DEPARTMENT OF VETERINARY PATHOBIOLOGY

Antifolates Against Mycobacterial Infections in AIDS

The purpose of this project is to develop new antimycobacterial agents for control and/or eradication of mycobacterial diseases. This project involves collaborations with a Crystallography Group and an Organic Chemistry group. This is a drug-design program based upon recombinant DNA technology, X-ray crystallography, organic synthesis and site-directed mutagenesis.

Sponsor: NIAID/NIH

PI: William W. Barrow, Rebecca J. Morton

SRI, Birmingham, Alabama: William J. Suling, Robert Reynolds

"In Vitro and Animal Models for Emerging Diseases and Biodefense," Part A: "*In Vitro* Screens for Antimicrobial Activity"

As directed by the NIH project officer, the College of Veterinary Medicine, Oklahoma State University, will develop, validate, and use *in vitro* assays to screen test substances for activity against emerging "infectious agents." The activity in this contract will involve the screening of compounds for antimicrobial activity against several biodefense-related select agents in Categories A and B. Materials for testing will be provided by NIAID, as described in RFP NIH-NIAID-DMID-03-39.

Sponsors: NIH, NIAID, DMID

PIs: W.W. Barrow, K. Clinkenbeard, R. Morton, J. Wyckoff

Narrow–Spectrum Drug Targets for *Bacillus anthracis*

The goal of this project is to show proof-of-principal for potential antimicrobial targets in a specific biosynthetic pathway of *B. anthracis* that will allow for the development of compounds that can be used to treat anthrax. The approach will utilize DNA recombinant technology to develop an enzyme assay that will be utilized for high-throughput screening (HTS) of potential inhibitors. A robotics system will be used to implement the HTS. A pharmaceutical company has agreed to supply the project with potential inhibitors, which will be screened in the recombinant enzyme assays.

Sponsor: NIAID/NIH

PI: William W. Barrow

A Cell Culture-Derived Vaccine for Anaplasmosis

Anaplasma marginale harvested from tick cell culture will be tested as an antigen for a new and improved vaccine for bovine anaplasmosis. This antigen should result in the development of a vaccine that is safe, easily standardized, and free of contaminating bovine cells and pathogens. A vaccine dose will be formulated and tested in cattle for vaccine efficacy.

Sponsor: Novartis Animal Vaccines, Inc., Oklahoma Center for the Advancement of Science and Technology

PIs: Edmour F. Blouin, Katherine M. Kocan, Jose de la Fuenta

Novartis Animal Vaccines, Inc.: Thomas Halbur, Virginia C. Onet

Cell Culture Derived Vaccine for Bovine Anaplasmosis

Anaplasma centrale will be propagated in tick cell culture. Organisms harvested from cell culture will be tested as a live vaccine for bovine anaplasmosis in South Africa and Israel. The cell culture derived *A. centrale* should result in the development of a vaccine that is safe, easily standardized, and free of contaminating bovine cells and pathogens. A vaccine dose will be formulated and tested in cattle for vaccine efficacy.

Sponsor: U.S.-Israel Cooperative Development Research (CDR) Program

PIs: Edmour F. Blouin, Katherine M. Kocan, José de la Fuente

Kimron Veterinary Institute, Israel: Varda Shkap

Onderstepoort Veterinary Institute, South Africa: Eric Zweygarth

Role of *Pasteurella (Mannheimia) haemolytica* Leukotoxin in Shipping Fever Pneumonia

Pasteurella haemolytica produces an exotoxin termed leukotoxin (LKT), which has been implicated as an important virulence factor in shipping fever pneumonia in cattle. LKT specifically intoxicates ruminant leukocytes and platelets, but LKT has limited or no effect on leukocytes from other species. Bovine CD18 acts as the species-specific and leukocyte-specific receptor for *P. haemolytica* LKT. Prior to exposure to LKT, CD18 was found to be evenly distributed around the periphery of the bovine leukocyte plasma membranes. Following one minute of exposure, LKT and CD 18 were found associated with patches of degenerative plasma membranes. No CD18 or LKT was observed in regions of intact plasma membranes. This supports LKT-induced capping of CD18. The rapid degeneration of the associated plasma membrane in these patches suggests that CD18 localization to these regions may play a role in lose of plasma membrane integrity.

Sponsor: Oklahoma Agricultural Experiment Station

PI: Ken Clinkenbeard

Networked Terrorism Detection System

The overall goal of this proposal is to develop highly specific approaches for detecting and identifying explosives, nerve gases, and BW agents using a unique amplifying fluorescent polymer (AFP) which will greatly increase the speed and sensitivity of detection of explosives and CBW agents and to apply this development to produce a continuous real-time microarray networkable detection system for use against terrorist threats. Central to the overall goal is the underlying hypothesis that AFP can be used as a sensor platform and functionalized with specific probes to detect in continuous real-time explosives and specific CBW agents at minimal effective levels. This hypothesis was tested for nitroaromatic and other explosives, a nerve gas agent, and three BW agents. The research conducted for this project: 1) developed and tested probes specific and selective for explosives and prototype CBW agents, 2) functionalized AFPs for detection of prototype agents, 3) produced and tested a prototype AFP microarray sensor for explosives and CBW detection, and 4) conducted refinement, application, and commercialization of our MIPT AFP microarray sensor.

Sponsors: Oklahoma City Memorial for the Prevention of Terrorism and National Institute of Justice, Nomadics, Inc.

PIs: Ken Clinkenbeard, Jerry Malayer, Rebecca Morton, John Wyckoff

Biological Warfare Agent Water Monitor

The threat from biological warfare (BW) agents in combat and terrorist scenarios makes the ability for rapid detection and identification of BW agents of great importance. Oklahoma State University (OSU) along with our commercial partner Nomadics, Inc. propose to adapt amplifying fluorescing polymer (AFP) technologies currently being developed for BW agent detection in aerosols for monitoring of water supplies for BW agents. Such a monitor is of great interest to the Joint Services Agent Water Monitor (JSAWM) project of the Army Soldier Biological and Chemical Command (SBCCOM). Hurdles for development of near-real-time continuous monitoring of water supplies for BW agents are requirements for high sensitivity and specificity for up to 20 agents monitored simultaneously with minimal use of consumable reagents. The approach proposed herein is novel using new technologies that can interrogate water supplies with a highly sensitive regenerable reagent adaptable for numerous BW agents. This concept was demonstrated utilizing a prototype portable BW agent water monitor for two BW agents, *Francisella tularensis*, the causative agent of tularemia, and the water-borne pathogen enterohemorrhagic *Escherichia coli* O157:H7 (EHEC). Central to the overall goal is the underlying hypothesis that AFP can be used as a sensor platform for BW agents in water supplies and functionalized with specific probes to detect in near-real-time BW agents at minimal infectious levels. This hypothesis was tested for two BW agents. The functionalized AFPs were incorporated into a recoverable microbead (MB) prototype monitor.
PI: Ken Clinkenbeard, Rebecca Morton, Jerry Malayer

Development of Aptamer Beacons to Lipopolysaccharide for the Real-time Sensing of BW Agents

Force protection is of utmost importance, but a lack of real-time sensing technologies for biological warfare (BW) agents leaves U.S. forces vulnerable to conventional as well as terrorist's BW attacks. The major barriers to real-time sensing of BW agents are: 1) a lack of robust probes for detection of BW agents, and 2) inadequate sensitivity of sensor platforms through which target probes transduce their detection signals. We propose to conduct a "proof-of-concept" project to marry a new type of probe technology with exceptional robustness termed aptamers with a newly conceived sensing platform with exceptional sensitivity termed AFP beacons to overcome the current barriers for developing real-time BW sensing. Aptamers are highly stable and specific oligonucleotides, which work like monoclonal antibodies (Mab), to bind directly to BW target agents. However, unlike Mab, aptamers have exceptional stability even under field conditions. Aptamer technology is only beginning to be applied to detection of infectious agents. We developed aptamers to surface-exposed targets on the prototype BW agent, enterohemorrhagic E coli. We will next demonstrate that these aptamers can be used to specifically detect BW agents. In future experiments, we will engineer our anti-E coli aptamers to act as beacons so that when the aptamer binds to its target, it will switch from an "off" to an "on" signal by turning on specific fluorescence directly. No consumable reagents are required. Once DEPSCoR proof-of-concept project is accomplished, we will transition to prototype sensor development using this technology.

Sponsor: Army Research Laboratory
PI: Ken Clinkenbeard.

Development of Aptamer Beacons for Antemortem Diagnosis of Chronic Wasting Disease

The goal of the project is develop the basic research in support of an aptamer beacon-based antemortem diagnostic test for the transmissible spongiform encephalopathy chronic wasting disease (CWD) of elk and deer. Currently available diagnostic tests are based on monoclonal antibodies. These tests are applicable for biopsy or postmortem samples, but may have limitations that preclude their further development as antemortem tests. The limitations of Mab-based tests may be overcome by a new molecular entity known as aptamers. In particular, aptamers can be manipulated using standard molecular biology techniques to act as beacons or molecular switches that turn “on” a fluorescent signal when they bind to their target. Aptamers are nucleic acids which serve as novel recognition molecule or probe that are highly specific for a wide range of targets. Attempts by others to use aptamers to distinguish normal hamster prion from its abnormal isoform were not successful. However, a novel aptamer selection strategy is proposed herein to overcome the problems of the original aptamer selection experiments. We propose to use a cross-over selection strategy. Initially, the aptamer selection target will be particular peptides of CWD prion thought to be exposed on abnormal isoform. The reduced aptamer pool will subsequently be selected against the insoluble plaques of the abnormal isoform isolated from brain tissue of affected elk or deer. Aptamer that specifically recognize the abnormal isoform of CWD prion will subsequently be engineered as aptamer beacons, a novel signaling aptamer that have a built-in molecular switch.

Sponsor: U.S. Army Medical Research and Materials Command, National Prion Research Program
PI: Ken Clinkenbeard.

Polymer-Based Yersinia Pestis Point-of-Case Diagnostics

The goal of the proposed research is to develop a highly sensitive and specific multi-locus array diagnostic for rapid identification of *Yersinia pestis*, the causative agent of plague. This infectious bacterial agent is on the NIAID category A priority list as a potential biological warfare (BW) agent. In order to confirm suspicions that clinical cases may be due to purposeful aerosol dissemination of *Y. pestis* and, as such, may be indicative of an imminent plague epidemic, and to enable healthcare professionals to instigate immediate and effective therapeutic intervention and control measures, a rapid, sensitive, and accurate method for early detection of disease is a high priority. To meet this diagnostic challenge, the detection technology will use a polymer that exhibits intrinsic amplification of fluorescence transduction events to rapidly identify species-specific genomic, proteomic, and lipo-oligosaccharide (LOS) markers of *Y. pestis*. This amplifying fluorescent polymer (AFP) will be fabricated as nanoparticles and functionalized for covalent attachment of quencher-labeled molecular or aptamer beacon probes, which will trigger amplified fluorescent responses when binding of the target to the probe causes dequenching of the polymer. We are confident that the amplification afforded by AFP will enable detection of target analytes in extremely low concentrations with minimal sample preparation, thus providing significant advantages over current microbiological and molecular diagnostic methods. Once proof of concept is established using standard microarray technology, the diagnostic platform will be integrated into a real-time, low-density, multi-locus array printed in a membrane sample delivery system that ultimately will be used to identify and discriminate between many different BW pathogens.

Sponsor: National Institute for Allergies and Infectious Disease, Nomadics, Inc.
PIs: Ken Clinkenbeard, Jerry Malayer

Ruminant B-Lymphocyte Yellow Fluorescent Protein Aggregation Bioassay for Elk Chronic Wasting Disease

The goal of the proposed research is to develop a cell culture model for elk chronic wasting disease (CWD) prion propagation that can be used as a bioassay for detecting CWD. CWD is a transmissible spongiform encephalopathy (TSE), caused by the mis-folding of a normal cell surface prion protein (PrP^{c}) through the interaction with infectious mis-folded and protease resistant prion protein (PrP^{res}). The PrP^{res} specific to CWD is PrP^{cwd}. CWD occurs in free ranging and captive elk and deer herds in several Rocky Mountain and Plains states. Although there is evidence suggesting that CWD cannot be transmitted to humans, this potential has not been thoroughly ruled out. Current infectivity bioassays involve the use of live animals, whereas the proposed cell culture model will reduce the need for animal experimentation to study the mechanism of prion infectivity and disease. In addition, a cell culture will be developed as an assay for the screening of infectious prions in veterinary medical samples from elk and deer. This assay system also has the potential for use in assessing therapeutic strategies. Our goal will be accomplished by bioengineering a bovine B-lymphocyte cell (B-cell) line to surface express elk PrP^{c} fused to yellow fluorescent protein (YFP). Like other glycosyl-phosphoinositol (GPI) anchored proteins, surface expressed YFP-PrP^{c} will have a dispersed distribution on these B-cells. Interaction of these B-cells with infectious, mis-folded, protease-resistant CWD prion (PrP^{cwd}) will induce conversion of the dispersed YFP-PrP^{c} to aggregated mis-folded YFP-PrP^{cwd} that will be detected by confocal microscopy as aggregated YFP-PrP^{cwd} on the B-cell surface.

Sponsors: US Army Research Office, Nomadics, Inc.

PIs: Ken Clinkenbeard, Jeff Blair

Shipping Fever: New Approaches to Understanding Prevention and Management

The objective is to determine infectious agents and host responses that cause respiratory disease and/or defend cattle from disease. Naturally occurring cattle diseases at Noble Foundation, privately owned, USDA, and OSU herds are being investigated. Emphasis is primarily on bovine viral diarrhea virus infection and pasteurellosis. Vaccination programs are being evaluated and new recommendations given.

Sponsor: The Noble Foundation, Ardmore, OK

PIs: Anthony W. Confer, S. Mady Dabo, Robert W. Fulton, Jerry W. Ritchey

Department of Veterinary Clinical Sciences: John G. Kirkpatrick, Robert A. Smith

Bovine Respiratory Disease: Risk Factors, Pathogens, Diagnosis, and Management

The project determines changing patterns, geographical differences, risk factors, and management practices related to bovine respiratory disease. The influence of various bacteria and viruses is studied. In addition, the pharmacokinetics and efficacy of newer therapies and new generation vaccines are evaluated. The host-pathogen relationship is characterized at the molecular level.

Sponsor: Oklahoma Agricultural Experiment Station
PIs: A. W. Confer, R. W. Fulton, R. J. Panciera, K. D. Clinkenbeard, R. J. Morton

***Mannheimia haemolytica* Outer Membrane Protein PlpE: Characterization of Epitopes Stimulating Homologous and Heterologous Serotype Protection**
This project is a molecular and immunologic approach to studying an immunologically important outer membrane protein of *M. haemolytica.* It compares the PlpE protein from serotypes 1, 2, and 6 with respect to important epitopes for immunity.
Sponsor: USDA CSREES, National Research Initiative Competitive Grant
PIs: A. W. Confer, Sahlu Ayalew

***Mannheimia haemolytica* Bacterin-Toxoid Efficacy Studies**
This project studies the efficacy of commercial *M. haemolytica* vaccines in an experimental challenge model.
Sponsor: Pfizer Animal Health, Lincoln, Nebraska
PIs: A.W. Confer, R.J. Panciera

Chimeric Vaccine for *Mannheimia haemolytica* Infection in Cattle
Recombinant DNA technology is being applied to induce a single chimeric protein that will stimulate immunity in cattle to *M. haemolytica* leukotoxin and the outer membrane.
Sponsor: Oklahoma Applied Research Program. Oklahoma Center for the Advancement of Science and Technology (OCAST).
PIs: A.W. Confer, Sahlu Ayalew

***Pasteurella multocida* OmpA: Functional Characterization**
This project studies the adherence and colonization properties of *Pasteurella multocida* OmpA. It is designed to investigate the specific role of *P. multocida* OmpA in the bacterium interaction with host cells and in the pathogenesis of the disease.
Sponsor: USDA CSREES, National Research Initiative Competitive Grant
PIs: S.M. Dabo, AW. Confer

Development of a Vaccine against *Ixodes scapularis* Infestations
Antigens identified in previous studies to induce protection against *Ixodes scapularis* tick infestations in mice will be used in vaccine formulations. The vaccine will be tested against the three tick stages: larvae, nymphs, and adults.
Sponsor: Pfizer Animal Health, Inc.
PIs: José de la Fuente, Katherine M. Kocan, Edmour F. Blouin, Consuelo Almazán

Molecular Diagnostic Tests for Simian Herpesviruses
The major danger to research and animal care personnel working with rhesus monkeys is monkey B virus (BV), a herpes virus that is transmitted by bites and scratches and is rapidly fatal in humans if not detected and treated rapidly. This project involves molecular characterization of BV and related viruses of other primates and application of these data to develop more sensitive and specific diagnostic tests that will permit rapid and reliable identification of BV infections in humans.
Sponsor: NIH, NCRR
PI: R. Eberle

Baboon Research Resource Program
Baboons are an important animal species used in biomedical research. This program will develop a breeding colony of baboons in Oklahoma and supports research aimed at improving the breeding efficiency of baboons in captivity, defining viruses that naturally infect baboons, and improving the basic well-being and behavior of captive-bred baboons.
Sponsor: NIH, NCRR
PIs: R. Eberle, A. Kocan, J. d'Offay
OUHSC: Gary White

Development of an SPF Baboon Colony
Indigenous viruses can have a major adverse effect on the results of biomedical research studies using animals, particularly where immunosuppression is involved. This program supports derivation of a colony of baboons that are free of all known herpesviruses and most retroviruses.
Sponsor: NIH, NCRR
PI: R. Eberle
OUHSC: Gary White

Bovine Viral Diarrhea Disease Virus (BVDV) Vaccines: Antibody Response to Heterologous BVDV Strains
The study will determine the range of heterologous immunity in calves receiving modified live virus (MLV) or killed BVDV vaccines. Currently there are two recognized antigenic types of BVDV, Type 1 and 2. The study will determine if these vaccines induce antibodies to various Type 1 and 2 viruses.
Sponsors: Grand Laboratories, Inc., Pfizer Animal Health
PIs: Robert W. Fulton, Anthony W. Confer

Genetic and Antigenic Variability of BVDV in Cattle Infections
Bovine viral diarrhea viruses (BVDV) isolates from the Oklahoma Animal Disease Diagnostic laboratory (OADDL) will be obtained from clinical/necropsy cases. The viruses will be typed as BVDV 1a, 1b, or 2. Potentially there will be additional typing and/or groups. Field isolates from naturally occurring disease including persistently infected (PI) cattle will be compared with vaccinal stains and standard reference strains. A phylogenetic survey of the BVDV subtypes from the field isolates, vaccinal strains, and reference strains will be performed to detect relationships among the virus and their genetic stability. Neutralization tests will be performed to compare the subtypes to the vaccinal strains. Potentially new subtypes may warrant additional subtypes in the vaccines.
Sponsor: Oklahoma Agricultural Experiment Station
PIs: Robert W. Fulton, A. W. Confer
Oklahoma Animal Disease Diagnostic Laboratory: J.T. Saliki

Antibiotic Administration and Vaccination with Live Bacterial Vaccine in Calves
This study will determine if an antibiotic given calves that have been administered avirulent *Pasteurella haemolytica* and *Pasteurella multocida* vaccine will decrease the immune responses to the immunogens. Calves will receive Micotil antibiotic and Once PMH *Pasteurella haemolytica* and *P. multocida* vaccine. The calves' sera will be tested for *P. haemolytica* and *P. multocida* antibodies.
Sponsor: ELANCO Animal Health, Division of Eli Lilly and Company

PIs: Robert W. Fulton, Anthony W. Confer

Molecular Diversity of Bovine Viral Diarrhea Viruses from Oklahoma Cattle
Bovine viral diarrhea viruses (BVDV) occur as biotypes, cytopathic (CP), and noncytopathic (NCP), and as genotypes 1 and 2. Certain BVDV disease forms occur with different biotypes/ genotypes. The molecular differences among biotypes/genotypes will be investigated by PCR and nucleic acid sequencing. Virulence markers of BVDV will be investigated.
Sponsor: Oklahoma Agricultural Experiment Station
PIs: Robert W. Fulton, Jean M. d'Offay, Anthony W. Confer, Jerry W. Ritchey
Oklahoma Animal Disease Diagnostic Laboratory: Jeremiah T. Saliki

Evaluation of Viral Vaccine Containing Infectious Bovine Rhinotracheitis Virus (IBRV), Bovine Viral Diarrhea Virus 1 and 2 (BVDV), Parainfluenza -3V (PI-3V), and Bovine Respiratory Syncytial Virus (BRSV) in Preventing Infection and Respiratory Disease in Cattle
The purpose of the study will be to determine if pre-weaning vaccination of ranch calves with viral vaccine: (1) reduces respiratory disease, and (2) reduces transmission of viruses in calves moved from auction markets and commingled with the fresh calves under feedlot conditions.
Sponsor: Fort Dodge Animal Health
PIs: Robert Fulton, A.W. Confer
Oklahoma Animal Disease Diagnostic Laboratory: J.T. Saliki
Department of Veterinary Clinical Sciences: D.L. Step

Vaccination of Ranch Calves with Modified Live Viral Vaccine: Effects on Viral Transmission on Commingled and Transported Calves
This study will determine if vaccination of ranch calves with a modified live viral vaccine will reduce respiratory disease and reduce transmission of viruses in the calves commingled with auction calves and moved to a feedlot.
Sponsor: Schering Plough Animal Health Corp.
PIs: Robert W. Fulton, Anthony W. Confer

Food Safety: Farm to Table
The long-term objectives are to develop methods for assuring the microbial safety of the food supply from farm to table. Focus is on control of *Salmonella* species and *Escherichia coli* O157:H7 associated with swine and cattle.
Sponsor: USDA/CSREES
PIs: Terry Lehenbauer
College of Agricultural Sciences and Natural Resources: Stanley Gilliland, Guolong Zhang, Peter Muriana, Siobhan Reilly

Prototype FSU Sensor Testing
Sensor technologies that have been developed in the former Soviet Union to detect biological threat agents are being evaluated for potential use in a fully integrated warning and response system to protect individuals, property, and the environment from the threats of accidental or terrorist release of biological agents. Our laboratory is involved in the selection, developmental assessment, and testing of such prototype detection systems.
Sponsor: General Atomics

PI: Rebecca J. Morton

Murine Model for Monkey B Virus Infection
Non-human primates are an integral part of biomedical research programs, particularly as animal models of human disease. As long as monkeys are used for research, zoonotic infection by simian viruses will continue to be an occupational hazard. Human infections by monkey B virus, a herpesvirus of macaques involve the central nervous system, when left untreated are usually fatal. The goal of this project is to establish and characterize, both pathologically and immunologically, a murine model for B virus infections. Establishment of a well characterized murine model system for B virus infection will serve as an important resource in which hypothesis-driven studies can be performed such as evaluation of anti-viral drugs, challenge assessment of potential vaccines, and investigation of the role of various viral genes/proteins in determining the pathogenic properties of these viruses.
Sponsor: National Institutes of Health
PI: Jerry W. Ritchey

A Study of the Viral, Bacterial, Mycologic, and Toxicologic Conditions Associated with Marine Mammal Strandings in the Gulf Coast of Mississippi and the Central Gulf Coast of Mexico
This is a comprehensive two-year study aimed at identifying the causes of stranding and death among dolphins in the Gulf coast of Mississippi. Viruses, bacteria, fungi, and toxins affecting dolphins in this region will be determined and detailed pathological lesions from dead dolphins will be described.
Sponsor: National Marine Fisheries Service (Prescott Grant Program)
PIs: Jeremiah T Saliki, Uriel Blas-Machado, Sandra Morgan, Ronald D Welsh
Institute of Marine Mammal Studies: Moby A. Solangi

On Watch for West Nile Virus in Oklahoma
This project aims to detect West Nile virus (WNV) in wild birds and use the information to assess the risk of West Nile to human health in various counties. There is an established correlation between intensity of WNV activity in wild birds and increased risk of human WNV infections.
Sponsor: Oklahoma State Department of Health
PI: Jeremiah T. Saliki

Functional Genomic/Proteomic Analysis of *Campylobacter* spp.
Campylobacter is the most common agent of gastroenteritis infection in humans. Despite an intensive research effort on understanding *Campylobacter* pathogenesis, conclusions on the exact mechanism of infection are extremely difficult to draw. This project proposes to investigate the mechanism of *Campylobacter* pathogenesis using functional genomic tools.
Sponsor: National Institutes of Health
PI: Alain Stintzi
Oklahoma University Health Science Center: John Iandolo

Response of *Campylobacter jejuni* to Host Temperature
The major goals of this project are to study the mechanism of *C. jejuni* adaptation to various host temperatures and characterize the interaction of *C. jejuni* with the chicken

gastrointestinal tract. This work will shed some light on the biology of bacterial adaptation to temperature changes and its role in the colonization and infection processes.
Sponsor: Oklahoma Center for the Advancement of Science and Technology
PI: Alain Stintzi

***Campylobacter C*olonization and Virulence Determinants**
The major goal of this project is to develop and use novel functional genomic tools to identify some of the *Campylobacter* colonization and virulence determinants. The identification of these *in vivo Campylobacter* determinants could significantly contribute to the development of more effective methods to diagnose, manage, and ultimately prevent *Campylobacter* infections.
Sponsor: National Institutes of Health
PI: Alain Stintzi

Cytokine Expression in Response to *Brucella* Vaccines
Cytokine responses induced by *Brucella* polysaccharides in blood cells from vaccinated and unvaccinated cattle and mice, unvaccinated pigs and humans were evaluated *in vitro*. Reverse transcriptase-polymerase chain reaction was used to detect cytokine mRNA. Development of a cytokine diagnostic profile indicative of induction of immunity for application to human vaccinates was attempted.
Sponsor: Canada Department of National Defense
PI: John H. Wyckoff III

Bovine T Lymphocyte Immunity to *Mycobacterium bovis* Stress Response Proteins
Synthetic peptides with sequences homologous to *Mycobacterium bovis* proteins will be used to develop better test reagents for field and laboratory diagnosis of bovine tuberculosis. Cattle immunized with *M. bovis* will be compared for responsiveness to these antigens and those of *M. avium* to determine both specific and cross-reactive responses.
Sponsor: Oklahoma Agricultural Experiment Station
PIs: John H. Wyckoff III
Department of Biochemistry and Molecular Biology: Richard Essenberg

Effector T Lymphocytes Provide Host Defense in Bovine Brucellosis
Heat shock protein-specific T lymphocytes derived from *Brucella abortus* vaccinated cattle will be characterized by flow cytometry for surface marker expression and cytotoxic function against infected monocyte-derived macrophages. Cytokine production by the T lymphocytes will be analyzed through RT-PCR. These studies will define a host defense effector mechanism against brucellosis.
Sponsor: United States Department of Agriculture
PIs: John H. Wyckoff III, Anthony W. Confer

DEPARTMENTOF PHYSIOLOGICAL SCIENCES

Active Site Studies of Human Sulfotransferases
The major goals of this project are to elucidate human sulfotransferase (SULT) chemical and kinetic mechanisms, to understand physiologic functions of SULTs, and to investigate their relevance to human health in physiologic and pathologic conditions. Research focus on: 1) mechanisms of enzyme catalysis, substrate inhibition, and product activation of

human SULTs; 2) kinetic characterization of polymorphic allozymes; and 3) oxidative regulation mechanisms of human SULTs.
Sponsors: National Institute of Health, Oklahoma State University
PI: Guangping Chen

Sulfotransferase Induction Mechanisms
Studies in this project focus on Sulfotransferases (SULTs) induction and their induction mechanisms. Rats, human hepatic carcinoma cell line (Hep G2), and human intestinal carcinoma cell line (Caco-2), will be used for these studies. Enzyme activity assay, Western blot, reverse transcription polymerase chain reaction (RT-PCR), site-directed mutagenesis, plasmid transfection, and small interfering RNA (siRNA) gene silencing methods will be used to determine the induction of SULTs and nuclear receptor-mediated SULT induction mechanisms.
Sponsor: Oklahoma State University
PI: Guangping Chen

Molecular Characterization of a Multidrug Resistance Mechanism Expressed by *Mannheimia haemolytica*
This research explores the involvement of a putative porin-mediated multidrug resistance system to survival of *Mannheimia haemolytica in vivo*, in the absence of antibiotics or in the presence of subtherapeutic and therapeutic levels of antibiotics.
Sponsor: Oklahoma Agricultural Experiment Station
PI: Cyril Clarke

In Vivo Emergence and Survival of MDR Salmonella
The long term goal of the project is to evaluate the contribution of the mar regulon to survival of Salmonella serovar *Typhimurium in vivo*, in the absence of antibiotics or in the presence of subtherapeutic and the therapeutic levels of antibiotics.
Sponsor: OCAST Health Research
PI: Cyril Clarke

Novel Biosensor for Detecting Antibiotic Resistance
The objective of this research is to develop a novel detection system utilizing an amplifying fluorescent polymer for rapid, highly sensitive, and selective detection of methicillin-resistant *Staphylococcus aureus* present in clinical exudates without prior culture and isolation of bacteria.
Sponsor: Nomadics, Inc/NIH
PIs: Cyril Clarke, Jerry R. Malayer

Regulation of Sperm Exocytosis
Mammalian sperm require a final maturation after ejaculation before they can fertilize eggs. This project examines the role of sterols and phospholipids in the control of intracellular pH and sperm fertilizing ability.
Sponsor: Oklahoma State University
PI: Nicholas L. Cross

Exercise-Induced Airway Injury in Horses

Similar to humans, it is possible that horses damage the lining of their airways during cold weather strenuous exercise. The goals of this project are to determine extent of airway mucosal injury secondary to cold weather exercise in horses, and identify medications that can decrease or prevent this injury.
Sponsors: United States Department of Agriculture, Oklahoma Agricultural Experiment Station, Thoroughbred Charities of America, National Institutes of Health
PIs: Michael S. Davis, Jerry Malayer, Katherine Williamson, Erica McKenzie
Animal Health Trust, Newmarket, United Kingdom: David Marlin
Department of Statistics: Mark Payton
Department of Veterinary Clinical Sciences: Todd Holbrook
Department of Clinical Sciences, Duke University: Wm. Michael Foster

Exercise-Induced Mucosal Dysfunction in Racing Sled Dogs
Racing sled dogs have a high prevalence of gastrointestinal and respiratory disease related to strenuous exercise. The goals of this project are to determine the causes of exercise-induced mucosal disease and identify methods of eliminating the disease in sled dogs, with an added goal of extending these findings to human athletes.
Sponsors: National Institutes of Health, Defense Advanced Research Projects Administration
PIs: Michael S. Davis, Katherine Williamson, Erica McKenzie
Department of Veterinary Clinical Sciences: Todd Holbrook
Texas A&M University: Michael Willard
Ohio State University: Kenneth Hinchcliff
Iditarod Trail Committee: Stuart Nelson, Jr.
Johns Hopkins University: Terence Risby

Molecular Mechanisms of Lung Surfactant Secretion
Lung surfactant is a surface-active material that stabilizes alveoli. It is synthesized and secreted by lung epithelial type II cells. The long-term objective of this proposed project is to elucidate the molecular mechanisms of lung surfactant secretion from type II cells including transport of secretory granules to and fusion with the plasma membrane. Deficiency of lung surfactant is the cause of respiratory distress syndrome in premature infants. Accomplishing the goals of this proposal may give a valuable insight to the therapy of pulmonary diseases such as neonatal respiratory distress syndrome.
Sponsor: National Institutes of Health
PI: Lin Liu

Mechanisms of Alveolar Epithelial Cell Differentiation
The goal of this grant is to understand molecular mechanisms of the differentiation of alveolar epithelial cells in isolated alveolar epithelial cells, fetal lung development, repair of injured lungs using in-house made 10K rat gene arrays, and RNA interference.
Sponsor: National Institutes of Health
PI: Lin Liu

Regulation of Lung Surfactant Secretion by Nitric Oxide
The goal of this project is to investigate NO signaling that leads to changes in lung

surfactant secretion. Two specific aims are proposed in the present application: 1) NO at low concentrations enhances lung surfactant secretion by stimulating guanylyl cyclase, raising intracellular cGMP levels and activating cGMP-dependent protein kinas; and 2) excess NO inhibits lung surfactant secretion by inactivating a-SNAP, a docking/fusion protein involved in lung surfactant secretion, via S-nitrosylation and/or nitration of the protein.
Sponsor: OCAST
PI: Lin Liu

Lung Epithelial Cell Differentiation and Oxidative Stress
The goal of this grant is to study the effect of oxidative stress on the differentiation of lung epithelial cells using DNA microarray
Sponor: American Heart Association
PI: Lin Liu

Minority Postdoctoral Supplement
The goal of this grant is to provide an opportunity for a minority postdoctoral fellow to participate in ongoing research projects and for career development experiences in preparation for an independent career in a health-related science.
Sponor: National Institutes of Health
PI: Lin Liu

Quantitative DNA Microarray: Application in Cardiopulmonary Diseases
The goal of this predoctoral fellowship is to develop a quantitative DNA microarray.
Sponor: American Heart Association
PI: Zhongming Chen

Characterization and Functional Studies of GABA-A Receptors in Alveolar Epithelial Cells
The goal of this predoctoral fellowship is to characterize GABA-A receptors and study their functions in lung epithelial cells.
Sponsor: American Heart Association
PI: Nili Jin

Nuclear Receptor Gene Expression in the Bovine Preimplantation Embryo Produced *In Vitro*
Objectives include the isolation of estrogen- and retinoic acid-responsive gene products in cattle from the developing fetal reproductive tract, adult female reproductive tract, and preimplantation embryo.
Sponsor: Oklahoma Agricultural Experiment Station
PI: Jerry R. Malayer

Development of Signal Amplification Technology for Detection of *L. monocytogenes*
Investigators from the College of Veterinary Medicine and Nomadics, Inc. are collaborating on a research project to develop rapid methods for detection of *Listeria monocytogenes* and other food-borne pathogens.
Sponsor: Nomadics, Inc., Oklahoma Center for the Advancement of Science and

Technology
PI: Jerry R. Malayer

Fluorescent Polymer Nanoparticle Gene Expression System
Investigators from the College of Veterinary Medicine and Nomadics, Inc. are working to use amplifying fluorescent polymer (AFP) nanoparticles to improve the sensitivity of nucleic acid hybridization assays by several orders of magnitude. This will directly impact gene expression studies by improving sensitivity and enabling quantitative comparison with reference samples.
Sponsor: Nomadics, Inc., National Institutes of Health
PI: Jerry R. Malayer

Biological Point Detection Based on the Amplifying Fluorescent Polymer Platform
Investigators from the College of Veterinary Medicine and Nomadics, Inc. are working to use amplifying fluorescent polymer (AFP) films and particles to improve the speed and sensitivity of nucleic acid hybridization-based detection assays by several orders of magnitude. This will directly impact detection of biological materials in the environment by improving sensitivity.
Sponsor: U.S. Army, Nomadics, Inc.
PI: Jerry R. Malayer

Electron Microscopy Laboratory
The Electron Microscopy Laboratory provides instruction in electron microscopy at the graduate level and offers a wide range of microscopy services including sample preparation, ultrathin sectioning, transmission and scanning electron microscopy, X-ray microanalysis, and confocal microscopy. Researchers may have all of the work done by lab personnel or do some or all of it themselves using lab equipment.
Sponsors: Oklahoma State University
PIs: Charlotte L. Ownby, Phoebe Doss, Terry Colberg

Presynaptic Modulation of Anticholinesterase Toxicity
The project evaluates contribution of presynaptic neurochemical mechanisms (e.g., in particular neurotransmitter release) in differential toxicity of organophosphorus insecticides.
Sponsor: National Institute of Environmental Health Sciences
PIs: Carey Pope, Jing Liu Pope, Lin Liu

Stress, Organophosphates, and Blood Brain Barrier Permeability
The project evaluates the ability of physical and chemical stressors to modify blood brain barrier permeability and the neurotoxicity of anticholinesterases.
Sponsor: United States Army
PIs: Carey Pope
Kennedy Krieger Institute, Johns Hopkins University: Joseph Bressler
LSU Health Sciences Center: Steve Pruett

DEPARTMENT OF VETERINARY CLINICAL SCIENCES

Analysis of Dogs with Sudden Acquired Retinal Degeneration Syndrome (SARDS)

SARDS is an acute retinal degenerative disorder of unknown etiology. It shares some similarities with cancer associated retinopathy in humans. Clinical evaluation of dogs with SARDS has included computed tomography, ultrasonography, and radiographic studies. The serum of SARDS dogs is being analyzed by Western blot for the detection of anti-retinal antibodies.
Sponsor: NIH
PIs: Dr. Margi Gilmour
OUHSC: Dr. Robert Anderson

College of Veterinary Medicine Biomedical Laser Laboratory
Since the establishment of the Biomedical Laser Laboratory within the College of Veterinary Medicine, research to establish protocols for clinical applications in veterinary medicine has been a primary objective. In addition, the use of laboratory models has resulted in transfer of technology to both industry and human medicine. Work will continue concentrating on the clinical applications of biomedical lasers coupled with collaborative research protocols involving basic scientists (engineers, physicists) and clinicians.
Sponsor: McCasland Foundation, Mercy Works Foundation
PI: Kenneth E. Bartels

Indocyanine Green and Immunoadjuvant in Selective Hyperthermia for Treatment of Mouse Melanoma
Hyperthermia has been proposed as a potential modality for cancer treatment due to the fact that tumor cells are more sensitive to temperature increase than normal tissue. Thermal effect alone often cannot achieve complete tumor eradication due to the incomplete malignant cell destruction since hyperthermia treatment alone often cannot discriminate normal tissue and cancerous tissue. Using *in situ* injected indocyanine green (ICG) and irradiation of 805-nm laser light, selective hyperthermia can be achieved. For increased selectivity, glycated chitosan (GC), a novel immunoadjuvant, will also be used with ICG for tumor injection, followed by the laser irradiation. Tumor profiles and mouse survival rates will be used to determine the effectiveness of the treatment.
Sponsor: Barbour Foundation
PIs: Kenneth E. Bartels
University of Central Oklahoma: Wei Chen

Photodynamic Therapy in Combination with Chemotherapy and Immunotherapy for Treatment of Metastatic Mammary Rat Tumors
Photodynamic therapy (PDT) can be an effective means for direct tumor destruction through its selective photochemical reaction. A common sensitizer used in PDT is 5-aminolevulinic acid (ALA). By itself, PDT using ALA can be effective in some cases, but not effective in others. To address this inconsistency, combinations of several drugs can result in synergistic or additive effects for certain types of cancer such as mammary carcinoma. Combining multiple tumor treatment modalities may create a type of “cocktail therapy” that could be successful for primary and metastatic disease caused by some types of cancer in both animals and human beings. It is hypothesized that the combination use of PDT and the glycated chitosan (GC) immunoadjuvant may significantly improve the efficacy of the treatment, particularly in treating metastatic tumors. It is further hypothesized that the concurrent use of PDT and a new chemotherapeutic agent, AEADA

(Bis-1,4 aminoethylamino-5,8-dihydroxyanthraquinone·2HCl), may enhance the systemic effect of cancer treatment. Combinations of these treatment modalities will be evaluated in the rat mammary tumor model.
Sponsor: Kleberg Foundation
PIs: Kenneth E. Bartels
University of Central Oklahoma: Wei Chen

Determination of Bloodflow of Aqueous Solutions Following Intramuscular Injection in the Green Iguana (*Iguana iguana*)
Reptiles have renal portal systems that conduct substances that are injected intravenously in the caudal portion of the body directly to the kidneys, thereby possessing an increased risk of organ damage with nephrotoxic substances. This study was undertaken to determine if the same was true for intramuscular injections using nuclear scintigraphy in a green iguana model.
Sponsor: College of Veterinary Medicine
PIs: Armando Burgos, John P. Hoover, Tawnia Zollinger

Pharmacokinetics of Single Dose, Topical Silamectin in America Bullfrogs (*Rana catesbeiana*)
Internal parasites are an important health management problem for captive amphibians. This study was undertaken to determine the pharmacokinetics of a single dose of the antiparasitic drug silamectin applied topically at an empirical dose in a bullfrog model and to assess if there were local or systemic adverse effects.
Sponsors: Oklahoma City Zoo, College of Veterinary Medicine
PIs: Jennifer D'Agostino, John P. Hoover
Oklahoma City Zoo: Gary West

Thyroid Hormone Levels in Captive Galapagos Tortoises (*Geochelone elephantopus*)
Hypothyroidism has been suspected in Galapagos tortoises that develop non-pitting myedema of the head and neck, and extremities. This study was undertaken to determine the thyroid hormone levels of affected and normal Galapagos tortoises.
Sponsor: Oklahoma City Zoo
PIs: Cynthia L DiGesualdo, John P. Hoover
Oklahoma City Zoo: Gary West
Oxford Biomedical Research: Thomas R Brown

Interactions of Viral Infection and Nitric Oxide within the Reproductive Tract of Horses and Cattle
This study includes investigations into the impact of viral infection on nitric oxide (NO) production within reproductive tract tissues. We are using animal disease models such as equine viral arteritis in the mare and bovine viral diarrhea virus in the cow, to investigate the mechanisms of viral infection within the ovary and how those interactions contribute to the pathogenesis of disease and the subsequent clearance of virus by the innate nitric oxide response to infection.
Sponsor: Oklahoma Agriculture Experiment Station
PIs: G. Reed Holyoak, Anthony W. Confer, Randy S. Lewis

Equine Arteritis Virus in the Persistently Infected Stallion
Investigations into differential density gradient centrifugation plus swim-up using various protocols will eliminate equine arteritis virus (EAV) from sperm of persistently infected stallions and whether this purified sperm can be artificially inseminated into naïve mares without risk of disease transmission to mare or offspring. We are also investigating through immunohistochemistry the location of EAV on equine spermatozoa and within semen fractions.
Sponsor: OSU College of Veterinary Medicine Summer Research, Bullock Endowed Chair for Equine Theriogenology
PI: G. Reed Holyoak

Investigations into Testicular Lesions in Adult Stallions
We are looking at the relationship of testicular lesions as found via micropathology compared to the numerical pixel value obtained via ultrasonographic examination.
Sponsor: Oklahoma Animal Disease Diagnostic Laboratory, Bullock Endowed Chair for Equine Theriogenology
PI: G. Reed Holyoak

Investigations into Acute Phase Proteins in the Equine Neonate
These investigations are determining the concentration of serum amyloid A (SAA) milk amyloid A (MAA) and C reactive protein (CRP) in equine colostrum and milk and investigate whether these proteins may be absorbed in the neonatal foal.
Sponsor: OSU College of Veterinary Medicine RED Account
PI: G. Reed Holyoak

Normal Blood Thiamine Levels of African Lions (*Panthera leo*) as Fed in North American Zoos
Thiamine (Vitamin B_1) deficiency may occur in large cats that are fed inappropriate diets resulting in neurologic signs that may include ataxia and seizure-like episodes. This study was undertaken to establish reference values for African lions held in North American zoos that were eating appropriate diets.
Sponsor: Department of Veterinary Clinical Sciences
PIs: John P Hoover, Cynthia L DiGesualdo

Effect of Vaccination Protocol on Virus/Bacteria-Specific Serum Antibody Level at Feedlot Entry, Morbidity and Mortality Rate, Productivity, and Carcass Value in Beef Calves – A Three-Year Study
The purpose of this study is to compare antibody response, morbidity and mortality rates, productivity, and carcass value of cattle vaccinated at different processing times, approximately 60 days of age, 30 days pre-weaning, and weaning, with a multi-valent modified live virus vaccine and *Mannheimia haemolytica / Pasteurella multocida* bacterin/ leukotoxoid.
Sponsor: Noble Foundation
PIs: John G. Kirkpatrick, D. L. Step

Development of an *In-Vivo*, Near-Real-Time Radiation Sensor for Use in Radiotherapy

Using thin silica fibers to transmit laser light, the radiation sensors might be able to detect and accurately measure the exact dose of radiation being delivered to a patient as that patient undergoes radiation therapy for cancer or other diseases. The measurement would take place during the radiation therapy procedure itself.
Sponsors: The Center for Sensors and Sensor Technology (CSST), The Oklahoma Center for Advancement of Science and Technology (OCAST)
PIs: R.J. Bahr
College of Arts and Sciences: S.W.S. McKeever

Comparison of Two Techniques of Uterine Tubal Occlusion in the Mare
For research purposes, and occasional clinical use, it is necessary to occlude either one or both uterine tubules in the mare. We are developing a method using minimal invasive surgery. Using a laser, the uterine openings to the tubes will be blocked.
Sponsor: Research Advisory Council
PI: David Moll

Photodynamic Inactivation of a RNA-Enveloped Virus in Goat Colostrum
This project will investigate the use of photodynamic inactivation to inactivate bovine viral diarrhea virus (BVDV) in goat colostrums. Photodynamic inactivation (PI) is the use of phenothiazine dye derivatives and fluorescent light to break up the viral genome. The anti-viral properties of phenothiazine dye derivatives have been known about for years. The use of methylene blue (phenothiazine dye derivative) and fluorescent light has, and is currently being used, to decontaminate human blood products. This procedure is proven effective in inactivating viruses such as HIV and hepatitis in human plasma and washed red cell suspensions.
Sponsor: CVM Office of Research
PIs: Kevin Washburn, Robert Streeter
Oklahoma Animal Disease Diagnostic Laboratory: Jeremiah T. Saliki
Veterinary Pathobiology: Terry W. Lehenbauer, Maria E. Prado

Evaluation of Enlarged Peripheral Lymph Nodes of the Bovine by Antemortem Tru-Cut Biopsy, Impression Smear, and Fine Needle Aspirate
Adult bovine with enlarged peripheral lymph nodes are frequently encountered in the day-to-day case load of food animal medicine. Malignant lymphoma is a common disease that presents as enlargement of the peripheral lymph nodes. However, a definitive test to determine whether the enlargement is due to neoplasia or inflammation is currently unavailable. In dogs, fine needle aspirates of an enlarged lymph node can be diagnostic of neoplasia. However, in the bovine, differentiating neoplastic from inflammatory lymph nodes from a fine needle aspirate is difficult. We have collected 25 cases over the last few years of cattle presented with enlargement of peripheral lymph nodes. Only animals diagnosed with a terminal illness (severe pneumonia, peritonitis, lymphoma etc.) were enrolled so they could be followed and eventually necropsied. Tru-cut biopsies, impression smears, and fine needle aspirates were obtained from each animal. A blinded anatomic and clinical pathologist examined these tissues and interpreted them as normal, inflammatory, or neoplastic. These interpretations will be compared to final necropsy results to determine the ability of any of these modalities to predict whether an animal with enlarged peripheral lymph nodes actually has malignant lymphoma.
Sponsor: CVM Office of

Research
PIs: Kevin Washburn, Robert Streeter
Department of Pathobiology: Tim Snyder, Jim Meinkoth

Pathologic Changes Associated With Injectable Carprofen in Pigeons (*Columba livia*)
Post operative pain management has become an important consideration in avian species. This study was undertaken to determine if there were any pathologic changes on necropsy and histologic examination following single and repeated injections of the nonsteroidal analgesic caprofen at an empirical dose.
Sponsor: College of Veterinary Medicine
PIs: Tawnia Zollinger, John P Hoover, Armando Burgos

Oklahoma Animal Disease Diagnostic Laboratory
The Oklahoma Animal Disease Diagnostic Laboratory provides accessible and accountable diagnostic service for Oklahoma veterinarians and animal owners in all 77 counties. Early detection of diseases provides the starting point for reducing their incidence and threat. The Laboratory also acts as a frontline sentinel for new and emerging diseases. OADDL promotes and protects the health and economic welfare of Oklahomans, supports the teaching and research missions of the OSU College of Veterinary Medicine, and conducts self-supported research aimed at developing more precise test procedures for commonly encountered, as well as emerging and foreign animal diseases, that may produce catastrophic losses (e.g., bovine viral diarrhea, malignant catarrhal fever, parvovirus disease, avian influenza, equine viral arteritis and encephalitis, and toxicoses related to oilfield wastes and agricultural chemicals). The Laboratory conducts research and diagnostic tests for morbillivirus infections of marine mammals. The Laboratory in conjunction with the Oklahoma State Department of Health is involved with surveillance of West Nile fever in Oklahoma horses and birds. The Laboratory maintains full accreditation by the American Association of Veterinary Laboratory Diagnosticians.
PI: Bill J. Johnson and staff