

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*Mycobacterium tuberculosis* is the bacterium responsible for the infection tuberculosis (TB), and is responsible for ~ 2 million deaths/year worldwide. Treatment of infections caused by *M. tuberculosis* requires multiple drugs and long-term therapy because of the resilience and high degree of antibiotic resistance associated with this organism. Consequently, new therapeutic alternatives are needed. The overall goals of our research is to better understand the mechanisms that contribute to the pathogenicity of mycobacteria, to identify and characterize novel drug targets, and to synthesize inhibitors with the potential to function as novel antibiotics. One potential defense mechanism our lab is interested in is mycothiol (MSH) biosynthesis. MSH is the unique thiol used by mycobacteria to protect against oxidative damage. Consequently, MSH may be one mechanism that enables mycobacterial survival in macrophages, thus implicating the enzymes involved in MSH biosynthesis as potential targets for drug development. We have successfully cloned and expressed three enzymes in this pathway (inositol synthase, Ino1, glycosyltransferase, MshA, metal-dependent deacetylase, MshB). Current research efforts are devoted towards the biochemical characterization of these enzymes.
Toxoplasma gondii, a protozoan parasite which annually infects 1.5 million people in the U.S., can have devastating effects on the developing fetus if infection occurs during pregnancy. Congenital toxoplasmosis results in stillbirths, hydrocephalus, blindness, mental retardation, and neurological and behavioral disorders. In mice, stimulating the maternal immune system during the periconceptual period dramatically reduces teratogen induced birth defects. The protective effects of maternal immune stimulation on infectious causes of birth defects are unknown and were investigated in this study. Mice were immune stimulated pre-breeding with either IFNγ or GM-CSF and were then infected orally with T. gondii tissue cysts on day 3 of gestation. Fetal malformations and mortality was determined on gestational day 17 from the following treatment groups: Control, IFNγ only, GM-CSF only, T. gondii only, IFNγ + T. gondii, and GM-CSF + T. gondii. Toxoplasma infection decreased the numbers of live fetuses from 93 percent in control litters to 39.7 percent in infected litters. In addition to reducing fetal mortality, immune stimulation decreased the percentage of fetuses dying in early pregnancy and increased the percentage dying in late pregnancy, thus increasing fetal survival as well as survival into late pregnancy. Toxoplasma infection resulted in death of the entire litter 47 percent of the time. Immune stimulation with IFNγ or GM-CSF reduced death of the entire litter to 6.7 percent and 25 percent of the time respectively. These promising results may lead to development of a treatment or prevention for congenital toxoplasmosis. Further studies are needed to determine whether improved gestational outcome translates into improved postnatal function. Supported by USDA AD-421 137196.
Development of a Photonic Biosensor Assay to Diagnose Francisella tularensis Infection

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The application of a photonic biosensor to diagnose the category-A select agent Francisella tularensis was investigated. Detection and differentiation of F. tularensis subspecies tularensis (strain TI0902 – Type-A) and subspecies holarctica (strain LVS – Type-B) were demonstrated using a single-mode multi-cavity fiber Fabry-Perot interferometric sensor. Sensors were prepared by depositing seven polymer bilayers onto the fiber tip followed by attaching one of two DNA probes: (a) 101-bp probe from the yhHW gene unique to Type-A strains, or (b) 117-bp probe of the lpnA gene, common to both Type-A and Type-B. The yhHW probe gave a signal only with Type-A strains and not with Type-B strains. Probe lpnA gave a positive signal with both Type-A and type-B. Specific detection of nanogram quantities of target DNA was seen, highlighting the sensitivity of the method when small quantities of target DNA were present. An optical fiber biosensor was also tested using a standard transmission mode long period fiber grating (LPFG) of length 15 mm and period 260 µm and the IgG fraction from antiserum to F. tularensis. Antibody thin films were first fabricated on planar substrates and the refractive index change that occurred upon antigen binding was evaluated using spectroscopic ellipsometry. Index changes on the order of 0.02 were observed, which correspond to typical LPFG wavelength shifts of 20-30 nm. The presence of the bacterium was detected based on the decrease of peak wavelength. These assays will fill a void that exists in rapid diagnosis of F. tularensis, but can also be applied to other agents.
Towards a Species-Selective Acetylcholinesterase Inhibitor to Control the Mosquito Vector of Malaria, *Anopheles gambiae*

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*Anopheles gambiae* is the major mosquito vector of malaria in sub-Saharan Africa. At present, insecticide-treated nets (ITNs) impregnated with pyrethroid insecticides are widely used in malaria-endemic regions to reduce infection, however the emergence of pyrethroid resistant mosquitoes has significantly reduced the effectiveness of the pyrethroid ITNs. An acetylcholinesterase (AChE) inhibitor that is potent for *A. gambiae* but weakly potent for the human enzyme could potentially be safely deployed on a new class of ITNs. In this paper we compare the structures of human, *A. gambiae*, *Drosophila melanogaster*, and *Torpedo californica* AChE. We also provide a preliminary pharmacological characterization of *A. gambiae* AChE, discuss structural features of *A. gambiae* and human AChE that could lead to selective inhibition of *Anopheles gambiae* AChE relative to human AChE.
Female mosquitoes of most species obtain amino acids and other nutrients needed for egg production from the blood of vertebrate hosts. Reproductive success of mosquitoes relies heavily on a repertoire of sexual, host-seeking, and pre-oviposition behaviors. These behaviors do not occur in female mosquitoes immediately after adult emergence. Circumstantial evidence has implicated insect juvenile hormone (JH) in the development of host-seeking behavior and female mating competence in some mosquito species. The roles of JH have not been definitely demonstrated, and little is known about how JH stimulates this maturation process at the molecular level. We have discovered that a group of mosquito genes, putatively encoding pheromone/odorant binding proteins and other proteins involved in sensory perception, become more active during the development of adult behaviors. We hypothesize that JH directs the behavioral development in the adult mosquito, at least in part, by activating the manufacture of components in the sensory systems that recognize relevant external stimuli. To test this hypothesis, we are constructing a transgenic mosquito strain that ectopically produces high level juvenile hormone esterase in the young adult female mosquitoes, presumably leading to rapid depletion of endogenous JH during the post-emergence behavior development. By comparison between the transgenic and control mosquitoes, we will (1) validate the roles of JH in the initiations of mating and host-seeking behaviors, (2) determine whether during adult development JH is essentially required for the enhanced expression of mosquito genes implicated in sensory perception.
Targeting mRNAs Involved in Viral and Neurodegenerative Diseases

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A central dogma of modern medicine is to use small molecules to specifically target the proteins or DNA associated with a particular illness. Until recent years, mRNA has not been a focus point for remediation. Currently, the therapy for neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, and Huntington's disease) only alleviates the symptoms but does nothing to treat cause of the disease. Thus, the progression of the disease is merely prolonged. The pathological hallmark of these diseases is the presence of oxidized protein aggregates in the brain termed Lewy bodies, and these plaques are composed primarily of a protein called α-synuclein. If the mRNA of α-synuclein can be specifically targeted and bound by a molecular entity, then the translation of that mRNA into protein could also be blocked. This would decrease the amount of α-synuclein available to form Lewy bodies, and the progression of the disease could be halted. We have used solid-phase combinatorial chemistry to generate large libraries of branched peptides (~250,000 members). These libraries are being screened in the solid phase for binding to the mRNA of α-synuclein as well as the mRNA encoding for HIV TAR, which is responsible for the viral replication of HIV. Molecules that are found to bind the target RNAs selectively will be resynthesized and subjected to various biochemical, biophysical and cellular assays.
Practice and Perceptions of Personal Protective Behaviors against Mosquitoes in Areas of Southwest Virginia

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Over the past decade, mosquito-borne diseases have become of more concern in Virginia. The state’s temperate climate is suitable for a wide array of mosquito species, several of which are capable of transmitting pathogens to humans. There are now four mosquito-borne illnesses in the state, eastern equine encephalitis, La Crosse encephalitis, West Nile fever and St. Louis encephalitis. Additionally, in 2002, two cases of malaria were reported. It is believed the malaria cases were contracted within the state. Within the region of southwest Virginia, La Crosse encephalitis and West Nile fever are most prominent. While only a handful of cases are reported each year, it is likely that these illnesses are here to stay. Several cases of La Crosse encephalitis have been reported in Tazewell and Wise counties in recent years, and Roanoke County has seen notable West Nile activity. These counties were chosen as study sites due to their exposure to these diseases. Surveys were conducted in several communities within these counties to assess knowledge levels of mosquito-borne diseases in the area, as well as practice and perceived effectiveness of personal protective behaviors (PPBs). Personal protective behaviors are actions individuals can take to protect themselves from being bitten by mosquitoes such as wearing repellent, removing standing water, etc. Responses are now being correlated to examine variance within and between communities to see how the use of PPBs may differ. This knowledge can then be used by local health departments to address specific issues and strengthen existing prevention programs.
Immunogenicity of Capsule-Like Material from *Francisella tularensis*

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*Francisella tularensis* is a category A select agent and the etiologic agent of tularemia in humans and animals. However, the virulence factors and surface antigens of *F. tularensis* have not been well characterized. Two carbohydrate antigens have been identified on this bacterium: the well-characterized lipopolysaccharide (LPS), and a recently isolated capsule-like material (CLM). The CLM can be observed as an electron dense layer surrounding individual cells, varies in size, and is not always present, suggesting it is upregulated under some environmental conditions. The CLM, which consists primarily of glucose, galactose, and mannose, was purified from an O-antigen deficient mutant that was subcultured repeatedly in minimal media to enhance CLM production. The purified CLM induced a moderate, but distinct immune response in BALB/c, C57/Black, and CD-1 mice, and a stronger response in rabbits in the presence of adjuvant. To further enhance immunogenicity, the CLM was conjugated to various immuno-stimulating proteins such as keyhole-limpet hemocyanin (KLH) and flagellin from *S. typhi*, both of which induced a protective immune response from BALB/c mice to *F. tularensis* live vaccine strain (LVS). Since the CLM has no reactive groups, CNBr was used to activate hydroxyl groups on the neutral polysaccharide. Further studies of these protein-CLM conjugates, including a conjugate using *F. tularensis* outer membrane protein, may have potential for use as a vaccine candidate.
Immunological and Molecular Diagnosis of Two Common Genera of Plant Viruses Using Membrane-Based Technologies

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Single-stranded RNA viruses of the genera Cucumovirus (Family Bromoviridae) and Potyvirus (Family Potyviridae) are two of the most important taxa of plant viruses worldwide. Diagnosis and characterization of these and other plant viruses have in the past involved traditional processes of collection and storage of samples, greenhouse culture and examination of biological properties, virus isolation and extraction, and purification of virus particles to enable the preparation of specific antibodies. The advent of enzyme-linked immunosorbent assay (ELISA) and reverse-transcription polymerase chain reaction (RT-PCR) have accelerated plant virus diagnosis, but are laborious, expensive, and not adapted to high throughput analyses of field samples. Modifications to the tissue blot immunosorbent assay (TBIA), a membrane-based ELISA, detect cucumovirus and potyvirus particles directly from infected leaves squashed or blotted to a nitrocellulose membrane (NCM). A modified Western blot procedure using virus- or genus-specific antibodies was used to detect and specifically identify several viruses. We have also shown that RT-PCR using specific primers produced amplicons of expected sizes from leaves pressed onto FTA® Plantsavers Cards (GE Whatman Inc., Florham Park NJ). Modifications of the Whatman FTA® protocol enabled us to apply RT-PCR directly from a NCM prepared for and/or used for TBIA, thus eliminating the need for RNA extraction or elution. All PCR amplifications from NCM blotted with cucumoviruses and potyviruses gave the expected sizes and sequences from cleaned RT-PCR products. Use of NCM allows multiple sampling in the field and extended storage, and eliminates the need for specific template preparation.
Development of Newcastle Disease Virus like Particles for Tumor Selective Targeting and Oncolytic Therapy

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According to the American Cancer Society (ACS), cancer accounts for approximately a quarter of all deaths in the United States. Many oncolytic virus therapies perhaps have greatest advantage such as NDV for rapid, rational design through recombinant DNA technology to facilitate the targeting of a broad spectrum of malignancies. Ligand directed, receptor mediated targeting is the common approach for targeting oncolytic viruses to specific cell types. NDV is a single-stranded RNA, negative polarity paramyxovirus and encodes for seven proteins from six genes. Three proteins constitute the nucleocapsid, nucleoprotein (NP), phosphoprotein (P) and large polymerase protein (L), and two membrane proteins, hemagglutinin-neuraminidase (HN) and fusion protein (F) which enables cell binding and fusion. NDV is naturally tumor-selective and inherently oncolytic and is shown to be safe even after repeated intravenous administrations in humans. Paramyxoviruses spread through infected cell surfaces and can produce virus particles as well as fuse with adjacent cells. Avian cells expressing viral proteins release VLPs and have protein ratios similar to those of the infectious virus with homogeneous densities differing only slightly from the authentic virus. Hence, here we will try to obtain VLPs by using NDV cDNA sequences encoding M, HN and F proteins subcloned into expression vector pCAGGS to generate pCAGGS-M, pCAGGS-HN and pCAGGS-F. DF-1 cells will be transfected with each of the three plasmids using Lipofectamine. The genome organization for NDV emphasize on the desired structural proteins to construct VLPs. Hence we attempt to use these Recombinant NDV VLPs with desired densities to mimic the NDV virus for specifically targeting tumor cells. In the future, we will use this recombinant NDV VLPs conjugated to the folate-PEG. This folate conjugated VLPs will be evaluated in-vitro for their tumor selective targeting in human cancer cell culture models and animal models by using a WST-1 assay.
Cross-species Infection of Specific-pathogen-free Pigs by a Genotype 4 Strain of Human Hepatitis E Virus

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Hepatitis E virus (HEV) is an important pathogen. The animal strain of HEV, swine HEV, is related to human HEV. The genotype 3 swine HEV infected humans and genotype 3 human HEV infected pigs. The genotype 4 swine and human HEV strains are genetically related, but it is unknown whether genotype 4 human HEV can infect pigs. A swine bioassay was utilized in this study to determine whether genotype 4 human HEV can infect pigs. Fifteen, four-week-old, specific-pathogen-free pigs were divided into three groups of five each. Group 1 pigs were each inoculated intravenously with PBS buffer as negative controls, group 2 pigs similarly with genotype 3 human HEV (strain US-2), and group 3 pigs similarly with genotype 4 human HEV (strain TW6196E). Serum and fecal samples were collected at 0, 7, 14, 21, 28, 35, 42, 49, and 56 days postinoculation (dpi) and tested for evidence of HEV infection. All pigs were necropsied at 56 dpi. As expected, the negative control pigs remained negative. The positive control pigs inoculated with genotype 3 human HEV all became infected as evidenced by detection of HEV antibodies, viremia and fecal virus shedding. All five pigs in group 3 inoculated with genotype 4 human HEV also became infected: fecal virus shedding and viremia were detected variably from seven to 56 dpi, and seroconversion occurred by 28 dpi. The data indicated that genotype 4 human HEV has an expanded host range, and the results have important implications for understanding HEV natural history and zoonosis.
**TABLE 1.** Detection of HEV RNA by RT-PCR in samples (fecal/serum) collected weekly from pigs inoculated with PBS buffer, with a genotype 3 human HEV, and a genotype 4 human HEV

<table>
<thead>
<tr>
<th>Group</th>
<th>Pig ID</th>
<th>positive (+) or negative (-) in fecal/serum samples at indicated vpi</th>
</tr>
</thead>
<tbody>
<tr>
<td>1^a</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>-</td>
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<tr>
<td></td>
<td>17</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>2^b</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>-</td>
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<tr>
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<td>33</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>-</td>
</tr>
</tbody>
</table>

- a: inoculated with sterile phosphate buffered saline, PBS (negative controls)
- b: inoculated with a genotype 3 human HEV strain US-2 (positive controls)
- c: inoculated with a genotype 4 human HEV strain TW6196E (experimental group)

**TABLE 2.** Quantification by real-time RT-PCR of HEV RNA in fecal samples collected every three days from pigs inoculated with a genotype 3 (group 2) and a genotype 4 (group 3) human HEV between 3 and 27 days postinoculation

<table>
<thead>
<tr>
<th>Days postinoculation</th>
<th>Group 2 - G3 US-2 Strain HEV RNA in fecal samples (log copies/mg)</th>
<th>Group 3 - G4 TW6196E Strain HEV RNA in fecal samples (log copies/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pig 25</td>
<td>pig 26</td>
</tr>
<tr>
<td>3</td>
<td>3.48</td>
<td>5.21</td>
</tr>
<tr>
<td>6</td>
<td>7.56</td>
<td>7.55</td>
</tr>
<tr>
<td>9</td>
<td>8.86</td>
<td>8.56</td>
</tr>
<tr>
<td>12</td>
<td>5.00</td>
<td>6.58</td>
</tr>
<tr>
<td>15</td>
<td>8.90</td>
<td>4.49</td>
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<tr>
<td>21</td>
<td>8.90</td>
<td>5.45</td>
</tr>
<tr>
<td>24</td>
<td>9.10</td>
<td>5.20</td>
</tr>
<tr>
<td>27</td>
<td>8.90</td>
<td>4.81</td>
</tr>
</tbody>
</table>

*: not detected
The availability of polytene chromosomes in *Anopheline* mosquitoes provides the unique opportunity for studying how differing levels of chromatin compaction affect functionality of chromosomal regions. However, current mosquito cytogenetic maps are either derived from low-resolution photo images or hand drawn chromosomes that in some cases vaguely represent polytene chromosomes seen under the microscope. The advent of digital imaging has given us the ability to create high resolution images that provide a better representation of the actual chromosome structure. Using a new technique for chromosome preparations, we have created an updated cytogenetic map of the major *Plasmodium falciparum* transmitting vector, *Anopheles gambiae*. This map incorporates an experimental high pressure treatment that increases the clarity of structures along the chromosome. We follow the normal procedure for creating chromosome squashes, spreading ovaries evenly across the slide and submersing them in a 50 percent propionic acid solution to remove excess proteins and cause surrounding tissues to become transparent. The process is modified by using a mechanical vise to apply additional pressure when flattening the chromosomes. The added pressure evenly squashes the slide, causing the entire slide to be on the same plane of focus. Using coordinates of *A. gambiae* Bacterial Artificial Chromosomes (BAC) clones that were previously hybridized to the polytene chromosomes, we have also integrated a crude 0.5 Mb coordinate map for each chromosomal arm. BAC clone locations were taken from Vector Base and only include clones that hybridized to specific loci. Some regions along the chromosomal arms either contain BAC clones that did not hybridize specifically enough to provide an accurate estimate of the clone’s location, or did not contain BAC clones at all and require additional mapping. This new map is being used to precisely map bands, interbands, and heterochromatin - euchromatin junctions to the genome assembly.
Characterization of the Structure and Expression of *Aedes aegypti* Hsp70 Genes

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While inducible promoters capable of controlling transgene expression in specific tissues have been characterized for *Aedes aegypti*, no whole body, whole life stage inducible promoter has been described for this mosquito. In order to identify such a promoter in *A. aegypti*, basic local alignment searches were performed using *Drosophila melanogaster* Hsp70 protein sequences compared to *A. aegypti* genome sequence. A family of six previously unannotated AaHsp70 genes was identified. The newly identified AaHsp70 genes were found to be organized into three pairs of nearly identical open reading frames, arranged within the pairs as inverts of each other, which mapped to a single supercontig. These AaHsp70 genes appear to be the result of an independent expansion after divergence from *D. melanogaster*. Northern analysis using a probe common to all six genes revealed expression at all life stages in a dose response manner with temperatures ranging from 35°C to 39°C. No expression was seen at 28°C, indicating heat induced expression. Northern analysis using probes generated from unique 3’ untranslated regions of each gene confirmed that at least five of the six genes are expressed. We performed 5’ and 3’ RACE to confirm the exact start and stop of transcription for each gene. Preliminary experiments show that upstream genomic fragments from several AaHsp70 genes drive robust expression of a marker gene in cultured cells. Future experiments will include further promoter testing in both cultured cells and transgenic mosquitoes.
Highly Species-Selective Acetylcholinesterase Inhibitors for Control of *Anopheles Gambiae*, the Mosquito Vector of Malaria

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Acetylcholinesterase (AChE) inhibition can present therapeutic or toxic effects, depending on the degree of inhibition. Thousands of structurally diverse aryl carbamate-based AChE inhibitors have been evaluated for use as treatments for Alzheimer’s disease (AD) memory loss (e.g. Rivastigmine) and insecticides (e.g. Propoxur). To combat emerging pyrethroid-resistant strains of *Anopheles gambiae* in sub-Saharan Africa, it would be useful to develop insecticide treated nets impregnated with carbamates. However, currently available insecticidal carbamates offer little selectivity for inhibition of *A. gambiae* AChE (AgAChE) over human AChE (hAChE). Since AgAChE has only 49 percent sequence identity to hAChE, we thus undertook selectivity-based optimization of the aryl carbamate pharmacophore, determining inhibition potencies at both AgAChE and hAChE. Contact toxicity to live *A. gambiae* was confirmed using the WHO protocol. These studies have culminated in the discovery of aryl carbamates possessing >1,000-fold selectivity for inhibition of AgAChE over hAChE.

![Chemical structures](image-url)  
Rivastigmine, human AChE inhibitor for AD memory loss  
Propoxur, insecticidal carbamate poorly selective for AgAChE  
AgAChE-selective carbamates, IC50 ratios 100-9,000
The Role of the Germination-Specific Lytic Enzymes CwlJ and SleB during Bacillus anthracis Spore Germination

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The endospore of *Bacillus anthracis* is the infectious agent responsible for causing anthrax. The structural characteristics of a spore enable it to withstand stresses that typically kill a vegetative cell. The spores remain dormant until nutritive signals induce them to germinate into vegetative bacilli. A late stage of germination requires degradation of the thick cortical peptidoglycan (PG) by germination-specific lytic enzymes (GSLEs). *B. anthracis* has at least three putative GSLEs based upon sequence similarity with known enzymes in other species: SleB, CwlJ1, and CwlJ2. The expression of cwlJ1 and sleB occurs 3.5 hours into sporulation, with transcription of cwlJ1 reaching levels 51-fold greater than sleB. Genetic analysis revealed that, typical to other known GSLEs, neither CwlJ1 nor SleB is required for vegetative growth, sporulation, or early germination. Late germination events however are affected when lacking either enzyme. Compared to wild-type, germinating spores without CwlJ1 suffer a delay in optical density and cortex PG release. The absence of SleB also yields a delay in cortex degradation. The gene encoding CwlJ2 is probably nonfunctional since a null mutation has no obvious consequence on any stage of the *B. anthracis* life cycle. HPLC and mass spectroscopy analysis revealed that SleB is responsible for lytic transglycosylase activity. CwlJ1 also clearly participates in cortex hydrolysis, but its specific mode of action remains unclear. Understanding the lytic germination activities that naturally diminish spore resistance can lead to methods that either inactivate the enzymes or prematurely induce them, thus greatly simplifying the process of cleaning anthrax contaminated sites.
Cytokine Bearing Influenza Vaccine: Adjuvant Potential of Membrane-bound Immunomodulators

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Influenza epidemics continue to cause morbidity and mortality within the human population despite widespread vaccination efforts. This, along with the ominous threat of an avian influenza pandemic (H5N1), demonstrates the need for a much improved, more sophisticated influenza vaccine. Our group has developed an *in vitro* model system for producing a membrane-bound Cytokine-bearing Influenza Vaccine (CYT-IVAC). Numerous cytokines are involved in directing both innate and adaptive immunity and it is our goal to utilize the properties of individual cytokines and other immunomodulatory proteins to create a more immunogenic vaccine. We have previously described construction of membrane-bound cytokine fusion constructs in which our cytokine of interest (mouse GM-CSF, mouse IL-2, mouse IL-4, and now CD40 ligand) is fused to the membrane anchoring regions of either the viral Hemagglutinin (HA) or Neuraminidase (NA). We have demonstrated that progeny virions, produced from influenza infected MDCK cells expressing membrane-bound cytokines, readily incorporate membrane-bound cytokines during budding and these cytokines on virus particles retain bioactivity following viral inactivation. *In vivo* vaccination studies in mice show enhanced antibody titers and improved survival following lethal challenge in those mice vaccinated with CYT-IVAC’s compared to the conventional wild-type vaccine without membrane-bound cytokines. In addition, the immune response induced by CYT-IVAC vaccination was skewed toward a balanced Th1/Th2 response compared to the Th2 dominated response induced with wild-type vaccination. Our focus has shifted as we have begun to evaluate the mechanism by which CYT-IVAC’s enhance the immune response. We are currently evaluating the effect of CYT-IVAC treatment on APCs and lymphocytes, both *in vitro* and *in vivo*, by assessing gene expression profiles and cell surface markers using RT-PCR and Flow cytometry, respectively. In addition we are comparing long-term memory induced by CYT-IVAC and wild-type vaccination. In conclusion, we have developed a novel methodology designed to introduce bioactive membrane-bound cytokines directly into virus particles in order to augment vaccine immunogenicity of inactivated whole virus influenza vaccines.
Inhibition of Reactive Oxygen Species (ROS) and Other Inflammatory Mediators by Cerium Oxide Nanoparticles

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Chronic inflammatory disease is a destructive and progressive pathogenic disorder characterized by simultaneous healing and injury of tissues and is a major mediator of autoimmune diseases. Alleviation of chronic inflammatory symptoms is achieved by reducing cellular levels of oxidative stress. Cerium oxide (CeO2) is a rare earth oxide that purportedly exhibits antioxidant and anti-inflammatory properties on the nano-scale. Nanoceria has auto-regenerative free radical scavenger properties from its valence and oxygen defects. The following investigation demonstrates the ability of nanoceria to scavenge the formation of reactive oxygen species (ROS) and inhibit inflammatory mediators in J774 A.1 murine macrophages. We showed that cells internalized nontoxic nanoceria and experience lower oxidative stress levels and inflammatory iNOS and COX-2 protein expression. We also examined nanoceria deposition in mouse tissues with no pathogenicity. Our studies suggest that cerium oxide nanoparticles may be used as a novel therapy for chronic inflammation.
Multi-drug Nanoplexes to Target Brucella melitensis Infection in BALB/c mice

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Brucellosis caused by a bioterrorism agent Brucella species remains one of the most common zoonotic diseases worldwide with more than 500,000 cases annually and billions of dollars of economic burden associated with treatment, hospitalization costs and loss of productivity. Although antibiotics are very effective in killing Brucella outside the body they are not efficient in crossing the cell membrane barriers inside the body and so are not very effective. In the present work we are focusing on the delivery of multiple drugs against these hidden pathogens by conjugating the antibiotics with the polymeric nano sized particles. These conjugated particles are considered as foreign by the animal and rapidly taken up by phagocytic cells, which are also sheltering the bacteria. The biocompatibility of these particles has been evaluated and their efficiency to deliver drugs has been tested in Brucella melitensis infected BALB/c mice. Administration of two doses of Streptomycin-Doxycyclin complex reduced Colony forming units (CFU) of B. melitensis in the spleens and livers of infected mice by 0.82 and 0.79 log compared to 0.51 and 0.42 log, respectively, with free drug. The results showing the comparative efficacy of these new multidrug-nanoplexes over the traditional drug therapy will be discussed.
Membrane Bound Immunomodulators to Enhance Influenza Virus Vaccines

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Influenza outbreaks are a continuous and significant problem afflicting ca. 5 percent - 20 percent of the general population, healthcare workers and researchers worldwide on a yearly basis. Annual vaccination is currently the most effective way to protect against influenza, but vaccine efficacy remains low in many high-risk populations. Increased efficacy in these groups will require adjuvant therapy. We have developed a cell culture based membrane-bound Cytokine adjuvanted Influenza Vaccine (CYT-IVAC) that presents a bioactive, membrane-bound cytokine on the surface of virus particles. Here, we report on our progress making CYT-IVACs bearing bioactive IL-12, IL-15 and Flt3L. This approach involved engineering cytokine fusion constructs encoding the mature forms of mouse Flt3L, mouse Interleukin 12 (mIL-12), mouse Interleukin 15 (mIL 15) and human Interleukin 15 (hIL-15) fused to the transmembrane and cytoplasmic tail domain of the influenza virus hemagglutinin glycoprotein. Resultant expression plasmids were stably transfected into MDCK cells, a virus permissive cell line and fusion construct protein expression at the cell surface was monitored by immunofluorescence microscopy. Stable transfectants were subsequently used to propagate influenza virus A/PR8/34 and released virus with incorporated membrane-bound adjuvant was harvested, purified and inactivated with β-propiolactone. Incorporation was demonstrated by western blot analysis of purified whole viral lysates. In *vitro* cytokine bioassays were employed to confirm that cytokines retained their bioactivity in our inactivated vaccines. Studies are underway to evaluate the ability of CYT-IVACs to confer protection against challenge and induce humoral and cellular immunity as well as to determine how CYT-IVACs influence dendritic cell maturation.
N-terminal Boronic Acid Peptide Inhibitors of Lon Protease

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Lon is a homo-oligomeric ATP-dependent serine protease that functions in the degradation of damaged and certain regulatory proteins. This enzyme has emerged as a novel target in the development of antibiotics because of its importance in conferring bacterial virulence. A C-terminal boronic acid peptide, MG262, [Z-(Lys)3-B(OH)2], is an effective inhibitor with a k of 6.6 nM. We have been developing a complementary approach of designing serine protease inhibitors using N-terminal peptidic boronic acids. Our first synthesized model peptide incorporating a racemic alanine monomer mimics the P1’ site of Lon with low micromolar inhibition (< 10 µM) and a differing selectivity than that of MG262, preferring the human over the bacterial La. With the effort of developing the structure-activity relationships of Lon, other peptide substrates have been synthesized which incorporate both natural and unnatural amino acid monomers at the Lon protease P1’ site. These putative inhibitors will be investigated to determine both the efficacy and selectivity against Lon.
Differential Potency of Bivalent Anticholinesterases as a Model for the Molecular Design of Selective Insecticides

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Acetylcholinesterase (AChE) is a target of conventional organophosphate and carbamate insecticides, which react with a conserved S200 residue in the active site, thereby causing toxicity to organisms. Due to the conserved nature of S200, conventional anticholinesterases are associated with cross-species toxicity. There is an urgent need to develop new selective chemicals with high insect toxicity and low mammalian toxicity with reduced propensity to elicit resistance. AChE has two ligand-binding sites, the peripheral aryl site (PAS) at the mouth, and the catalytic active site (CAS) at the bottom of a deep narrow (20 angstrom) active site gorge. Tacrine dimers are bivalent inhibitors whose dual binding property on AChEs vary with tether length, and therefore serve as useful probes of AChE gorge geometry. We used tacrine dimers with linkers C2-C12 to systematically probe the Drosophila melanogaster, Anopheles gambiae, Blattella germanica and human AChE gorge. We screened for both differential potency and dual binding ability of the bivalents in the AChEs. Tacrine dimers were found to be more potent to vertebrate and Drosophila AChEs, compared to that of other insects. The tether length dependence index (monomer IC50/the most active dimmer IC50) was lowest in Anopheles and Blattella compared to other organisms. AChE sequences were analyzed using Clustal-W, to map candidate amino acid residues that are unique to insect AChE and hence possible targets for selectophore design. These structural data should prove useful for the molecular design of selective insecticides.
Studies of Dipicolinic Acid in Clostridium perfringens

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Dipicolinic acid (DPA) is a major component of all bacterial spores, commonly composing 5 percent - 10 percent of the spore dry weight. Spores with decreased levels of DPA have been shown to be highly sensitive to wet heat. In spores produced by species of Bacillus, DPA is produced in the mother cell by the products of the spoVF operon. The bacterium Clostridium perfringens is the leading cause of acute food poisoning (AFP) in the U.S. each year. AFP is caused when C. perfringens spores contaminate food and possess enough heat resistance to survive the cooking process. The surviving spores germinate and multiply when the food cools and cause the condition when consumed. C. perfringens does not possess a clear homologue for the spoVF operon, yet produces DPA. Elucidation of the mechanisms by which DPA is produced by this organism may lead to methods of decreasing the occurrence of AFP.
Stereoselective Alkylation of Chiral \(\beta\)-Borylated Esters

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The use of boron in medicinal agents has increased significantly over the last decade resulting in Velcade\textsuperscript®, the first FDA approved chiral boron containing drug treatment for multiple myeloma. Currently, the use of chiral boronic esters to enhance stereochemistry of molecules through intramolecular asymmetric induction is absent in the literature. We report the development of a novel reaction for the \(\alpha\)-alkylation of chiral \(\beta\)-borylated esters via intramolecular asymmetric induction. Standard deprotonation with LDA of chiral \(\beta\)-borylated esters leads to the formation of an intramolecular five-membered ring “boron-ate” species affording chiral alpha, beta-substituted boronic esters. This reaction is powerful in that a wide range of chiral \(\beta\)-borylated ester substrates can be employed, possessing varying degrees of substitution and steric bulk, including cinnamates and crotonates. Initial results show up to 60 percent yield with moderate diastereomeric ratio (dr) of products (3:1). Optimization of reaction conditions will provide higher yields and enriched DE ratios. This novel reaction allows for facile production of chiral boryl products that can be integrated into biological agents, such as transition state analogues, anticancer agents, and also used as intermediates in organic synthesis.
Delivery of Aminoglycoside Using Novel Nanoplexes against a Salmonella Mouse Infection Model

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A novel methodology for incorporating gentamicin into macromolecular complexes with anionic homo- and block copolymers via cooperative electrostatic interactions was developed and investigated in this study. Block copolymers of poly(ethylene oxide-b-sodium acrylate) (PEO-b-PAA- +Na) or poly(ethylene oxide-b-sodium methacrylate) (PEO-b-PMA- +Na) was blended with PAA- Na+ and complexed with the polycationic antibiotic gentamicin. The gentamicin nanoplex made with the PEO-b-PMA- +Na/PAA- +Na (PMPG) and the analogous nanoplex with the PEO-b-PAA- +Na/ PAA- +Na (PAPG) showed mean intensity average diameters of 120 and 90 nm and zeta potentials of -17 and -11 mv, respectively. PAPG and PMPG can incorporate up to 20 percent - 25 percent by weight of gentamicin which is 20 - 25-fold higher than previously reported studies. Drug release of gentamicin from the PAPG and PMPG nanoplexes at the physiological pH was relatively slow. The efficacy of nanoplexes as drug delivery systems for treating intracellular Salmonella in a mouse model at doses similar to the free gentamicin experiments resulted in reduced numbers of viable bacteria in the liver and spleen at a specific time point of treatment in this study. Polymeric nanoplexes developed by this novel technology have the potential to be developed into specific targeting agents for intracellular pathogens, and enhance our ability to minimize development of drug resistant pathogens.
Efficacy of an Attenuated *Francisella tularensis* Type A O-antigen Mutant Combined with a Lipopolysaccharide-Protein Conjugate for Protection of Mice against *F. tularensis* Type A Challenge

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*Francisella tularensis* is a gram-negative coccobacillus, the etiological agent of tularemia, and a category A biowarfare agent. The live vaccine strain (LVS) of *F. tularensis* is attenuated in humans, but remains virulent for mice, depending on challenge route and strain of mouse. Lipopolysaccharide (LPS) O-antigen mutants of LVS are highly attenuated in mice and have been shown to provide protection against challenge with LVS or type B strains. We generated an O-antigen mutant (MU2) of a type A clinical isolate (TI0902) with a mutation in the O-antigen locus gene *wbtK*. Complementation of the *wbtK* gene in trans restored O-antigen synthesis. MU2 was completely attenuated in mice at 10^7 colony forming units (CFU). In initial experiments, intranasal immunization (IN) of BALB/c mice twice with 10^6 CFU of the mutant did not protect the mice against intranasal challenge with ~10X the LD50 of type A strain SCHU S4. However, when the immunization route was intradermal (ID), some protection against SCHU S4 was provided following ID challenge. When mice were immunized twice with MU2 combined with native LPS conjugated to keyhole limpet hemocyanin there was significant (P > 0.001) protection following challenge with SCHU S4 compared to sham-immunized controls. Immunization and challenge routes were also important variables that influenced the level of protection. Mice immunized IN [conjugate vaccination] and then challenged IN showed greater resistance against disease than mice immunized ID and than challenged IN. Our results indicate that the combination of an attenuated mutant with the conjugated antigen that the mutant is lacking may provide improved protection against Type A *F. tularensis* infection.
The Enemy of My Enemy is My Friend: an Unconventional Approach to Control of Foodborne Salmonella

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Salmonella is the second most common cause of foodborne illness in the United States. It causes an estimated three million illness and 500 deaths annually, costs $3 billion, and is becoming increasingly antibiotic resistant. Bacteriophage Felix O1 is specific for and lethal to most members of the genus Salmonella. A cocktail of whole phage applied to the surface of meat and meat products has proven effective as a means of microbial control for Listeria, another common foodborne pathogen. Other studies have demonstrated that purified lysin is lethal to Gram-positive bacteria when applied externally. The aim of this study is to determine the efficacy of the Felix O1 purified lysin and holin proteins as a means of control of Salmonella in raw and ready-to-eat poultry products. The lysin gene, lys, was identified in a previous study. A potential holin gene, rIIA, was identified by partial sequence homology to other Myoviridae holins using the BLAST algorithm. Lys and rIIA were amplified by PCR, cloned into the expression vector pRSETA, and transformed into Mach 1 chemically competent E. coli cells. We are in the process of characterizing the clones for their expression of lysin and holin proteins. They will be purified and tested in a controlled study of infected poultry meat in order to determine their efficacy in reducing the Salmonella load. Results of current Felix O1 lysin and holin cloning and protein expression efforts will be discussed.
Detection Assays for *Escherichia coli* 0157:H7, *Salmonella enterica*, and *Campylobacter jejuni* in Bovine Fecal Samples

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Three major pathogens associated with gastroenteritis are *Escherichia coli* O157:H7, *Salmonella enterica*, and *Campylobacter jejuni*. These organisms can lead to hospitalization or in young, elderly or immunocompromised individuals complications from these infections can result in death. Molecular detection assays such as PCR can provide rapid qualitative results to determine the absence or presence of a pathogen in an environmental matrix. A multiplex PCR reaction was designed for these pathogens using specific primers for *invA*, *rfbE*, *cdtB* and 16SrRNA will be useful in screening for multiple organisms in a single sample. The primers were determined to be specific for *S. enterica*, *E. coli* O157:H7, and *C. jejuni* respectively. Amplification of the 16s rDNA serves as an internal control, indicating the PCR reaction is not inhibited. The primers were tested using pure cultures and cattle feces. Cattle feces obtained from grab samples were inoculated with 106 CFU cocktail consisting of two strains of *E. coli* 0157:H7, *S. enterica* sv. Typhimurium LT2, *S. enterica* sv. Kentucky and *C. jejuni*. Amounts of DNA and suitability of the DNA for downstream PCR were determined for using three different DNA extraction kits, UltraClean Fecal DNA kit (MoBio®), ZR Fecal DNA kit (Zymo®) and PrepMan® Ultra Sample Preparation Reagent, DNA was extracted, quantified and used for PCR. PCR products were viewed by electrophoresis on a 1.5 percent agarose gel. This multiplex reaction will allow for a rapid, cost-effective determination of the presence of three food-borne pathogens in pre-harvest animals. Rapid identification of pathogens pre-harvest will enable interventions that may inhibit transfer to carcasses, and ultimately reduce the cases of food-borne illness. While the PrepMan® reagent yielded the most DNA it was unable to be used for PCR due to the high concentration of inhibitors.
Bump-hole Reoptimization of the Tacrine Pharmacophore Achieves Selective Inhibition of Anopheles gambiae Acetylcholinesterase

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Acetylcholinesterase (AChE) inhibition can present therapeutic or toxic effects, depending on the degree of inhibition. 9-Amino-1,2,3,4-tetrahydroacridine (tacrine) was the first drug approved for the treatment of Alzheimer’s-related memory loss, other AChE inhibitors have been used as pesticides and chemical warfare agents. With the goal of developing new insecticides to control the malaria-transmitting mosquito, Anopheles gambiae, we undertook a reoptimization of the tacrine pharmacophore to attain high selectivity for inhibition of mosquito AChE. By exploring structural modifications of tacrine known to reduce affinity for human AChE as well as through dimerization of the most active compound, we discovered an inhibitor that exhibits a greater than 1000-fold selectivity for the mosquito enzyme over the human enzyme.
Deans’ Forum on
INFECTIOUS DISEASES

Molecular Pathogenesis
Characterization of Pathogenicity and Development of Live, Attenuated Vaccines against *Burkholderia mallei*

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*Burkholderia mallei* is the etiologic agent of glanders in solipeds (horses, mules and donkeys), and incidentally in carnivores and humans. The importance of the putative carboxy-terminal processing protease (CtpA) and the putative Wzt hydrophilic protein of the lipopolysaccharide ABC-2 transporter complex to pathogenicity of *B. mallei* was investigated. A sucrose-resistant strain of *B. mallei* was constructed by deleting a major portion of the sacB gene of the wild type strain ATCC 23344 by gene replacement, and designated as strain 23344ΔsacB. A portion of the *ctpA* gene (encoding CtpA) of strain 23344ΔsacB was deleted to generate strain 23344ΔsacBΔctpA. In contrast to the wild type, the *ctpA* mutant 23344ΔsacBΔctpA displayed altered cell morphologies with partially or fully disintegrated cell envelopes, and less ability to survive in J774.2 murine macrophages. A portion of the *wzt* gene (encoding Wzt) of strain 23344ΔsacB was deleted to generate strain 23344ΔsacBΔwzt. The LD$_{50}$ of the wild type, the sacB mutant, and the wzt mutant strains in CD1 mice when inoculated intraperitoneally were 5.9 x 10$^5$, 6.6 x 10$^5$, and 9.1 x 10$^5$ cfu, respectively. When the CD1 mice injected with saline or a non-lethal dose of the wzt mutant were subsequently challenged intraperitoneally with 11.2 times the LD$_{50}$ of strain ATCC 23344, 20 percent and 87.5 percent respectively survived longer than 15 d post-challenge. Our findings suggest that CtpA is important for maintaining cell morphology and intracellular survival, whereas Wzt is important for *in vivo* pathogenicity of *B. mallei*. The wzt mutant is attenuated and protects CD1 mice against glanders.
Biochemical and Structural Identification of a Novel Kynurenic Acid Producing Enzyme, Kynurenine Aminotransferase III

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Kynurenine aminotransferase III (KAT III) has been considered to be involved in the production of mammalian brain kynurenic acid (KYNA), a compound playing a role in protecting neurons from over-stimulation by excitatory neurotransmitters. The enzyme was named based on its high sequence identity with mammalian KAT I, but its activity to kynurenine and its structural characteristics have not been established. This study concerns the biochemical and structural characterization of mouse KAT III (mKAT III). In this study mKAT III cDNA was amplified from a mouse brain cDNA library and its recombinant protein expressed in insect cell protein expression system. Through biochemical characterization we found that mKAT III was able to efficiently catalyze the transamination of kynurenine to KYNA. mKAT III has optimum activity at relative basic conditions around pH 9.0 and at relatively high temperature around 50 – 60 °C. In addition, mKAT III is active to a number of other amino acids. Its activity to kynurenine is significantly decreased in the presence of methionine, histidine, glutamine, leucine, cysteine and 3-hydroxykynurenienine. Through macromolecular crystallography we determined the mKAT III crystal structure and its structures in complex with kynurenine and glutamine. These structural data revealed that the overall architecture of mKAT III, and its cofactor binding residues and active center residues. This is the first report concerning the biochemical characteristics and crystal structures of KAT III enzymes, which provide a biochemical and structural basis towards understanding the overall physiological role of mammalian KAT III in vivo and insight into controlling the levels of endogenous KYNA through modulation of the enzyme in the mouse brain.
Identification of an *Histophilus somni (Haemophilus somnus)* Exopolysaccharide, a Component of the *H. somni* Biofilm

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*Histophilus somni (Haemophilus somnus)* is a gram-negative coccobacillus responsible for respiratory disease and other systemic infections in cattle. We now report the production of a polysaccharide by *H. somni* that was produced primarily under growth restricting stress conditions, such as during anaerobiosis, stationary phase, or in hypertonic salt. The polysaccharide was isolated in greatest quantity from the sedimentary cell mass matrix (biofilm), during growth under low oxygen tension. The polysaccharide was purified by extraction of the biofilm with 45 percent phenol, digestion with RNase, DNase, and proteinase K, and ultracentrifugation to remove lipooligosaccharide. Immuno-transmission electron microscopy revealed that the polysaccharide was not closely associated with the cell surface indicating that it was an exopolysaccharide (EPS). However, antibodies to the EPS did bind to the biofilm matrix of *H. somni*. Electrospray-mass spectrometry and gas liquid chromatography analysis indicated that the EPS was composed of mannose and galactose in a ratio of about 3:1, and the mannose-specific lectin Morinda M also bound to the biofilm matrix. Most strains of *H. somni* tested produced an EPS, determined by enzyme-linked immunosorbent assay, but the amount of EPS produced or the antigenic epitopes varied between strains. Antibody titers to the EPS were higher in cattle after experimental challenge than prechallenge. However, antibodies to EPS did not passively protect mice against lethal systemic challenge. A DNA locus of about 19 kb that contains genes with homology to carbohydrate biosynthesis genes from capsule and EPS loci of other bacteria was identified that may be responsible for synthesis of this EPS. Thus, the *H. somni* EPS appears to be produced primarily under growth conditions that favor biofilm formation, and is a component of the biofilm matrix.

**Fig. 1.** Biofilm formation by *H. somni* strain 738 after 72 h of growth in supplemented Terrific broth. Bacteria were grown as planktonic cells in a starter culture of 50 ml and then transferred to 1 L of filtered Terrific broth supplemented with TMP, Trizma base, and glucose in a 1 L bottle. The bottle was rotated slowly at 70 rpm and 37°C. After 24 h the turbid broth became clarified and the cell mass settled to the bottom as a biofilm. Maximum amounts of EPS could be isolated from this biofilm after at least 3 days growth.

**Fig. 2.** Immuno-transmission electron microscopy. IgG from antiserum to Cetavlon precipitates washed cell supernatant was prepared in rabbits, and incubated with whole cells that were gently scraped off plates, followed by Protein-A gold particles. The dark particles binding to the extracellular matrix (arrows) are Protein A-gold particles binding to immunoglobulins. Note that none of the Protein A-gold particles are binding to the cell membrane, but are bound to extracellular material shed from the cell. More of this extracellular material is present off cells grown anaerobically (Fig. 2A) than off cells grown in CO₂ (Fig. 2B).
Isolation and Characterization of a Capsule-Like Material from *Francisella tularensis*

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*Francisella tularensis* is a category A select agent and the etiologic agent of tularemia in humans and animals. A capsule-like structure has been described on this bacterium using electron microscopy, although such a component has not been isolated. The capsule-like material (CLM) can be observed as an electron dense layer surrounding individual cells, varies in size, and is not always present. A small amount of non-lipopolysaccharide (LPS) carbohydrate-rich material was extracted from *F. tularensis* using aqueous phenol. To increase the amount of CLM synthesized, the bacteria were subcultured daily in synthetic media, followed by growth on defined medium for five days at 32°C in 6 percent CO₂. To simplify separation from LPS, an O-antigen deficient mutant of *F. tularensis* was used for CLM isolation. The CLM was extracted from the bacteria with 0.5 percent phenol, and the thick, sudsy extract was treated with nucleases and Proteinase K, and contaminants removed by ultracentrifugation and gel filtration chromatography. The purified CLM contained octadecanoic acid, glucose, galactose, and mannose and was void of quinovosamine and KDO, both unique to the LPS. The electrophoretic profile of the CLM appeared as a high molecular size, ladder-like material. Affinity purified antibodies to the CLM bound to material on and off the cell surface, as determined by immuno-electron microscopy. The purified CLM induced an immune response in BALB/c, C57/Black, and CD-1 mice, and a strong response in rabbits in the presence of adjuvant. Allelic exchange mutagenesis of two genes (FTT0797-0798) in an apparent carbohydrate locus resulted in a mutant devoid of CLM, as determined by electron microscopy and antigen purification. Complementation of these genes in trans restored CLM synthesis. Further characterization of this novel polysaccharide will be necessary to determine its usefulness in diagnostic assays and vaccines.

![Figure 1](image1.png)

**Figure 1.** Electron micrograph of parent strain mutant WbtIG191V_P17, glycosyl transferase mutant WptIG191V_P17,gT, and complemented strain WbtIG191V_P17,gT+[gT+]. A, The parent strain has an electron dense layer surrounding the cells; B, mutant WptIG191V_P17,gT had no layer or extracellular material surrounding the cells; C, complemented strain WbtIG191V_P17,gT+[gT+] expressing extracellular material.

![Figure 2](image2.png)

**Figure 2.** Alcian-Blue/silver stain of CLM. Lanes: 1, LVS LPS (20 µg); 2, Actinobacillus pleuropneumoniae capsule (20 µg); 3, LVS WbtIG191V_P17 capsule (20 µg). The CLM was separated through a 25 percent polyacrylamide native gel at 30 V cm⁻¹ following pre-electrophoresis for 7 hr. The gel was fixed in the dark in 0.5% alcian blue, a glycolipid stain, for 30 min and destained in water for 30 min. The gel was then stained and capsule visualized using the Bio-Rad silver stain kit (Bio-Rad).
Plasmodium falciparum Aminopeptidase P: Localization, Biochemical Properties and Determinants of Dual Targeting

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Plasmodium falciparum possesses a homolog of aminopeptidase P, a widely distributed enzyme that cleaves the first amino acid from peptides with the sequence X-Pro, where X is any amino acid. P. falciparum aminopeptidase P (PfAPP) differs from other known homologs in that it contains a 149 amino acid N-terminal extension (NTE) preceding the catalytic and oligomerization domains. Localization experiments with anti-PfAPP antibodies indicate that the enzyme is concentrated in the food vacuole with a lower amount in the cytosol. The size of the major vacuolar PfAPP isoform suggests that the NTE has been removed by vacuolar peptidases. Both native vacuolar and recombinant PfAPP (lacking the NTE) co-migrate on a gel filtration column with apparent dimeric structures. Recombinant PfAPP hydrolyses peptide substrates containing an X-Pro amino terminus at the acidic pH of the food vacuole. As our attempts to disrupt the PfAPP gene have been unsuccessful, we have sought to diminish PfAPP levels by tagging PfAPP with a destabilized form of the FK506 binding protein (the FKBP12 “destabilization domain”). Although the presence of the destabilization domain reduces PfAPP levels in the food vacuole and cytosol, parasites growth in culture is unaffected. These data suggest that the residual levels of PfAPP are sufficient for normal growth and development. To investigate the mechanism of dual targeting of PfAPP to the food vacuole and cytosol, we have expressed fragments of the NTE fused to yellow fluorescent protein in P. falciparum. We find that the first 40 residues of the NTE, which contain a putative signal peptide for translocation into the endoplasmic reticulum, are sufficient to specify dual targeting.
Activation of AMPK Inhibits Inflammation In MRL/lpr Mouse Mesangial Cells

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Recent reports show that 5-amino-4-imidazole carboxamide riboside (AICAR), a pharmacological activator of AMP-activated protein kinase (AMPK), inhibits the LPS-induced production of pro-inflammatory cytokines. MRL/MPJ-Faslpr (MRL/lpr) mice show an intrinsic decreased threshold for the production of inflammatory mediators when stimulated. In our current studies, we sought to determine if AMPK activation would inhibit inflammatory mediator production in stimulated kidney mesangial cells. Cultured mesangial cells from MRL/lpr mice were treated with AICAR and stimulated with LPS/IFN-gamma. AICAR dose dependently decreased inducible nitric oxide synthase (iNOS), COX-2, and interleukin-6 (IL-6) production in LPS/IFN-gamma-stimulated mesangial cells. Mechanistically, AICAR inhibited the LPS/IFN-gamma-stimulated PI3K/Akt signaling inflammatory cascade but did not affect LPS/IFN-gamma-mediated I-kappa-B phosphorylation or NF-kappa-B (p65) nuclear translocation. Treatment with the adenosine kinase inhibitor 5'-iodotubercidin blocked the ability of AICAR to activate AMPK and prevented AICAR from inhibiting the LPS/IFN-gamma-stimulated PI3K/Akt pathway and attenuating iNOS expression. Taken together, these observations suggest that AICAR inhibits Akt activation through AMPK activation and may serve as a potential therapeutic target in inflammatory diseases.
Characterization of Outer Membrane Proteins from *Francisella tularensis* LVS and O-Antigen Deficient Mutants

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The major outer membrane proteins of *Francisella tularensis* live vaccine strain (LVS) and three O-antigen deficient mutants were analyzed to assess the contribution O-antigen to outer membrane integrity. The protein-enriched outer membranes (PEOM) of a chemical mutant (LVSMuGB), a spontaneous grey colony variant (LVSGV), and a deletion mutant (LVS\_wbtI\_D191V) were compared. Bacterial cells were grown to mid-log phase in Chamberlain's synthetic medium. The separation of outer membranes from inner membranes and periplasmic proteins proved to be atypically difficult. Therefore, outer membrane proteins were biotin-labeled to aid in identifying proteins accessible on the cell surface. The biotinylated cells were lysed by sonication to isolate crude outer membranes. Unlysed cells were removed by low speed centrifugation and the total membrane fractions were recovered by discontinuous sucrose density gradient centrifugation. The PEOM were subsequently separated from the inner membranes by continuous sucrose density gradient ultracentrifugation for 96 hours. Protein fractions were collected from the bottom of each tube starting at an OD280 of 0.05, separated by SDS-PAGE (non-reduced), and the proteins detected by silver stain and by streptavidin-HRP Western blot to identify biotin-labeled proteins. Immunoreactive proteins from extracts were detected using a polyclonal rabbit antiserum to LVS. The PEOM were separated much more efficiently from inner membranes in the sucrose gradients from each of the O-antigen deficient mutants, indicating that the O-antigen interferes with PEOM separation.
Figure 2. Silver stain and Western blot of sucrose gradient fractions of LVS mutant strain Mu1. Lanes: 1, molecular weight marker; 2, total membrane fraction; 3-10, sucrose gradient fractions 1-8. Lanes 2-8 contain 2 μg protein or 20 μl of sample. All samples are non-reduced. Protein bands of 50, 45, 28, 20, and 17 kDa are clearly visible in the first few fractions with Mu1, but do not appear until the later fractions in LVS and Vb1p<sup>Δ</sup> mutants. Protein bands of 113, 106, 74, 64, 50, and 64 kDa are clearly evident in Mu1, but not in the parent strain by Western blot.

Figure 3. Silver stain and Western blot of sucrose gradient fractions of LVS mutant strain Vb1p<sup>Δ</sup>+. Lanes: 1, molecular weight marker; 2, total membrane fraction; 3-10, sucrose gradient fractions 1-8. Lanes 2-8 contain 2 μg protein or 20 μl of sample. All samples are non-reduced. Protein bands of 116, 106, 74, 64 and 54 kDa appear prominently in early fractions in the silver stain and Western blot. These higher molecular weight proteins appear in the silver stain of the LVS parent, but in lesser amounts and are not evident (biotinylated) in the Western blot.

Figure 4. Silver stain and Western blot of sucrose gradient fractions of LVS mutant strain LVS<sup>Δ</sup>LVG. Lanes: 1, molecular weight marker; 2, total membrane fraction; 3-10, sucrose gradient fractions 1-8. Lanes 2-8 contain 2 μg protein or 20 μl of sample. All samples are non-reduced. These results are very similar to results seen with mutant Vb1p<sup>Δ</sup>.

Figure 5. PEDM samples after continuous sucrose gradient separation and during fraction collection. Vb1p<sup>Δ</sup> and LVS<sup>Δ</sup>LVG samples precipitated after total membrane isolation and again during PEDM protein isolation. Some sonication was required to resolubilize the precipitates from these 2 mutants. LVS and Mu1 remained in solution during the entire procedure.
Conversion of NO₂ to NO by Reduced Coenzyme F420: A Strategy of Mycobacterial Survival against Nitrosative Stress

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Mycobacteria contain F420, a deazaflavin derivative, which acts as a hydride transfer coenzyme for an F420-specific glucose-6-phosphate dehydrogenase (Fgd) in these bacteria. Reactions that utilize Fgd-generated reduced F420 (H₂F420) in the mycobacteria are unknown. In this study, H₂F420 was found to be oxidized by NO only in the presence of oxygen. We demonstrated that NO₂, produced from NO and O₂, was the oxidant. UV-visible spectroscopic and NO-sensor based analyses proved that H₂F420 reduced NO₂ to NO, this conversion is a two-electron transfer process. Activated macrophages produce NO, which in acidified phagosomes is converted to NO₂. By converting more potent anti-mycobacterial NO₂ back to NO with H₂F420, pathogenic mycobacteria decrease the effectiveness of antibacterial action of macrophages.
Biofilm Formation by *Histophilus somni*: Molecular Investigation and Implications for Bovine Respiratory Disease

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*Histophilus somni* (*Haemophilus somnus*) is an etiologic agent of pneumonia and systemic disease in bovines, and has been shown to form a biofilm *in vitro* and *in vivo*. The sum of our previous investigations on biofilm formation by *H. somni* demonstrated that: a) the amount, stability, and architecture of the biofilm formed *in vitro* varied between pathogenic strain 2336 and commensal strain 129Pt, and b) the biofilm formed in the natural host (bovine) following respiratory and systemic infection strongly suggest that a biofilm may contribute to the disease process, and may be important for harboring and protecting other bovine respiratory disease complex pathogens from innate and adaptive host defenses. *H. somni* is the only animal pathogen for which genome sequences of a pathogenic strain and non-pathogenic strain are available. The availability of these sequences have been utilized to investigate the genes involved in the expression of virulence factors such as lipooligosaccharide (LOS) synthesis and phase variation, exopolysaccharide (EPS) biosynthesis, immunoglobulin binding proteins, quorum sensing, mannose synthesis, twitching motility, and restriction modification systems. We have now determined the expression of specific genes in the genomes of strains 2336 and 129Pt at different time points during biofilm development and the planktonic state using real time qRT-PCR. Gene expression analysis will help to determine, in part, which genes are responsible for virulence factors that are necessary for biofilm formation and/or contribute to disease.
Members of the *Anopheles gambiae* complex have remarkably distinct ecological adaptations, behaviors, and degrees of vectorial capacity. Inferring phylogenetic relationships in the complex is crucial for identifying the genomic changes associated with the origin and loss of epidemiologically important traits. However, the high level of sequence similarity, genetic introgression, and shared molecular ancestral polymorphisms makes reconstruction of the *A. gambiae* complex phylogeny difficult. Phylogenetic relationships among the members of species complexes can be inferred from the distribution of fixed chromosomal inversions if outgroup arrangements are known (Fig. 1). The aim of this work is to test a possibility of determining ancestral autosomal arrangements in the *A. gambiae* complex using outgroup chromosomes and a combination of bioinformatic and cytogenetic approaches. The minimum number of inversions between members of the *A. gambiae* complex and the outgroup species *A. funestus* and *A. stephensi* was calculated using the Multiple Genome Rearrangements (MGR) and Sorting Permutation by Reversions and block-INterchanGes (SPRING) programs (Fig. 2). The physical mapping of *A. merus* chromosomes identified molecular coordinates of the proximal 2Ro+ inversion breakpoint in *A. gambiae*. DNA probes from 2La+ and 2Ro+ inversion breakpoints of the *A. gambiae* were mapped to the *A. stephensi* chromosomes (Fig. 3, Fig. 4). The results suggest that the *A. gambiae* complex shares the 2La and 2Ro arrangements with the outgroup species. Assuming monophyletic origin of the inversions, this study concludes that physical mapping of ingroup and outgroup species can be used for identifying inversion breakpoints and ancestral autosomal arrangements within species complexes.

![Fig. 1. A hypothetical phylogenetic tree of the A. gambiae complex based on the ancestry of the 2La arrangement. The known chromosomal arrangements that support this tree are shown on the edges.](image-url)
Fig. 2. Trees recovered by MGR (A) and SPRING (B) programs showing that the 2Rop has a smaller distance to A. stephensi and A. funestus 2R than 2R+. The number of rearrangements that occurred on each edge is shown. A-A. arabiensis, G-A. gambiae. A4 and A5 are putative ancestral species.

Fig. 3. FISH of 146D17 labeled with Cy5 (A) and 131F22 labeled with Cy3 (B) performed on the chromosomes of A. stephensi. Arrows point at the hybridization signals.

Fig. 4. FISH of AGAP002934 (A, C) and AGAP002935 (B, D) with A. stephensi chromosomes. Arrows point at the hybridization signals. A and B show the banding pattern of the chromosomes counterstained with the fluorophore YOYO-1. C and D show fluorescence due to hybridization. The images are inverted.
Structural Organization of the Malaria Mosquito Heterochromatin

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The development of new genome-based vector control strategies requires detailed knowledge about the organization and function of the mosquito genome. Heterochromatin is a functionally important and rapidly evolving part of the chromosome. Anopheline mosquitoes represent an ideal system for studying the structure and evolution of the heterochromatin because of the presence of polytene chromosomes in several tissues and great variability in heterochromatin among species. Two different morphological types, condensed/dark granulated (alfa) and diffuse/light granulated (beta) heterochromatin, have been identified in polytene chromosomes of ovarian nurse cells in A. gambiae and A. stephensi. Only diffuse beta type heterochromatin forms large visible attachments of the chromosomes to the nuclear envelope. The goal of this study was to characterize the two types of heterochromatin using immunostaining and bioinformatic analysis. Immunostaining of chromosomes using antibodies against Drosophila Heterochromatin Protein 1 (HP1) and nuclear envelope protein lamin Dm0 revealed co-localization of these proteins in most of the heterochromatic and euchromatic sites. The total number of sites was 128/158 in A. gambiae and 266/268 for in A. stephensi chromosomes for HP1/lamin, respectively. Surprisingly an alternative pattern of protein localization has been detected between the species: both proteins were concentrated in all pericentromeric areas in A. gambiae, but in internal regions in A. stephensi chromosomes. No antibodies have been detected in pericentromeric alfa heterochromatin of 2R, 3R and 3L chromosomal arms in A. stephensi and intercalary alfa heterochromatin of A. gambiae. Gene density, AT, and repetitive element content were analyzed in the assembled part of the A. gambiae heterochromatin (13.3 Mb) and in the euchromatin (219 Mb) using Biomart, ATCONTENT, and RepeatMasker programs respectively. All heterochromatic regions had higher AT content and five times lower gene density than in euchromatin. Analysis of transposable elements and tandem repeats revealed the major difference in proportion of DNA transposons between two types of heterochromatin. These findings suggest alfa and beta types of the heterochromatin may have different functions in the mosquito genome.
Patterns of Gene Inactivation in *Brucella* Species and their Correlation with Virulence Phenotype

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Pseudogenes are the remnants of genes that have lost their function through mutations in their coding sequence which interfere with translation or in regulatory elements which prevent their transcription or interfere with pre-mRNA processing. In effect, pseudogenes represent nature’s own gene-knockout experiments. As a result, it is instructive to look at closely-related organisms to identify cases where orthologous genes are active in some species and inactive in others. This provides a gene-centric view of polymorphisms which can be correlated with phenotypic variation between the organisms. Software was developed to construct a complete set of orthologous (pseudo)genes given representative members and display the information as a table of ortholog groups vs. genomes, overlaid with the functional state of each locus. To test the software, we examined the eight fully-sequenced genomes from the alpha-proteobacteria genus *Brucella*, a group of endosymbiotic livestock pathogens and potential biowarfare agents. Generally speaking, pseudogenes are identified as a by-product of functional gene annotation. In contrast, we sought to use known pseudogenes in one *Brucella* species to identify pseudogenes in the remaining species by sequence similarity. We started with corresponding DNA sequences of 1174 pseudogenes (from all *Brucella*) and aligned them to the eight genomes using BLASTN with stringent cutoffs. This resulted in 4289 distinct alignment regions divided into 549 homologous groups. Of the alignments, 2690 (63%) corresponded to known (functional) genes and 1599 to pseudogenes, of which 425 (27%) were newly identified. The overall pseudogene frequency in the eight Brucellae was 6 percent. Of the eight *Brucella* strains in this study, all are virulent except *B. abortus* S19. One might therefore expect genes responsible for virulence to either be functional in S19 and non-functional in the remaining species or vice versa. Of the 10 loci that meet these criteria, seven corresponded to proteins of known function, including several membrane-bound transporters and enzymes of intermediate metabolism. Perhaps the most interesting candidate is a DedA-family integral membrane protein with homology to the eukaryotic SNARE-associated Golgi protein. This protein, inactive in S19, could play a role in the way in which *Brucella* evades degradation when living in its host macrophage.
Comparative Genomics of Brucella, an Intracellular Pathogen from the Prokaryotic Order Rhizobiales

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There are now eight sequenced genomes in the genus Brucella, creating an opportunity for a comprehensive review of this group. Structural comparisons among these genomes showed strong co-linearity in the first chromosome and rearrangements, some of them species-specific, on the second chromosome. Genomic components including protein coding genes and different RNA classes were compared and were all similar between the different genomes, but there was variation in the number of pseudogenes. Differences associated with the “rough” phenotype of B. ovis, which lacks genes essential for O-polysaccharide synthesis, were identified. In addition, key components of the α946, ketoadipate pathway that is found in all Brucella species and in a number of other members of the Rhizobiales order have been lost in B. suis ATCC 23445. Ortholog groups were created with these Brucella genomes and with 27 other genomes within the order Rhizobiales. Protein families that the Brucella genomes share with their fellow Rhizobiales, as well as proteins unique to the genus and those that shared with other bacteria outside of the Rhizobiales were analyzed, and core genes and those laterally transferred were identified. The protein sequences from 2475 ortholog families that contain all representatives from all Brucella genomes were used to create a phylogenetic tree that also includes proteins from Bartonella quintana, Ochrobactrum anthropi, and Mesorhizobium loti.
Expression, Purification and Biochemical Characterization of a Flavin Adenine Dinucleotide-Dependent Monoxygenase from *Mycobacterium smegmatis* Involved in Mycobactin Biosynthesis

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Iron is an essential nutrient for nearly all living organisms. In humans, iron is sequestered by iron binding proteins such as transferrin, lactoferrin and hemoglobin and is therefore unavailable to invading microbial pathogens (Figure 1). To meet the cell’s optimal iron needs of ~10-6 M, many pathogenic bacteria have evolved a competitive and selective iron acquisition system (1,2). They synthesize and secrete low-molecular-weight iron chelators, called siderophores, to scavenge iron from the host. Through a series of specific membrane transporters, the siderophore-iron complex is then transferred into the bacterium cytoplasm, increasing the iron concentration to levels optimal for bacterial proliferation (2,3). Among the numerous natural siderophores known, the most documented are the mycobactins, a distinct class of amphiphilic siderophores produced by the human pathogens *Mycobacterium tuberculosis* and *Mycobacterium leprae*, the causative agents of tuberculosis and leprosy, respectively (4,5). Most siderophores are synthesized via non-ribosomal peptide synthetases and the functional groups that chelate iron are a combination of carboxylates, catecholates, and hydroxamates. The hydroxamate functional groups are commonly derived from amino acids, such as L-lysine or L-ornithine. The committed step of siderophore biosynthesis is the hydroxylation of the terminal amino group by a flavin-dependent monoxygenase (Figures 2,3) (4). In *Mycobacterium* spp., this monoxygenase enzyme is called MbtG, and it has been shown to be essential in *M. tuberculosis* and *M. smegmatis*, making this enzyme an attractive target for anti-mycobacterium drug discovery (6). In this work, we present the cloning, expression, purification, and biochemical characterization of MbtG from *M. smegmatis*.

Anti-inflammatory Activity of Curcumin in a Murine Model of Autoimmune Glomerulonephritis

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Curcumin, a polyphenolic compound present in turmeric (*Curcuma longa*), has been shown to possess potent anti-inflammatory properties and prevent renal injury in various animals. In our present studies, we sought to determine if curcumin might show efficacy in autoimmune lupus nephritis. C57BL/6 mice were given curcumin by oral gavage at either a low dose (100 mg/kg) or high dose (300 mg/kg). After a 2.5 week pretreatment period with curcumin, disease was induced by injecting anti-glomerular basement membrane antibody (anti-GBM) intravenously and LPS intraperitoneally. As experimental controls, three other groups of C57BL/6 mice received either the high dose of curcumin only, anti-GBM/LPS only, or remained untreated. Animals continued receiving oral curcumin daily for two weeks and were then sacrificed for tissues analysis. Serum and urine samples were collected and assessed for cytokine production and proteinuria. RT-PCR was performed on splenic tissue for Foxp3 induction, and kidney tissue for COX-2, iNOS TNF-α, and IL-6 induction. Kidney sections were evaluated for structural integrity, IgG deposition, and complement activation. To complement our *in vivo* studies, kidney mesangial cells from C57BL/6 mice were stimulated with LPS/IFN-γ following curcumin treatment and inflammatory mediator production determined. Through both the *in vivo* and *in vitro* model, we anticipate showing curcumin administration inhibits the progression of induced lupus-like autoimmune glomerulonephritis.
Quorum Sensing Relating to Infectious Bacteria

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We present a computational analysis of small RNAs (sRNAs) and target interactions in quorum-sensing. The analysis includes the effects of multiple sRNAs which have recently been shown to play a critical role in quorum sensing in Vibrio spp. Although the sRNAs are highly homologous across species, their associated luminescence phenotypes differ dramatically: in V. harveyi, the sRNAs act additively; however, in V. cholerae, the sRNAs act redundantly. Corresponding to the sRNAs acting additively or redundantly, we show how changing a single parameter causes dramatic differences in luminescence phenotypes within our model. We also show that as key parameters are changed, the sRNA-target response can change from graded to switch-like. Finally, we use the model to understand features of luminescence phenotypes for different mutants in V. harveyi and V. cholerae and make testable predictions for future experiments.
Estrogen Modulates Innate Immune System by Altering TLR4-Mediated Events

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Gender has been considered an important contributory factor in the incidence and severity of multiple diseases, such as infectious and autoimmune diseases. Females are in general less prone to infectious diseases, in both prevalence and intensity, over males partly because of the protective effects of estrogen on the immune system. Effector cells of innate immune system particularly macrophage and dendritic cells recognize pathogen associated molecular patterns (PAMPs) expressed by microbes and clear these by various mechanisms such as phagocytosis and lysis of infected cells. Toll like receptors (TLR) on effector cells of innate immune system recognize PAMPs such as LPS, CpG during infection and initiate signaling pathways which culminate in the increased expression of immune and inflammatory genes e.g. IL-27, IL-6, IL-23, and aid in activation of adaptive immune system. However, it is still unclear how estrogen modulates responsiveness of effector cells of innate immune system to PAMPs. To address this question, splenocytes from estrogen- and placebo-treated mice were briefly stimulated in the presence of LPS to study the role of estrogen in TLR4-mediated signaling events. It was observed that estrogen upregulates expression of TLR4 mRNA and proinflammatory biomolecules, such as IL-6, and MCP-1. These results suggest that estrogen plays an important role in modulating the responsiveness of effector cells of innate immune system to pathogen-associated molecular patterns and therefore, reduces the susceptibility of females to infectious diseases.
Generation and Analysis of Acylated Homoserine Lactone-Insensitive Variants of the Quorum Sensing Regulator EsaR

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Production of exo/capsular polysaccharide (EPS) is naturally repressed at low cell densities via the quorum-sensing regulator EsaR in the corn pathogen *Pantoea stewartii* subsp. *stewartii*. However, at high cell densities when high concentrations of its cognate acylated homoserine lactone (AHL) signal are present, EsaR is inactivated and derepression of EPS production occurs. The mechanism that enables EsaR to respond to AHL in a manner opposite to that of most LuxR homologues remains elusive. Hence, we have utilized a random mutagenesis genetic approach to isolate EsaR* variants that are immune to the effects of AHL. Error-prone PCR was used to generate the desired mutants, which were subsequently screened for their ability to repress transcription in the presence of AHL. Following sequencing, site-directed mutagenesis was used to generate all possible mutations of interest as single, rather than multiple amino acid substitutions. Several individual amino acids playing a critical role in the AHL-insensitive phenotype have been identified and mapped onto a homology model of EsaR. The role of these residues in AHL binding and DNA binding is being examined so that a model describing the molecular basis of the AHL responsiveness of EsaR may be developed.
The *Bacillus anthracis* SleL (YaaH) Protein is an N-Acetylglucosaminidase Involved in Spore Cortex Depolymerization

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*Bacillus anthracis* spores, the infectious agent of anthrax, are notoriously difficult to remove from contaminated areas because they are resistant to many eradication methods. These resistance properties are due to the spore dehydration and dormancy, and to multiple protective layers surrounding the spore core, one of which is the cortex. In order for *B. anthracis* spores to germinate and resume growth, the cortex peptidoglycan must be depolymerized. This study reports analyses of *sleL* (*yaaH*), which encodes a cortex lytic enzyme. Inactivation of *sleL* does not affect vegetative growth, spore viability, or the initial stages of germination including dipicolinic acid release. However, mutant spores exhibit a slight delay in loss of optical density when compared to wild-type spores. Mutants also retain more diaminopimelic acid and N-acetylmuramic acid during germination than wild-type spores, suggesting that the cortex peptidoglycan is not being hydrolyzed as rapidly. This finding is supported by HPLC analysis of peptidoglycan structure used to confirm that SleL acts as an N-acetylglucosaminidase. When *sleL* is inactivated, the cortex peptidoglycan is not depolymerized into small muropeptides but instead is retained within the spore as large fragments. Spores germinating in the absence of the *sleL*-encoded N-acetylglucosaminidase must be relying on other cortex lytic enzymes to break down the cortex peptidoglycan.
Functional Expression and Purification of UDP-Galactopyranose Mutases from *Leishmania major*, *Aspergillus fumigatus* and *Trypanosoma cruzi*

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Galactofuranose (Galf) is a major component of glycolipids and glycoproteins found at the cell surface of *Leishmania major*, *Trypasonoma cruzi*, and *Aspergillus fumigatus* and plays an important role in pathogenesis. Parasites from the genus *Leishmania* are the etiological agents of leishmaniasis, a disease whose manifestations in humans range from mild cutaneous lesions to fatal visceral infections. *T. cruzi* is a parasite that causes Chagas’ disease, which can lead to swelling and inflammation of the heart. *A. fumigatus* is a fungus that infects people with compromised immune systems leading to Aspergillosis. There are approximately 25 million people infected with these diseases and more than 100,000 people die every year from them. Despite the significant health threat posed by these diseases, there are no efficacious drug treatments; therefore, new therapeutic treatments are urgently needed. UDP-Galactopyranose mutases (UGM) catalyze the conversion of UDP-galactopyranose to UDP-galactofuranose, which is the committed step in the biosynthesis of Galf. Since Galf is absent in humans, this enzyme is an attractive target for the identification of novel antiparasitic and antifungal drugs. In this work we present the cloning, expression, and preliminary characterization of the recombinant UGMs from *L. major*, *T. cruzi*, and *A. fumigatus*. The eukaryotic enzymes were successfully expressed in *E. coli* and were purified to homogeneity. All of the recombinant enzymes contained a tightly bound FAD cofactor and are active. This work sets the stage for future mechanistic and structural studies that will aid in the identification of inhibitors of UGMs which might serve as drugs for the treatment of leishmaniasis, Chagas’ disease, and Aspergillosis.
The Hypervariable Region (HVR) in the Open Reading Frame 1 (Orf1) of HEV is Dispensable for Virus Replication

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Hepatitis E virus (HEV) is an important but understudied human pathogen. Comparative genome sequence analysis revealed a hypervariable region (HVR) with extensive sequence variations in the ORF1 of HEV, not only among the four major HEV genotypes and avian HEV, but also within each genotype. The observed sequence variations in the HVR suggest that this region may be dispensable for virus replication. To elucidate the role of HVR in HEV replication, we constructed a deletion mutant hHVRd with aa residues 747-761 deleted in the HVR of a genotype 1 human HEV replicon expressing GFP protein. Replication of the mutant was evident in Huh-7 cells transfected with in vitro RNA transcripts of the mutant as evidenced by the expression of GFP. To confirm in vitro results, we further constructed three avian HEV mutants with various deletions in the HVR: aHVRd1 (Δaa 557-641), aHVRd2 (Δaa 557-585) and aHVRd3 (Δaa 612-641). Chickens intrahepatically inoculated with the capped RNA transcripts from aHVRd2 and aHVRd3 developed active viral infection as evidenced by seroconversion to avian HEV antibodies, viremia and fecal shedding. To further confirm the avian HEV results, we subsequently constructed four additional HEV mutants using the backbone of the genotype 3 swine HEV infectious clone: sHVRd1 (Δaa 712-790), sHVRd2 (Δaa 722-781), sHVRd3 (Δaa 735-765) and sHVRd4 (Δaa 712-765). Mutants sHVRd2, sHVRd3 and sHVRd4 were shown to tolerate deletions and were infectious in pigs intrahepatically inoculated with the capped RNA transcripts from these mutants. The data obtained from this study indicates that the HVR region of HEV is not required for HEV replication *in vitro or in vivo.*
Multiple Lipid Kinase (MuLK) Inhibitors for Prostate Cancer Therapy

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Prostate cancer is the most common cancer in American men and the second leading cause of cancer deaths. As with other types of cancer, cell signaling by lipids is important in progression of the disease. The phospholipids such as lysophosphatidic acid and phosphatidic acid are known to be important players in the pathogenesis of cancer. These phospholipids are the result of phosphorylation of monoacyl and diacylglycerols by acylglycerol kinase (AGK). AGK, also known as MuLK, has been shown to be highly expressed in cancer cell lines and is crucial in the initiation and progression of prostate cancer. We have been interested in developing selective inhibitors of MuLK as possible therapy for prostate cancer. We have been synthesizing libraries of 1-(para-octylphenyl)-4-substituted cyclohexane derivatives to define the structure-activity relationships of MuLK. The synthesis and activity of these compounds will be discussed.
Expression of Type IV Pilin Proteins from the Gram-Positive Pathogen Clostridium perfringens in Neisseria gonorrhoeae Changes the Tropism from Human Genito-Urinary Tract Cells to Mouse Muscle Cells

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Clostridium perfringens is an anaerobic, gram-positive bacterium that causes a range of diseases in humans, including lethal gas gangrene infections and food poisoning. We have recently shown that all three sequenced strains of C. perfringens move across the surface of agar plates using a unique TFP-mediated social motility that has not been previously described. Based on sequence homology to pilins in Gram (-) bacteria, C. perfringens appears to have two pilin subunits, PilA1 and PilA2. Structural prediction software indicates PilA1 is similar to the pseudopilin found in Klebsiella oxytoca, while PilA2 is more similar to true pilins found in the Gram (-) pathogens P. aeruginosa and N. gonorrhoeae. N. gonorrhoeae is a Gram negative pathogen with TFP that have been well characterized. Strains of N. gonorrhoeae that are genetically deficient in the native pilin, PilE, but supplemented with PilA1 and PilA2 of C. perfringens, have been constructed. Both strains produced pili that were visible using electron microscopy. Genetic competence was not restored by either pilin. Twitching motility was restored to a nonmotile PilE- strain when induced to express PilA2, the pilin predicted to have a structure similar to the pilin in N. gonorrhoeae, but the strain expressing PilA1 remained nonmotile. Attachment to a cell line derived from human genito-urinary epithelial cells, which requires TFP in N. gonorrhoeae, was not restored by expression of either C. perfringens pilin. However, attachment was seen with the N. gonorrhoeae strain expressing PilA2 to a mouse myoblast (muscle) cell line. This correlates with the type of host cells (i.e., muscles) that C. perfringens is likely to encounter in a gangrene infection, which is defined as an invasive infection of muscle tissue. These findings provide insight into the ability of C. perfringens to adhere to and move along muscle fibers in vivo, which may provide a therapeutic approach to limiting this rapidly spreading and highly lethal infection.
Structural Organization and Evolutionary Plasticity of the Malaria Mosquito Genome

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An. gambiae, An. funestus, and An. stephensi are primary malaria vectors and are members of different series of the subgenus Cellia. These species are highly polymorphic for inversions that are distributed non-randomly on five chromosome elements. The goal of this study was to determine the rates of inversion fixation and to identify molecular features that correlate with unequal rates of karyotype evolution. We mapped 326 An. stephensi, An. funestus, and An. gambiae cDNA and BAC clones to A. stephensi polytene chromosomes and used 231 uniquely located markers for comparative mapping with An. funestus, and An. gambiae. The comparative mapping has clearly demonstrated a striking contrast among chromosome arms in length of conserved segments. There are small conserved blocks (< 2 Mb) on arm 2R and large conserved blocks (up to 8 Mb) on 3R and 3L of An. gambiae. There were significantly more synteny blocks shared among all three species than synteny blocks shared between only a pair of species. This suggests existence of functional gene clusters that constrain chromosomal breakage. The analysis using the Genome Rearrangements In Man and Mouse (GRIMM) program revealed that the X chromosome has the highest rate of inversion fixation whereas autosomes vary in the inversion density: 2R>2L>3R>3L. Another remarkable observation was a significant positive correlation between polymorphic inversions and fixed inversion on autosomes. The analysis of the An. gambiae genome revealed a significant negative correlation between the number of fixed inversions and the density of Matrix/Scaffold Attachment Regions (M/SARs) suggesting a role of nuclear architecture in determining the chromosome specificity of rearrangement rates. In addition, we found a positive correlation between the rates of inversion fixations and the simple repeat content on five chromosomal arms. This suggests involvement of simple DNA repeats in facilitating rearrangements.
Defining the CsrA Regulon in *Vibrio fischeri* and its Interactions with the Quorum Sensing Network

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CsrA is a post-transcriptional global regulator of carbon utilization that plays a key role in metabolic changes in the cell, and transitions from exponential to stationary growth phase. In *Vibrio fischeri*, we have shown that CsrA activity is controlled by two small RNA's called csrB1 and csrB2, which act to bind multiple copies of the CsrA protein, thus rapidly counteracting CsrA regulation of other genes. Using a combination of computational analysis and experimental validation, we are currently working to better define the CsrA-binding regions in mRNA in order to refine our computational search program, and identify novel targets of *V. fischeri* CsrA. In an attempt to directly identify CsrA interactions in *V. fischeri*, RNA pull-down analysis is being used to identify mRNA that is co-purified with CsrA. Also, using the computational predictions as a guide, transcriptional and translational fusions to Gfp are being constructed to examine expression levels of potential CsrA-regulated genes and validate the predictions. Due to the fact that quorum sensing in *V. fischeri* also has global effects, we hypothesize that there are significant interactions between the quorum sensing and CsrA networks that determine how the cell will respond to its environment and localized cell density. In other organisms such as *V. cholerae* and *Pseudomonas aeruginosa*, some of the links between the quorum sensing and CsrA networks have been demonstrated, but there are potentially many more interactions that have yet to be discovered.
Deans’ Forum on
INFECTIONOUS DISEASES

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