**In Vitro and Animal Models for Emerging Diseases and Biodefense, Part A: “In Vitro Screens for Antimicrobial Activity” - Drug-Screening Contract**

The CVHS is currently using in vitro assays to screen test substances for activity against emerging “infectious agents”. Work involves screening of compounds for antimicrobial activity against several biodefense-related bacterial pathogens and drug resistant strains in Categories A, B, and C. Testing materials are submitted through NIAID. Work also includes screening of a 10,000 compound diversity library for leads on new drugs as well as a natural product marine library. The work performed under this IDIQ contract is part of the NIAID Biodefense Research Resources Program and supports research being conducted by scientists working in this area.

**Sponsors:** NIH/NIAID/DMID, Contract HHSN266200400004I.

**PI/PD:** William W. Barrow, Ph.D., Professor and Sitlington Chair in Infectious Diseases.

**Broad-spectrum Antifolates for Treatment of Drug Resistant Bacillus anthracis - Grant**

This is a multidisciplinary partnership grant involving the Departments of Veterinary Pathobiology (CVHS-VP) and Chemistry at OSU, Sapient Discovery (San Diego, CA), and Lovelace Respiratory Research Institute (LBERI) (Albuquerque, NM). Co-Investigators are Drs. Christina Bourne and Phil Bourne (CVHS-VP), Drs. Darrell Berlin and Richard Bunce (Chemistry/OSU), Dr. Kal Ramnarayan (Sapient), and Dr. Michelle Valderas (LBERI). The goal is to develop a new assemblage of antimicrobials for the treatment of inhalation anthrax. The iterative process will involve a combination of traditional drug design methods, including crystallography, molecular biology and medicinal chemistry as well as in silico discovery tools and animal models.

**Sponsors:** NIH/NIAID/DMID, Grant RO1-AI090685-01, Aug, 2010-July, 2015.

**PI/PD:** William W. Barrow, Ph.D., Professor and Sitlington Chair in Infectious Diseases.

**Commercial Assay for Prion Disease Detection**

The goal of the research is to develop a cervid (white-tailed deer, mule deer, or elk) cell culture model system for prion conversion that can be used for detection of prions. To accomplish this, a library of transformed cervid cell lines was established from primary cultures of brain, spleen, lymph node and bone marrow. Exposure of these transformed cell lines to brain from elk with chronic wasting disease (CWD) demonstrated conversion normal prion protein to mis-folded disease associated prion protein. The goal for this research is to use the cervid cell lines previously selected to develop and optimize a commercial assay for antemortem and environmental detection of CWD prions. Following optimization, the assay will be validated, accuracy and sensitivity determined and compared with currently available assays.

**Sponsors:** Oklahoma Center for the Advancement of Science and Technology Oklahoma Applied Research Support Program

**PI/PDs:** Ken Clinkenbeard
DNA Solutions, Inc: Brandt Cassidy

**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 relationships are characterized at the molecular level.
**Sponsor:** Oklahoma Agricultural Experiment Station  
**PI/PDs:** A. W. Confer, R. W. Fulton, S. Ayalew

**M. haemolytica Chimeric Protein Vaccine for Delivery of Multiple Outer Membrane Protein and Leukotoxin Antigens**

Using immunoproteomic approach, outer membrane protein antigens that are important in stimulating immunity to *M. haemolytica* were identified and sequenced. The genes were then cloned and expressed for functional studies of antibodies to these important antigens. Studies will incorporate new epitopes into chimeric vaccines to stimulate immunity against the bacterium.  
**Sponsors:** USDA CSREES, National Research Initiative Competitive Grant  
**PI/PDs:** A. W. Confer, S. Ayalew

**Comparison of *Mannheimia haemolytica* Isolates from Cattle in Australia with U.S. Isolates**

*Mannheimia haemolytica* is the most common bacterial agent of bovine respiratory disease. This study is examining *M. haemolytica* isolates from across the United States to isolates obtained from Australia, with the goal of determining whether vaccine products licensed for use in the US may be expected to be effective in other geographic regions. Genotyping techniques as well as antigenic comparisons will be made to determine diversity and effectiveness of immune response to the vaccine at neutralizing the isolates.  
**Sponsor:** Pfizer Animal Health  
**PI/PDs:** A. W. Confer, J. D. Taylor

**Prevalence of *Brucella canis* Infection in a Fighting Pit Bull Population**

*Brucella canis*, the causative agent of canine brucellosis, is a zoonotic disease that causes abortion and infertility in dogs and flu-like illness, lymphadenopathy and recurrent fevers in humans. This study proposes to determine the prevalence of *B. canis* infection, using serology and polymerase chain reaction, among a population of >400 fighting pit bulls and associated breeding animals. This could assist in determining the public health risk of dogfighting operations and unregulated dog breeding operations.  
**PI:** Tamara Gull

**Characterization of Tick Genes Involved in the Tick Developmental Cycle and Transmission of the Cattle Pathogen, *Anaplasma marginale***

The vectorial capacity of ticks for *Anaplasma marginale* is most likely dependent upon both tick cell and pathogen interactions that involve pathogen adhesion proteins, tick cell receptors and a series of tick cell proteins that mediate the trafficking of the pathogen throughout ticks. In this research we will identify and characterize genes differentially expressed in tick cells in response to infection with *A. marginale*. These key genes will then be tested as vaccine antigens aimed at reducing the vectorial capacity of ticks for transmission of *A. marginale*.  
**Sponsor:** Oklahoma Agricultural Experiment Station  
**PI/PDs:** OSU Center for Veterinary Health Sciences, Katherine M. Kocan, José de la Fuente, Edmour F. Blouin

**Functional Genomic and Proteomic Analysis of Differential Gene Expression in Tick Cells in Response to Infection with the Cattle Pathogen *Anaplasma marginale***

Control of ticks and tick-borne pathogens by vaccination will avoid the use of acaricides that result in selection of drug-resistant ticks and environmental pollution. We have demonstrated the feasibility of developing vaccines targeted at the reduction of tick infestations and interruption of pathogen
transmission. In this research we will identify genes expressed by tick cells in response to *A. marginale* infection that may prove to be useful in vaccine development.

**Sponsor:** Oklahoma Agriculture Experiment Station, Animal Health Funds  
**PI/PDs:** Center for Veterinary Health Sciences, Jose de la Fuente J, Katherine M. Kocan, Edmour F. Blouin

### Characterization of Tick-protective Antigens and Development of a Vaccine for the Control of Lone Star Tick Infestations on Cattle

The Lone Star tick (LST), *Amblyomma americanum*, is the primary pest of cattle in central and eastern U.S. Four key LST tick protective antigens identified previously will be characterized and tested as vaccine candidate antigens. Control of ticks on cattle, without the use of acaricides, will avoid selection of drug-resistant ticks and environmental pollution, and contribute to the overall reduction of LST populations.

**Sponsor:** USDA – National Research Initiative Competitive Grants Program  
**PI/PDs:** José de la Fuente, Katherine M. Kocan, Edmour F. Blouin

### Acquisition, Cultivation and Characterization of Canine Geographic Isolates of *Anaplasma phagocytophilum* and *Ehrlichia canis*

Canine geographic isolates of *Anaplasma phagocytophilum* and *Ehrlichia canis* will be obtained, isolated in tick cell culture and characterized.

**Sponsor:** Pfizer Animal Health, Inc.  
**PI/PDs:** Kocan KM, Blouin EF, de la Fuente J, Little SE

### Defining the Mechanism of RNA Interference in Tick Cells.

The goal of this study is to identify genes involved in RNA interference (RNAi) in ticks. Genes involved in the tick RNAi machinery will be identified in cultured tick cells and then selected genes will then be tested in ticks. After injection of ticks with complementary double stranded RNA for gene silencing of target genes, the ticks will be allowed to feed to test for the effect of gene silencing on the tick feeding, mortality and reproduction.

**Sponsor:** OSU Center for Veterinary Health Sciences, Research Advisory Committee  
**PI/PDs:** Ruchira Mitra, Katherine M. Kocan, José de la Fuente, Victoria Naranjo, Edmour F. Blouin.

### Walter R. Sitlington Endowed Chair in Food Animal Research

Funds generated by this endowed chair support the overall Tick and Tick-borne Pathogen Vaccine Development Laboratory for the conduct of the various research projects.

**Sponsor:** Center for Veterinary Health Sciences  
**PI/PD:** Katherine M. Kocan

### Proposal for Enhancement of Infrastructure for the National Tick Research & Education Resource (NTRER): Establishment of the NTRER Core Tick Research Facility

A Core Tick Researcher Facility was renovated and established to (1) provide a tick holding facility for ticks collected from the field or being used in research and (2) to provide critical equipment for tick research.

**Sponsor:** Office of the Vice President for Research, Oklahoma State University  
**PI/PDs:** Kocan KM, Shaw E, Dillwith J, Jaworski D, Reiskind M, Talley J.

### 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  
**PI/PDs:** R. Eberle, J. d’Offay, M. Reichard, 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/PD:** 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 and several subgenotypes of BVDV: BVDV1a,2a,1b, and 2b.. The study will determine if these vaccines induce antibodies to these four BVDV subtypes.

**Sponsors:** Novartis Animal Health, Pfizer Animal Health, Fort Dodge Animal Health  
**PI/PDs:** Robert W. Fulton, Anthony W. Confer

**Bovine Viral Diarrhea Virus: Diversity of BVDV Strains and Impact on Diagnosis, Vaccinations, and Control Programs**
Bovine viral diarrhea viruses (BVDV) isolates from persistently infected (PI) cattle will be obtained from feedlot cattle. The isolates will be subtyped by sequencing a region of the 5’-UTR. Subgenotypes to be detected will include those of worldwide epidemiology. The BVDV subgenotypes expected in North America are: BVDV1a, 1b, 2a, and 2b. Molecular diagnostic tests including reverse transcriptase PCR and real time PCR will be evaluated to detect these diverse BVDV subgenotypes in peripheral blood, serums and ear notch samples of infected animals. Cytopathic BVDV 1b strains as potential vaccine strains will be sequenced to permit detection by genomic tests allowing differentiation of vaccine strains from field strains in vaccinated animals. The prevalence of PI/PD animals in Oklahoma beef herds will be determined using immunohistochemistry and antigen capture ELISA (ACE) on ear notches. The PI/PD strains will be subtyped. Vaccination records will be obtained to determine use of BVDV vaccines or lack thereof in herds with PI/PD animals. Potentially new subtypes may warrant additional subtypes in the vaccines.

**Sponsor:** Oklahoma Agricultural Experiment Station  
**PI/PDs:** Robert W. Fulton, A. W. Confer. D.L. Step

**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 Mannheimia haemolytica and P. multocida vaccine. The calves’ sera will be tested for M. haemolytica and P. multocida antibodies.

**Sponsor:** ELANCO Animal Health, Division of Eli Lilly and Company  
**PI/PDs:** Robert W. Fulton, Anthony W. Confer
Bovine Viral Diarrhea Virus (BVDV) in Oklahoma Beef Operations: Impact of Infections and Economic Benefits to Control Programs
This study will identify infected beef breeding herds and develop a control program for beef herds stressing biosecurity and vaccination. Samples to be tested will be ear notches for the immunohistochemistry and antigen capture ELISA (ACE) using the newborn calves. BVDV isolates will be collected from infected animals and subtyped. Stocker cattle entering the OSU Sparks facility will be tested as well. Educational programs will be provided for producers regarding BVDV control. The enhanced economic benefit for marketing BVDV free stocker calves will be investigated.
Sponsor: Team Initiative Program, Oklahoma Agricultural Experiment Station
PI/PDs: Robert W. Fulton, Clint Krehbiel, DL Step

Evaluation of Viral Vaccine Containing Infectious Bovine Rhinotracheitis Virus (IBRV), Bovine Viral Diarrhea Virus 1 and 2 (BVDV), Parainfluenza -3V (PI/PD-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.
PI/PDs: Robert W. Fulton, A.W. Confer

Rapid Diagnosis of Viruses Involved in Bovine Respiratory Diseases
The purpose of the study will be to determine if use of reverse transcriptase PCR testing aids in the diagnosis of viruses involved in bovine respiratory disease. Samples including nasal swabs, serums, and lung samples collected at necropsy will be tested for viruses using the PCR tests for bovine viral diarrhea viruses, bovine herpesvirus 1, bovine respiratory syncytial virus, and bovine coronavirus. The tests results will be compared to use of standard tests including cell culture isolation for viruses. Genomic tests have advantages as cell culture tests are not often rewarding for viral identification. A higher recovery rate for these viruses is expected by PCR.
Sponsors: Oklahoma Agricultural Experiment Station, 1433 Animal Health Research
PI/PD: Robert W. Fulton

Bovine Viral Diarrhea Viruses (BVDV) for Evaluation of Antigen Capture ELISA (ACE)
This study will provide samples from bovine virus diarrhea virus (BVDV) infected animals to be used to evaluate an antigen capture ELISA (ACE) test. BVDV is represented by four different subgenotypes in the United States, BVDV1a, 1b, 2a, and 2b. The ACE test is currently used to detect persistently infected (PI/PD) cattle by testing for BVDV antigen in fresh ear notches collected in PBS. By using field isolates as compared to known reference strains maintained in research laboratories, the ACE test should be validated against current naturally occurring field strains.
Sponsor: IDEXX Laboratories
PI/PD: Robert W. Fulton

Generation of Samples for Use in Comparative Studies on Different BVDV Test Platforms
This study will provide bovine viral diarrhea virus (BVDV) positive tissue samples for use in evaluating real time PCR tests to detect persistently infected (PI/PD) cattle. Samples will include ear notch samples from PI/PD cattle infected with BVDV 1a, 1b or 2a viruses. These cattle will represent naturally occurring PI/PD cattle maintained for this study. The samples will be sent to reference laboratories (state and university) providing BVDV diagnosis by real time PCR. The samples will be tested by at least four reference laboratories, Attempts will be made to determine the endpoint whereby samples can be
pooled to test for positive animals samples.

**Sponsor:** USDA, ARS, National Animal Disease Center (NADC)
**PI/PD:** Robert W. Fulton

**Efficacy of an Experimental IBR-BVD-PI/ PD3-BSRV-VL5 Vaccine in Protecting Pregnant Heifers and Fetuses Against a Bovine Viral Diarrhea Virus Type 1b Challenge**

This objective of this study is to demonstrate efficacy of a IBR, BVDV, PI/ PD3V, BRSV, Campylobacter fetus, and leptosPI/PDral bacterin prebreeding vaccine in protecting pregnant heifers and their fetuses against challenge exposure to seeder calves persistently infected with BVDV 1b at approximately 75 days of gestation.

**Sponsor:** Pfizer Veterinary Medicine Research and Development
**PI/PDs:** Robert W. Fulton, Bill J. Johnson, DL Step, Clint Krehbiel

**Bovine Coronavirus: Role in Respiratory Disease**

Bovine respiratory diseases (BRD) also referred to as “Shipping Fever” represents significant losses to cattlemen. There are several viruses contributing to the BRD which has a polymicrobial etiology. These include bovine herpesvirus, parainfluenza-3 virus, bovine respiratory syncytial virus, and bovine viral diarrhea viruses along with *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni,* and *Mycoplasma spp.* The bovine coronavirus (BCV) has emerged as another virus which contributes to BRD. Measuring the BCV role in BRD has been hampered by lack of tools for the study of this virus both in vitro and in animal studies. The purpose of this study will be to develop means of propagating the BCV in cell cultures, developing and validating a serologic test for BCV antibodies to demonstrate active infections, developing an immunohistochemistry assay to detect BCV in infected cells and tissues, and a challenge model to demonstrate the disease induced by BCV. An attempt will be made to develop live and inactivated BCV vaccines for cattle.

**Sponsors:** Oklahoma Agriculture Experiment Station, USDA Section 1433 Animal Health Research Funds
**PI/PDs:** Robert W. Fulton, A.W. Confer, R. Eberle, D.L. Step

**Bovine Coronavirus in Respiratory Disease: Methods of Study, Pathogenesis, and Development of Challenge Model for Efficacy Studies**

Bovine coronavirus (BCV) represents an infectious agent contributing to bovine respiratory disease (BRD) in cattle. This study will investigate the role of BCV in clinical cases of BRD by viral isolations from clinically ill cattle, and the use of serology to detect active infections in commingled cattle under feedlot conditions. Serologic tests will include ELISA and viral neutralization tests to detect BCV antibodies. BCV isolated from affected cattle will be characterized as BCV and propagated with viral quantification. A challenge model will be developed to measure infectivity and pathology caused by BCV in susceptible cattle. This challenge model will be used to measure resistance to BCV in cattle immune and susceptible to BCV.

**Sponsor:** Pfizer Veterinary Medicine Research and Development
**PI/PDs:** Robert W. Fulton, Anthony W. Confer, Richard Eberle, D.L. Step

**Veterinary Medical Diagnostic Program**

The Veterinary Medical Diagnostic Program at the Oklahoma Animal Disease Diagnostic Laboratory (OADDL) serves to: 1) investigate and document the types of injuries sustained by horses involved in horse racing and in race training related activities on racetracks that fall under the jurisdiction of the Oklahoma Horse Racing Commission (OHRC); 2) monitor this population of migrating horses for the presence of any epizootic disease(s) that may pose a threat to the Oklahoma horse industry; and 3) evaluate the overall effects of all other aspects (including diet and stress) of racing and race training on
the health and well being of Oklahoma’s racehorses. This program is the result of an alliance formed between the OHRC and OADDL.

**Sponsors:** Oklahoma Horse Racing Commission, Oklahoma Bred Program.

**PIs:** Bill J. Johnson, Grant B. Rezabek

**Epidemiology of American Canine Hepatozoonosis: The Susceptibility of Selected Prey of Carnivores to Infection with *Hepatozoon americanum* and the Role of Vertical Transmission**

*Amblyomma maculatum* has been identified as the definitive host and vector of *Hepatozoon americanum* in dogs throughout the southern U.S. Transmission to dogs, an obligate intermediate host, occurs through ingestion of the infected tick. However, dogs are rarely infested with any of the 3 feeding stages of the tick. Immature ticks, stages that acquire the parasite, feed preferentially on smaller mammals and ground-dwelling birds; therefore, dogs are an unlikely source of infection for the tick definitive host. A naturally reservoir for the parasite has not been identified. However, cystozoites, a quiescent, infectious stage of the parasite, develops in the tissues of experimentally exposed laboratory rodents, making canine exposure through predation of infected prey (potential paratenic or facultative intermediate hosts of *H. americanum*) a possible mode of transmission. The primary goals of this research are: 1) to determine the natural vertebrate reservoirs for *Hepatozoon americanum*, 2) to determine the susceptibility of select vertebrate species known to be preferred hosts of immature *Amblyomma maculatum* to infection with *H. americanum*, 3) to determine the susceptibility of common prey of canids to infection with *H. americanum*, and 4) to determine if vertical transmission from canine dam to offspring occurs. Experimental transmission trials to laboratory raised rodents, birds and lagomorphs will be conducted. Comparisons of *Hepatozoon* isolates from wild-caught vertebrates to canine isolates will be made through morphology, histopathology and DNA sequencing. Vertical transmission will be investigated through the natural birth of puppies from a chronically infected carrier dog and monitoring of the offspring by weekly DNA analysis of the puppies’ blood. Identifying the natural reservoirs of the parasite and methods of transmission will help implement preventative measures for susceptible dogs to a non-curable, potential fatal parasitic disease.

**Sponsor:** OSU Center of Veterinary Health Sciences, Research Advisory Committee

**PI/PDs:** Eileen M. Johnson, Kelly E. Allen, Roger J. Panciera, Susan E. Little, Sidney A. Ewing

**Natural History of Borrelia lonestari**

This project examines the natural maintenance cycle of *Borrelia lonestari*, a putative agent of southern tick-associated rash illness (STARI) or “southern Lyme disease” in lone star ticks, white-tailed deer, and other wildlife species. The work involves both evaluation of naturally infected ticks and wild animals and experimental confirmation of the proposed maintenance cycle.

**Sponsor:** NIH NIAID

**PI/PDs:** Susan E. Little; Co-PI/PDs: OSU/CVM, Edmour Blouin, Kathy Kocan; UGA, Michael Yabsley, Kevin Keel

**Diagnosis of Borrelia lonestari**

This project seeks to develop better diagnostic assays for *Borrelia lonestari*, a putative agent of southern tick-associated rash illness (STARI) or “southern Lyme disease.” Research projects include experimental infection of white-tailed deer and rabbits and development of microbiologic, molecular, and serologic techniques to identify infected animals with the ultimate goal of developing diagnostic assays for use in people.

**Sponsor:** NIH NIAID

**PI/PDs:** Susan E. Little UGA: Michael Yabsley
Transmission of *Ehrlichia canis* by *Rhipi/PDcephalus sanguineus*

This project examines the transmission dynamics at play as *Ehrlichia canis* is moved between dogs by the brown dog tick *Rhipi/PDcephalus sanguineus*.

**Sponsor:** Bayer Animal Health.

**PI/PDs:** Susan E. Little, Kathy Kocan, Eileen Johnson, Sidney Ewing

*Ehrlichia ewingii* Infection and Exposure Rates in Dogs

*Ehrlichia ewingii* commonly infects dogs in areas of the US where lone star ticks predominate. This project seeks to document the prevalence of *E. ewingii* infection and exposure in dogs from the Ozark Plateau and use samples acquired from naturally infected dogs to refine diagnostic assays for *E. ewingii* and other, closely-related rickettsial pathogens of dogs and people.

**Sponsor:** IDEXX

**PI/PDs:** Susan E. Little, Sidney Ewing, Jim Meinkoth

Assembly of Human Respiratory Syncytial Virus (HRSV)

HRSV infects virtually every person on the planet and presents a serious, worldwide, disease burden. Available therapies are not adequate, and a better understanding is needed of the virus life cycle. In this project, the molecular mechanisms of virus assembly are investigated, and the role of the viral matrix and transmembrane glycoproteins therein determined. With an improved understanding of virus assembly, the project aims to: 1) control the virion assembly process such that the quality of anti-HRSV vaccine preparations can be improved, and 2) create new concepts for antiviral therapies.

**Sponsor:** Oklahoma Center for the Advancement of Science and Technology (OCAST)

**PI/PD:** Tom Oomens

Efficacy of Nucleoside Analogs for Treatment of Monkey B Virus Infection in Mice

Monkey B virus (BV, *Cercop/PDthecine herpesvirus*-1) is an α-herpesvirus indigenous to macaque monkeys that cause serious, often fatal disease when transmitted to humans. Typically, BV infections involve researchers and animal technicians that work with macaque monkeys and acquire the infection via bites or scratches from monkeys shedding BV; therefore, BV is the major occupational biohazard concern for persons working with and around macaque monkeys. The optimal therapy for human BV infections remains unknown. The goal of this project is to use a murine BV model system to compare several existing anti-herpetic drugs to identify the one that is most efficacious.

**Sponsor:** ACLAM Foundation

**PI/PD:** Jerry Ritchey, R Eberle, Lara Maxwell

Diversity of Bacterial Isolates from the Nares of Healthy and Sick Calves

The project will determine the genetic diversity of bacteria isolated from nasal passages of both healthy calves and those suffering from bovine respiratory disease (BRD). The goal is to determine whether a single clone typically dominates or if a variety of strains of a given species can be expected. If most or all isolates represent a single clone, culture of nasal swabs may be used in both diagnostic and research efforts of BRD. If a variety of strains are frequently present, nasal swabs would have limited usefulness, as it is not practical to examine all strains present in each calf.

**Sponsor:** Oklahoma State University Center for Veterinary Health Sciences

**PI/PDs:** J. D. Taylor, A. W. Confer, D. L. Step
Mechanisms and Functions 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) effect of clinical widely used drugs on human sulfotransferase catalytic activities. (3) oxidative regulation mechanisms of human SULTs.

Sponsor: NIH
PI/PD: Guangping Chen
Paul Cook, University of Oklahoma

Stress Regulation of Sulfotransferases
The aims of this research project are to investigate how sulfotransferase (SULT) expression and activity are regulated by various stressors including oxidative stress, physical stress, and chemical stress. The relationships between the changes of SULT expressions and the changes of various biosignaling molecule levels as well as nuclear receptor expression levels will be investigated to understand the mechanisms.

Sponsor: NIH
PI/PD: Guangping Chen

Cancer Drugs Induction of Human Sulfotransferases
Studies in this project focus on sulfotransferase (SULT) induction by cancer drugs. Rats, human hepatic carcinoma cell line, Hep G2, and human intestinal carcinoma cell line, Caco-2, are used for these studies. Enzyme activity assay, Western blot, RT-PCR, site directed mutagenesis, plasmid transfection, small interfering RNA (siRNA) gene silencing, promoter gene deletion, DNA footprinting, and electrophoretic mobility shift assay are used to determine the SULT gene regulation and nuclear receptor mediated SULT induction mechanisms.

Sponsors: American Cancer Society, State of Oklahoma
PI/PD: Guangping Chen

Effect of Selected Nutritional Components on Immunity to Haemonchus in Small Ruminants
Internal parasitism causes a significant loss in the small ruminant industry of Oklahoma and other regions of the USA and worldwide. The proposed project will address this problem through characterizing the mechanisms of immune responses and attempting to manipulate specific cytokine expression to enhance resistance to parasites in small ruminants. The outcome of this research will lead to a better understanding of the interrelationship between immunity and parasitism and a precise immunity-enhancing strategy for parasite control in ruminants.

Sponsor: USDA
PI/PDs: Guangping Chen
Zaisen Wang, Langston University

Role of GABA Receptors in Fetal Lung Development
The goal of this seed grant is to study role of GABA receptor in fetal lung development.
Sponsor: Center of Veterinary Health Sciences, Oklahoma State University
PI/PD: Narendranath Reddy Chintagari (Mentor: Lin Liu)
**Capacity for Respiratory-based Thermoregulation in Brachycephalic Breeds**
Brachycephalic dogs are believed to be less capable of thermoregulation due to the conformation of their upper airways, leading many airlines to prohibit shipping of these dogs. This study will objectively characterize the thermoregulatory capacity of these dogs relative to dogs with longer noses to determine more precisely the appropriate guidelines for shipping.

**Source:** American Kennel Club Canine Health Foundation

**PI/PD:** Michael Davis

**Muscle Adaptations Permitting Fatigue-resistant Exercise**
Exercise capacity is limited by either the ability to supply muscle with substrates for the production of energy, or the ability of the body to eliminate the waste products of energy production. In the case of endurance exercise, the availability of macromolecules for oxidation is typically the limiting factor in a thermoneutral environment. This study will determine the mechanisms by which availability of macromolecular substrates can be increased to facilitate improved exercise endurance.

**Source:** Army Research Office Division of Life Sciences, Department of Defense

**PI/PD:** Michael Davis, Guangping Chen
Peter Hoyt, Department of Biochemistry
Ray Geor, Michigan State University
Kenneth W. Hinchcliff, University of Melbourne
Arend Bonen, University of Guelph
Ken Bartels, Department of Veterinary Clinical Sciences

**Combinatorial Drug Screening to Identify Strategies to Enhance Ground Troop Readiness at High Altitude**
Acute exposure to high altitude confers a disadvantage to individuals needing to perform physical tasks, when compared to individuals who have acclimatized to high altitude. The goal of this study is to develop novel compounds that will accelerate the acclimatization process to low ambient oxygen levels, with subsequent improvement in exercise performance.

**Source:** DARPA-DSO (Subcontract through University of Colorado)

**PI/PD:** Michael Davis, Lara K. Maxwell
Jerry Ritchey, Veterinary Pathobiology

**Physiology and Pathophysiology of Equine Athletic Performance**
Superior athletic performance in horses requires optimal physiological adaptation in the musculoskeletal and cardiopulmonary systems, and improvements in these systems often lead to improved performance. Conversely, disease in these systems will invariably cause decreased performance, and in some cases the exercise itself is the cause of the disease. The goals of this program are to investigate the mechanisms underlying the physiological adaptation to exercise in horses, identify methods that will improve the horse's adaptation to exercise, and determine strategies that can prevent exercise-induced disease. (2729)

**Sponsor:** Oklahoma Agricultural Experiment Station, Oxley Chair in Equine Sports Medicine

**PI/PDs:** Michael Davis

**The Role of P2X7R-Mediated Purinergic Signaling in Alveolar Epithelial Cell Death**
The goal of this predoctoral fellowship is to study functional roles of P2X7R in alveolar epithelial cells.

**Sponsor:** American Heart Association, South Central Affiliate, predoctoral fellowship #09PRE2300211

**PI/PD:** Yujie Guo (Mentor: Lin Liu)
**Genetic Modifiers of Organ Dysfunction in PKD**
This proposal’s focus is on the role that proteoglycans and inflammation have on renal and liver dysfunction in polycystic kidney disease. These experiments utilize available multiple mouse models from collaborators in combination with mutant mice we’ve made to examine lesion severity.
**Sponsor:** Oklahoma Center for Advancement of Science and Technology  
**PI/PD:** Myron Hinsdale

**Extracellular Determinants of Polycystic Kidney Disease Severity**
The focus of this grant is to study the role that proteoglycan levels have in lesion severity in PKD and specifically autosomal recessive polycystic kidney disease.
**Sponsor:** NIH  
**PI/PD:** Myron Hinsdale

**Adult Stem Cell Proteoglycans and Emphysema**
The focus of this grant is to study the role that stem cell extracellular matrix proteoglycans have in the healing process of lung disease.
**Sponsor:** Oklahoma Center for Adult Stem Cell Research  
**PI/PD:** Myron Hinsdale

**Regulation of MicroRNA-124 in the Lung**
The goal of this seed grant is to study how miRNA-124 is regulated.
**Sponsor:** Center of Veterinary Health Sciences, Oklahoma State University  
**PI/PD:** Chaoqun Huang (Mentor: Lin Liu)

**Mechanisms of Alveolar Fluid Transport**
The goal of this project is to investigate the roles of chloride channels of alveolar epithelial type I and type II cells in fluid secretion of fetal lungs, and in maintaining fluid homeostasis of adult and injured lungs.
**Sponsor:** National Institutes of Health R01 HL083188  
**PI/PD:** Lin Liu

**Role of MicroRNAs in Bronchopulmonary Dysplasia**
The major goal of this project is to identify microRNAs involved in bronchopulmonary dysplasia using microRNA microarray and examine their functions.
**Sponsor:** National Institutes of Health R21HL087884  
**PI/PD:** Lin Liu

**MicroRNA Expression Profiling in Idiopathic Pulmonary Fibrosis**
The goal of this project is to identify and characterize microRNAs changed in idiopathic pulmonary fibrosis using the IPF patient lung samples from the Lung Tissue Research Consortium.
**Sponsor:** National Institutes of Health R03HL095383  
**PI/PD:** Lin Liu

**MicroRNAs and Bovine Respiratory Disease**
The major goal of this project is to examine microRNA expression in LPS-challenged bovine microphages as it relates to bovine respiratory disease.
**Sponsor:** U.S. Department of Agriculture 2009-035505-05855  
**PI/PD:** Lin Liu
**Paracine Regulation of Lung Surfactant Secretion**
The goal of this grant is to understand how type I cells regulate surfactant secretion in type II cells.
**Sponsor:** RAC, Center for Veterinary Health Sciences, OSU
**PI/PD:** Lin Liu

**Reprogramming of Adult Lung Cells for Cell-based Therapy**
The goal of this grant is to convert adult lung cells into stem cells and use them for treating COPD.
**Sponsor:** Oklahoma Center for Adult Stem Cell Research
**PI/PD:** Lin Liu

**Regulation of Placenta Growth Factor by Hemodynamics and Reactive Oxygen Species**
Ischemic cardiovascular disease is highly prevalent in diabetics and accounts for most of the mortality in this patient group. Stimulation of collateral artery growth (arteriogenesis) to create a physiological “bypass” is a promising treatment concept; however, greater understanding of the mechanisms controlling arteriogenesis is needed before such treatments are a reality. This research will investigate fundamental mechanisms regulating the key arteriogenic growth factor PLGF as a basis for developing new, noninvasive treatments for ischemic CVD. A variety of *in vitro* and *in vivo* model systems will be used to identify key regulatory mechanisms controlling PLGF expression in health and disease.
**Sponsors:** National Institutes of Health
**PI/PD:** Pamela G. Lloyd

**Endothelial Progenitor Cell Survival and Function in Emphysema: Role of VEGF-A/PLGF Signaling**
Endothelial progenitor cells (EPC) are a type of adult stem cell that is implicated in vascular growth and repair. Chronic obstructive pulmonary disease (COPD) is a progressive lung disease characterized by enlargement of airspaces and loss of lung microvasculature. Since lung epithelial and endothelial cells engage in signaling crosstalk, we hypothesized that unbalanced endothelial growth factor expression may contribute to the pathogenesis of COPD. The goal of this project is to determine whether altered VEGF-A/PLGF ratios contribute to EPC homing, migration, and/or survival in lungs of mice with experimental emphysema.
**Sponsor:** Oklahoma Center for Adult Stem Cell Research (OCASCR)
**PI/PD:** Pamela G. Lloyd

**Role of Vitamin A in Modulation of Gene Expression in the Cumulus-Oocyte Complex**
The central hypothesis of this work is that specific patterns of early gene expression in the cumulus-oocyte complex during the maturation phase affects subsequent developmental capacity; these patterns are subject to influences of the local environment and can be characterized by examining the stimulatory effect of certain nutrient, including vitamin A (9-cis Retinoic Acid (RA)). It follows that identification of the positive effects of 9-cis RA will provide a view of a transcript profile having positive influence on subsequent embryo development. The effect of 9-cis RA stimulation on maternal transcriptional activity and transcript profile, and the impact on the oocyte transcript profile will be separated by analysis of the cumulus cells alone, the oocyte alone, versus the cumulus-oocyte complex as a whole, using subtractive hybridization. These results are expected to yield a better understanding of the early transcriptional profile and those genes critical to developmental potential. Benefits may include targets for testing developmental potential of preimplantation embryos and better production methods.
**Sponsors:** Oklahoma Agricultural Experiment Station
**PI/PDs:** Jerry Malayer
Initiating Factors of Neurodegeneration
Parkinson’s Disease and age are both characterized by a loss of functional dopamine-producing neurons. Several aging factors have been implicated in the development of dopaminergic neuronal loss including misfolding of a nerve protein known as alpha-synuclein (a-syn). The long-term goal of our research program is to understanding how aging results in the accumulation of misfolded a-syn and a loss of functional neurons and why the process is accelerated in those individuals with Parkinson’s Disease using two novel animal models; spontaneous neurodegeneration in aged baboons and a highly prevalent neurodegenerative disease of aged horses.

Sponsor: NCRR, NIH 1K01RR023946-01A1
PI/PD: Dianne McFarlane

The Effect of Season, Latitude and Breed on Equine Pituitary Function
Seasonal variation in pituitary activity, resulting in increased production of pars intermedia (PI) hormones in the fall, occurs in many species including the horse, presumably to prepare them metabolically and physically for winter. It is unknown what triggers seasonal PI activation, and when in the year it begins. We hypothesize that hormone release from the healthy equine pituitary pars intermedia is regulated by seasonal change in day length. Therefore, we expect plasma concentrations of PI hormones to increase starting in July, when day length begins to shorten. We also expect that horses residing in latitudes further from the equator, where the seasonal change is more extreme, to have a more pronounced seasonal hormone increase compared to those residing closer to the equator. Finally, we expect thrifty breeds (ponies, Morgan horses) to have a more robust seasonal increase in PI hormone concentration compared to light breeds and horses with PPID to have no seasonal hormone variation.

Sponsor: American College of Veterinary Internal Medicine Foundation
PI/PD: Dianne McFarlane

Role of R2X7 Receptor in Acute Lung Injury
The goal of this student seed grant is role of P2X7 receptors in lung injury.

Sponsor: Center of Veterinary Health Sciences, Oklahoma State University
PI/PD: Amarjit Mishra (Mentor: Lin Liu)

Presynaptic Modulation of Anticholinesterase Toxicity
The project evaluates the role of endocannabinoid signaling in differential toxicity of organophosphorus insecticides.

Sponsor: National Institute of Environmental Health Sciences
PIs: Carey Pope, Jing Liu Pope

Chemical Forensics for Toxic Chemicals and Explosives
This project develops approaches for detecting toxic chemicals on synthetic surfaces.

Sponsor: Department of Defense
PIs: Carey Pope, Jing Liu Pope

Effect of Hyperlipidemia on Signaling Pathways Mediating Exercise-induced Vascular Remodeling
Exercise training is a highly recommended treatment for patients with ischemic vascular disease, including peripheral artery disease (PAD) and coronary artery disease (CAD). However, most studies investigating the effects of exercise training on vascular remodeling have been done in young, healthy animals that do not closely mimic the condition of the average human patient with PAD or CAD. Thus, the goals of this project are to determine whether exercise training activates angiogenic signal
transduction and induces improvements in capillarity and blood flow in hyperlipidemic animals to the same extent as previously shown in healthy animals.

**Sponsor:** OSU CVHS Research Advisory Committee  
**PI/PD:** Jennifer Shaw (Mentor: Pamela G. Lloyd)

**Crosstalk of miR-21 and TGF-B/BMP Signaling Pathway in Bronchopulmonary Dysplasia**

The goal of this seed grant is to study the role of miR-21 in BPD

**Sponsor:** Center of Veterinary Health Sciences, Oklahoma State University  
**PI/PD:** Dong Xi (Mentor: Lin Liu)

**An Acquisition for a SELDI ProteinChip Reader**

This shared equipment proposal will support the acquisition of SELDI proteomics technology for users at Oklahoma State University.

**Sponsor:** National Science Foundation  
**PI/PD:** Wei Yin, Mechanical & Aerospace Engineering  
**Co-I:** Pamela G. Lloyd

**DEPARTMENT OF VETERINARY CLINICAL SCIENCES**

**Veterinary Medicine Biomedical Laser Laboratory**

Since the establishment of the Biomedical Laser Laboratory within the OSU Center for Veterinary Health Sciences, 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.

**Sponsors:** McCasland Foundation, Kerr Foundation  
**PI/PD:** Kenneth E. Bartels

**Transrectal Near-infrared Optical Tomography for Prostate Imaging**

This project is a collaboration between OSU colleges (School of Electrical and Computer Engineering and the Center for Veterinary Health Sciences), and the University of Oklahoma Health Sciences Center. It involves development of a trans-rectal ultrasound probe using an animal model to image and differentiate malignant tumors in the prostate.

**Sponsor:** U.S. Army Prostate Cancer Research Program  
**PI/PDs:** Kenneth E. Bartels, Reed W. Holyoak, Jerry W. Ritchey

**Assessment of *Bdellovibrio bacteriovorus* Strain 109J as an Alternative Treatment for Infectious Bovine Keratoconjunctivitis**

Ocular infections with the infectious bovine keratoconjunctivitis (IBK) agent *Moraxella bovis* (*M. bovis*) are associated with significant economic loss in the cattle industry. Although antibiotic therapy is the treatment of choice for IBK, treatment failures are common and current vaccines are not optimally effective. As a result, our laboratory has been actively investigating the therapeutic potential of *Bdellovibrio bacteriovorus* 109J (*B. bacteriovorus*), as a new treatment for IBK. The objectives of this study are 1) to evaluate whether *B. bacteriovorus* can be trained to kill *M. bovis* as effectively as its normal prey *E coli*, and 2) to determine how long *B. bacteriovorus* maintain its viability in bovine tears. Our work is expected to lay the foundation for further definitive research on the development and
preparation of *B. bacteriovorus* ophthalmic formulation, and dosing interval for the treatment of IBK in a field setting.

**Sponsors:** Oklahoma State University’s Veterinary Research Scholars program (supported by NIH, Merial Veterinary Scholars program, Morris Animal Foundation, Center of Veterinary Health Sciences)

**PI/PDs:** Melanie J. Boileau  
Veterinary Pathobiology: Kenneth D. Clinkenbeard

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**Bacteremia Following Regional Intravenous Perfusion of the Distal Limb in Cattle with Deep Digital Sepsis**

The goal of the research is to determine if regional intravenous perfusion (RIVP) employed on cattle with deep digital sepsis results in bacterial translocation into the pedal circulation, and then to determine which bacterial pathogens are most commonly responsible for the resultant pedal bacteremia. Additionally, the antimicrobial susceptibilities will be determined for bacterial pathogens cultured from the pedal circulation following aseptic RIVP in cattle with clinically diagnosed deep digital sepsis.

**Sponsors:** OSU Center for Veterinary Health Sciences, Research Advisory Committee  
**PI/PDs:** Robert Streeter, Katie Simpson

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**Interactions of Viral Infection and Nitric Oxide within the Reproductive Tract of Horses and Cattle**

During the normal physiologic breeding season ovarian/follicular dynamics and associated changes within the reproductive tract will be assessed via transrectal ultrasonography and plasma concentrations of estradiol, progesterone, FSH and LH will be through the follicular phase and periovulatory phase to ovulation. At specific phases of the reproductive cycle, immunolocalization techniques will be utilized to determine the sites of NOS isoform active in the follicular wall of mare ovaries following tissue excision. Western blot and mRNA RT-PCR analyses of follicular fluid and follicle wall tissues will be used to quantitate NOS isoform expression associated with various sized follicles during the folliculogenic and ovulatory periods. Specific NOS isoform activity will be determined in granulosa cells from various sized follicles. Granulosa cells will be aspirated from mare ovaries during the follicular phase of the estrous cycle. As follicles reach each specific size range, follicular fluid will be aspirated for granulosa cell harvest. Specific NOS activity will be assessed relative to follicle size as will plasma and follicular fluid hormone concentrations and NO metabolite concentrations. Follicular fluids and plasma without extraction will be analyzed and quantified for estradiol, progesterone, prostaglandin F2 alpha, prostaglandin E2, and FSH and LH respectively radiolmmunoassay. Twenty breeding age mares, seronegative for anti-EAV antibodies will be used in this study. During the normal physiologic breeding season parallel samples will be taken of buffy coats and nasopharyngeal (NP) swabs for virus isolation. Ovaries and oviducts will be obtained from the remaining control and infected mares at various stages of the pre and periovulatory period during the viremic phase of infection. After removal the ovaries and oviducts, specific NOS isoform presence and staining intensity will be investigated using in-situ immunohistochemistry relative to virus immunolocalization and histopathology. Clinical signs of infection, follicular dynamics and ovarian edema will be assessed via ultrasonography and plasma concentrations of estradiol, progesterone, FSH and LH will be assessed twice daily for all mares. Specific NOS isoform activity will be determined in granulosa cells from various sized follicles during the acute phase of EAV infection. During the normal physiologic breeding season mares will be synchronized and exposed to a field isolate of EAV (KY-84). Granulosa cells will be aspirated from their ovaries during the follicular phase of the estrous cycle. As follicles reach each specific size range follicular fluid will be aspirated for granulosa cell harvest. Virus isolation, specific NOS activity, plasma and follicular fluid hormone concentrations and NO metabolite concentrations will be assessed relative to follicle size and clinical signs of infection. Follicular fluids and plasma without extraction will be analyzed and quantified for estradiol, progesterone, prostaglandin F2 alpha, prostaglandin E2, and FSH and LH respectively.
Quantitative Analysis of the Vascular Supply of the Feline Tibia

Tibial fractures are the second most common long bone fracture type in the cat. Clinical observations suggest that tibial fractures in cats are prone to delayed and non-union. Although a poor blood supply to the tibia has been anecdotally reported as a significant risk factor for delayed union and non-union complications, there exists no detailed description of the vascular anatomy of the feline tibia. The aims of this study are: to quantitatively describe the vascular supply to the tibia, to determine if the vascular supply is significantly different from that of dogs, and if the vascular supply changes with age.

Sponsors: OSU, Center for Veterinary Health Sciences, Department of Veterinary Clinical Sciences; Cohn Family Chair for Small Animals

PI/PDs: Mark Rochat, Danielle Pawloski, Jerry Ritchey, Mark Payton

The Effects of Intervertebral Disk Calcification in Chondrodystrophic Dogs on the Success of the Percutaneous Thoracolumbar Intervertebral Disk Ablation Procedure

Intervertebral disk disease results in herniation of the disk and variable neurologic impairment, with some dogs suffering permanent paralysis. The percutaneous laser disk ablation (PLDA) procedure has been recommended as a minimally invasive procedure to reduce the risk of recurrence in dogs with thoracolumbar disk disease. A holmium yttrium aluminum garnet (Ho:YAG) laser has been utilized for the PLDA procedure and shown to significantly reduce the risk of recurrence, with recurrence seen at sites with calcified disks identified radiographically following the procedure. This study will evaluate the effects that intervertebral disk calcification has on laser-tissue interactions via measurement of optical tissue spectral reflection characteristics, evaluate the ability of radiographs to identify significant disk calcification based on histopathology and optical spectral characteristics, as well as determine if increasing the fluency (time / power) in the current laser protocol results in more successful ablation of calcified discs.

Sponsors: Kerr Foundation, OSU Department of Veterinary Clinical Sciences

PI/PDs: Kenneth Bartels, Kelci McKeirnan, Melanie Breshears, Mark Rochat

Statistics: Mark Payton

Electrical and Computer Engineering: Daqing Piao, Katherine Jiang

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). OADDL is a member of the National Animal Health Laboratory Network and does surveillance testing for Avian Influenza, novel H1N1, exotic Newcastle Disease and Classical Swine Fever.
in Oklahoma livestock and birds. The Laboratory maintains full accreditation by the American Association of Veterinary Laboratory Diagnosticians.

**PI/PDs:** Bill J. Johnson and staff