

The top half of the cover features three microscope objective lenses from a Zeiss microscope. The central lens is in focus, showing the text 'ZEISS', 'Germany', and 'NEOFLUAR'. The lenses are set against a dark blue background.

# insight

College of Veterinary Medicine and Biomedical Sciences

Fall 2008

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**CVMBS  
Research  
Goes Global**

The bottom half of the cover shows a satellite view of the Earth from space, focusing on the continents of Europe, Africa, and Asia. The Earth is set against a clear blue sky.

Colorado State University



Lance Perryman, DVM, PhD

welcome

## Dear Friends,

In this edition of *Insight Magazine*, you'll have the opportunity to learn about some of the most innovative global and national collaborative research projects at the College of Veterinary Medicine and Biomedical Sciences. In today's rapidly advancing world of research, it is the rare laboratory that can do it all – in fact, those laboratories may now be extinct.

Partnerships, both private and public, are essential to bring the best, the brightest, and the latest to our research laboratories, optimizing our resources and enhancing our ability to improve human and animal health.

Colorado State University has championed this new model of research and technology transfer with its SuperCluster concept. A SuperCluster is a multidisciplinary alliance that integrates experts from many fields with the goal of improving quality of life. The College is home to two of the three University SuperClusters, one in infectious disease and one cancer research. But that model has taken root organically throughout the College, and mini-Clusters are forming as laboratories spread out from their home bases to outposts around the world.

In avian influenza, four years ago Dr. Kristy Pabilonia, working with Dr. Hana Van Campen, pioneered the Avian Influenza Surveillance Program in Colorado. Working with producers and the state veterinarian, the program monitored poultry in Colorado to detect any early signs of an outbreak. Dr. Mo Salman, with the Animal Population Health Institute at CSU, has ties around the globe because of his work in epidemiology. He recruited Dr. Pabilonia to conduct avian epidemiology workshops in other parts of the world. From there, Dr. Pabilonia set up research partnerships in Indonesia, where avian influenza is endemic. She is in the process of establishing a veterinary research facility not only to help the local population, but to provide

a study base for the CSU team abroad where avian influenza is readily found and studied.

There are many stories like Dr. Pabilonia's. Dr. Varalakshmi Vissa works with laboratories in China, Brazil, India, and other exotic locales conducting leprosy studies and tracking down leprosy cases. Dr. Richard Bowen collaborates with multiple government agencies conducting investigations into rabies and animal populations that act as reservoirs for the virus. The Equine Orthopaedic Research Center is pulling in human resources from England, Scotland, and Australia to study the impact of joint shape on disease and injury, a study with immense possibilities for human medicine. Almost anywhere you go on the planet, you'll find a CVMBS connection.

As our research programs grow, so does our investment in laboratories, equipment, and the finest faculty and staff. The College continues to lead similar veterinary and biomedical colleges in federally funded research, particularly with large programs in infectious diseases receiving substantial grants from the National Institutes of Health. In addition, private grants are having a greater impact, including grants from the Bill and Melinda Gates Foundation Grand Challenges. The Grand Challenges most recently awarded \$1.25 million for cutting-edge tuberculosis drug research at the Mycobacteria Research Laboratories. In our last fiscal year, the College's research expenditures were \$64 million.

I hope you enjoy this edition of *Insight* and come away with a greater understanding of the global nature of our research programs. It is through reaching out in cooperation to the world that truly great breakthroughs in research will happen, and we will all reap the benefits.

Best Regards,

Lance Perryman, DVM, PhD  
Dean, College of Veterinary Medicine  
and Biomedical Sciences

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# insight

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## 2 Dr. Vissa fights leprosy

CVMBBS researchers are leading global efforts to help the World Health Organization eliminate an ancient disease that was once so feared its sufferers were banished to island colonies and leprosaria.



## 18 Bats provide clues to rabies

While bats play an important role in the environment, they also are a reservoir of some of the most deadly infectious diseases known to humans. A CVMBBS research team is trying to find out how and why.



## 20 Mares shed light on infertility

A collaborative research project reveals new aspects of reproductive aging in the horse, providing insights into oocyte quality that will be directly translatable to the treatment of human infertility.



From left:  
Rama Murthy Sakamuri,  
We Lei, and Vara Vissa

# Efforts hope to eliminate ancient disease



Far from Dr. Varalakshmi Vissa's office at Colorado State University, a young girl is examined for possible nerve damage and skin lesions at the Eversley Childs Sanitaria on the tiny island of Cebu in the Philippines. Though worlds apart, Dr. Vissa and the girl are linked together by an ancient mycobacterium that causes a disease once so feared its victims were banished from family, friends, and communities.

Known more commonly in world health circles as Hansen's disease, leprosy still evokes a strong reaction and continues to challenge scientists and physicians who had hopes of eliminating the affliction globally by the year 2000, later revised to 2005. With the introduction of a highly effective multidrug-treatment program in the early 1980s, an incurable disease became curable. The goal of elimination was achieved worldwide (with global rates of 1 case per 10,000 inhabitants), but at a national level there remain 12 endemic countries where leprosy rates are four times higher than the World Health Organization's standard for elimination. In 2004, the global incidence of new cases of leprosy was estimated to be just over 400,000 (two to three million people are estimated to be permanently disabled because of the disease).

*Mycobacterium leprae*, the causative agent of leprosy, is proving to be tenacious as are the medical limitations, geographic inaccessibility,

and social stigmas which help maintain leprosy's status as a public health threat. Despite advances in treatment, early diagnosis remains problematic (when the disease is treated subclinically the risk of long-term disfigurement or disability drops dramatically). A multi-national approach, incorporating laboratories from around the world including the Mycobacteria Research Laboratories at CSU, might just be the golden arrow that finally takes down the disease some medieval societies considered a Purgatory on Earth.

"We are collaborating with laboratories all over the world studying disease transmission modalities, epidemiology, drug resistance, incorporating molecular tools such as DNA fingerprinting, and more," said Dr. Vissa, an Assistant Professor in the Department of Microbiology, Immunology and Pathology and member of the Mycobacteria Research Laboratories (MRL) since 1994. Dr. Vissa is originally from India where leprosy is endemic and still above WHO elimination levels. "We are looking at *M. leprae* not only from a molecular level, but also from population studies that examine leprosy rates within families and villages, the incidence of relapse and re-infection, and most likely forms of transmission. Despite the fact that leprosy has

"I feel my work with leprosy is not only a challenge, but an obligation."



From left, standing: Rama Murthy Sakamuri, Reem Al-Mubarak, We Lei; sitting: Vara Vissa, Jason Rivest

been with us for centuries, there is still much we don't understand. I feel my work with leprosy is not only a challenge, but an obligation."

In 2001, the *M. leprae* genome was sequenced and researchers had a new tool with which to work. New drug targets, diagnostic antigens, and in vitro manipulations offered novel areas for research breakthroughs, as did the investigation into genomic markers that distinguish one bacterium from another. But researchers also realized that in order to advance the understanding of *M. leprae* and leprosy, laboratories needed to be connected and communicating. From her office, Dr. Vissa is at the center of a global virtual leprosy research community. A grant from the National Institutes of Health is enabling her to establish a formal communications network, as well as provide seed money for collaborative experiments. She also is a member of an international consortium known as "Initiative for Diagnostic and Epidemiological Assays for Leprosy" (IDEAL) that was awarded funds by the Heiser Program for Research in Leprosy and Tuberculosis for developing and testing molecular and immunology reagents.

"One of our small pilot projects is looking at prevalence and new case detection in villages and family groups on Cebu Island," said Dr. Vissa. "We are trying to design more controlled experiments that look at epidemiology and transmission. For example, we know prolonged

and high frequency contact increases the risk of infection, but what about the workplace, water, the respiratory route? We know that *M. leprae* is not a highly contagious bacterium, and needs a susceptible host, but we still don't know exactly how it is transmitted or what all the reservoirs of infection may be. Part of what we are trying to do is track the disease to see if we can determine the most likely route of transmission."

Among the laboratories and organizations collaborating with the MRL are Beijing Tropical Medicine Research Institute in China, Instituto Colombiano de Medicina Tropical, Thai National Institutes of Health, Blue Peter Research Center and Stanley Brown Laboratories in India, Leonard Wood Memorial in the Philippines, Oswaldo Cruz Institute (IOC) – Oswaldo Cruz Foundation (Fiocruz) in Brazil, Yonsei University in Korea, and the National Hansen's Disease Program in Baton Rouge. In addition to collaborative research projects, Dr. Vissa coordinates campus visits for other leprosy researchers to assist them in learning how to work through and set up experiments, do DNA testing, collect samples, and other practical skills. Dr. Vissa continues to learn new skills to share with her colleagues, including tutoring from Dr. William Black, also with MIP, on how to manage and interpret multilocus variable number tandem repeats (VNTRs). Dr. Vissa's mentor, and longtime

founder/director of the Leprosy Resource Service at CSU, is Dr. Patrick Brennan, who continues to provide leadership and direction to the group's leprosy research programs.

"There are so many new tools we can incorporate into our research that a major part of our efforts is to teach others how to use and gain maximum benefit from these tools," Dr. Vissa said. "We don't have formal meetings, other than when we see each other at conferences, but provide a lot of support and information via e-mails and the Web, as well as bringing our expertise to colleagues in other parts of the world. We are trying to make this as interactive as possible, given the diversity of cultures and time zones."

Dr. Vissa spends time visiting other laboratories including travel to India for the recent International Leprosy Congress and, last year, a trip to China to explore the challenges of biomedical research and refining plans of how she can help. Growing up in India, witnessing the devastation of leprosy firsthand, for Dr. Vissa it's not only what she wants to do, it's what she has to do.



Leprosarium at Agua de Dios, Cundinamarca, Colombia.



## Leprosy Facts

- *Mycobacterium leprae*, the causative agent of leprosy, was discovered by G. H. Armauer Hansen in Norway in 1873, making it the first bacterium to be identified as causing disease in humans.
- Only 5 to 10 percent of the human population is susceptible to infection by *M. leprae*, the rest are immune. Armadillos are the only other mammals known to be naturally infected by *M. leprae*.
- *M. leprae* is not a highly contagious bacterium.
- *M. leprae* is an intracellular bacterium that doesn't adapt to other environments and cannot be grown in vitro (test tube). Live bacterium is harvested from armadillos and the foot pads of genetically engineered nude mice to provide study materials.
- Leprosy Research Support at Colorado State University is federally funded and charged with providing leprosy research materials (from whole cells to subcellular fractions) worldwide to leprosy research laboratories.
- In the 1940s, the development of the drug dapsone led to the first effective treatment of leprosy. *M. leprae* quickly evolved antibiotic resistance and by the 1960s the anti-leprosy drug became ineffective.
- Anti-leprosy drugs clofazimine and rifampicin were used in the 1960s and 1970s, followed by the development of a combined three-drug therapy intended to prevent the development of drug resistance. This treatment is still in use today.
- Without treatment, leprosy can be progressive, causing permanent damage to the skin, nerves, limbs, and eyes. Loss of sensation in the skin can lead to damage through injury that doesn't register with pain in the body.
- Leprosaria (leper hospitals), and leper colonies, isolated victims from the general population as leprosy was thought to be highly contagious. Even today, the social stigma associated with leprosy prevents many victims from seeking medical attention for this curable disease.



Dr. Steven Dow





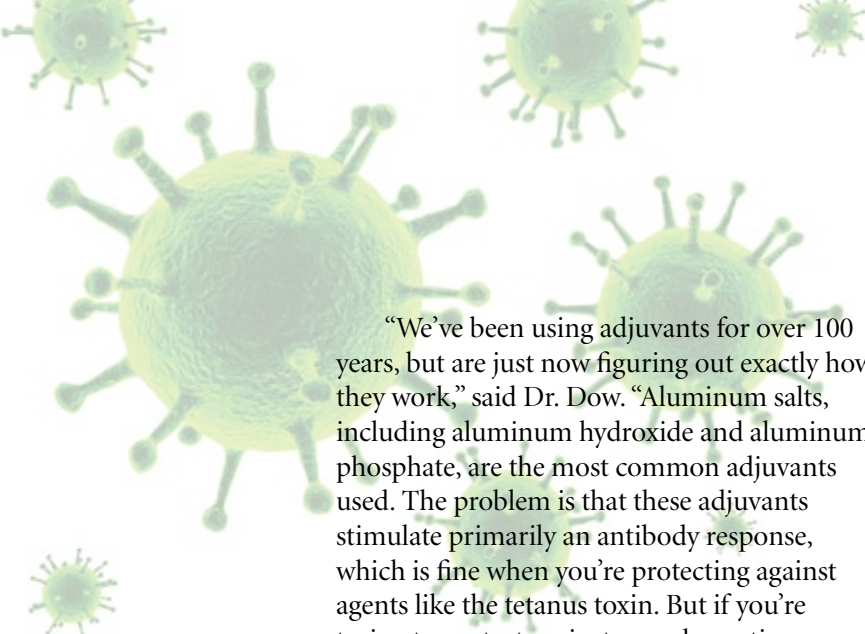
# Liposome complex holds great potential

The research team at National Jewish Medical and Research Center was investigating gene therapy options for cancer treatment, but things were not going as planned. The liposomal complex they were using as a delivery vector to introduce therapeutic genes into the target cells, was eliciting a very strong immune response and muddying the waters of their investigation. The research team tried to work around the immune response, keeping their focus on gene therapy, but to no avail – until it occurred to the team that they might just have something more here, not gene therapy, but a powerful immunostimulant with many potential uses in medicine.

“The immune response was a side effect of the gene therapy delivery system we were working with,” said Dr. Steven Dow, a Professor in the Department of Clinical Sciences and in the Department of Microbiology, Immunology and Pathology at Colorado State University, and a former director of the National Jewish research team. “We were taught that the gene delivery vectors were inert, but we found that instead they were very stimulating to the immune system. We realized that instead of working around what we thought was a problem, the delivery system might be ideal as an adjuvant for vaccines and for use as an immunotherapeutic. It was pure serendipity – most discoveries in science are not made because they are thought through, but because someone is a good observer.”

In 2001, Dr. Dow left National Jewish to join the faculty at the College of Veterinary Medicine and Biomedical Sciences, but his research partnerships as a result of his work with the cationic liposome DNA complex (CLDC) continue to expand, particularly in the use of cationic lipid adjuvant systems to increase the efficacy of vaccines and immunotherapeutics. Adjuvants are critical components of vaccines, helping to stimulate the innate immune response which in turn activates the adaptive immune response. The innate immune system is comprised of cells and mechanisms that defend the host from infection in a general manner without conferring long-lasting immunity. The adaptive immune response is put into play by the innate immune system and enables the host to recognize and remember specific pathogens, conferring protective immunity and preparing the body for future challenges.

The adaptive immune response builds immunity over time to multiple challenges which is why, in normal situations, young children do not have protective immunity to the degree that adults do. Vaccines help to build that immunity by challenging the immune system with attenuated or killed pathogens, generating a protective immune response without harming the host. Adjuvants such as CLDC activate the innate immune response by mimicking natural infection, triggering a low level of inflammation, and an improved T-cell and B-cell response.



“We’ve been using adjuvants for over 100 years, but are just now figuring out exactly how they work,” said Dr. Dow. “Aluminum salts, including aluminum hydroxide and aluminum phosphate, are the most common adjuvants used. The problem is that these adjuvants stimulate primarily an antibody response, which is fine when you’re protecting against agents like the tetanus toxin. But if you’re trying to protect against complex antigens such as tuberculosis, cancer, and HIV, you need more than an antibody response, you need an adjuvant that will elicit a T-cell response as well

which is what we are seeing with CLDC.”

“All these partnerships allow a fuller exploration of the biomedical applications of CLDC, ultimately benefiting human and animal patients with improved preventive and therapeutic care.”

In addition to using CLDC as a vaccine adjuvant, researchers are investigating the complexes for use in immunotherapy. The first human clinical trials started in June with flu vaccines incorporating the new adjuvant. In October, researchers will begin a study in hepatitis C patients using the complex as an immune system stimulant and in

patients with acute myelogenous leukemia. The potential uses for the complexes are varied, as evidenced by the current number of research projects and clinical trials.

Dr. Dow works with colleagues at: National Jewish on cancer and asthma vaccines; University of Colorado Health Sciences Center on formulation development; University of California at Davis and Stanford University on flu studies (which, if successful, could help to dramatically increase the country’s flu vaccine stores); National Institutes of Health on a herpes vaccine study; Penn State University Medical Center on a cancer study in humans with AML; University of Utah

on immunotherapy for viral infections; and CSU’s Rocky Mountain Regional Center of Excellence for Biodefense and Emerging Infectious Diseases on a number of agents including arboviruses, Eastern and Western equine encephalitis viruses, and West Nile virus, LaCross virus, *Francisella tularensis*, and *Mycobacterium tuberculosis*. He works with colleagues in his own lab on bacterial diseases including *Burkholderia mallei* and *pseudomallei*, *Yersinia pestis*, and *Francisella tularensis*.

Dr. Dow also is a member of the Animal Cancer Center, where CLDC complexes are being investigated for use in tumor immunotherapy and tumor vaccines. In addition, he collaborates with Dr. Michael Lappin and other colleagues in the Department of Clinical Sciences on studies of CLDC immunotherapy for feline and canine viral and fungal diseases.

“We are doing a lot of translational research here and working at the Veterinary Teaching Hospital provides a tremendous advantage in advancing these studies rapidly,” said Dr. Dow. “We can quickly move from the laboratory into clinical trials, and from there take the next step into human medicine, benefiting both animals and humans.”

The CLDC technology serves as the core technology for the start-up company Juvaris Biotherapeutics based in San Francisco. Juvaris is pursuing the use of CLDC as both a vaccine adjuvant and as a stand-alone immunotherapeutic. Recently, Bayer Animal Health licensed the CLDC technology from Juvaris Biotherapeutics for use in animal health applications. Since coming to CSU, Dow and his research partners have had numerous patents filed through the Colorado State University Research Foundation.

“It appears that there are many possible applications for the CLDC immunotherapy technology,” said Dr. Dow. “All these partnerships allow a fuller exploration of the biomedical applications of CLDC, ultimately benefiting human and animal patients with improved preventive and therapeutic care.”

# Innovative TB drug research



The Mycobacteria Research Laboratories is investigating the questions of why tuberculosis often recurs in a person with the infection, and why tuberculosis treatments work so slowly. A recent award from the Bill and Melinda Gates Foundation will help the laboratory to build on new discoveries that question long-held beliefs about recurrence and treatment. The \$1.25 million grant, part of the Gates' initiative to develop faster and more effective tuberculosis treatments, was awarded to Dr. Ian Orme, a Professor in the Department of Microbiology, Immunology and Pathology and member of the Mycobacteria Research Laboratories.

The grant allows Dr. Orme and his research team to pursue the ever-present question facing tuberculosis researchers regarding why it takes nine to 12 months of antibiotics to kill tuberculosis and why, even after such extensive treatment, many patients relapse.

"We think it is because current drugs cannot reach all of the tuberculosis bacterium in an effective manner," Dr. Orme said. "We know that it kills about 90 percent of the bacteria within the first few weeks of treatment, but the remaining 10 percent persists and is very hard to kill. As yet we know extremely little about these persistent bacteria. But we think we may have now discovered how it might be hiding from the drugs."

Dr. Orme's team has new data that shows the persistent bacteria can be found in very discrete areas within lesions in the lung. The bacteria also may exist in a state that tuberculosis experts have previously not considered: the bacteria may be forming microscopic clusters in a biofilm. A biofilm is a thin layer of material that encapsulates the bacteria and protects it from outside elements

– in this case, those elements include medications aimed at killing the bacteria.

Tuberculosis researchers around the world have only recently begun to suspect that the tuberculosis bacterium can form a biofilm. Dr. Orme's research team now has new information suggesting that the biofilm forms about two to three months after infection. According to Dr. Orme, because the drugs cannot get to the bacterium that causes tuberculosis, the persistent bacteria remain in the host and cause relapses after treatment is completed.

The Gates Foundation grant will fund a closer look by Dr. Orme and his team at the area of tissue in the lungs where these bacteria hide, to see if new drugs can kill the persistent bacteria before the biofilm forms or alternatively disrupt the formation of the biofilm to prevent it from protecting the bacteria.

For the past 10 years, the Mycobacteria Research Laboratories has managed the National Institutes of Health's drug compound testing program for tuberculosis, testing more than 85,000 potential drug compounds since 1997. The laboratory tests new compounds being investigated as potential TB treatments by other universities and by pharmaceutical companies. Compounds being looked at by pharmaceutical companies, other universities and Colorado State researchers are tested in the program. The laboratory has developed numerous tests and models to research tuberculosis drugs today, including specialized tests that facilitate screening large numbers of compounds within shorter time frames.



Dr. Richard Slayden

# MDR-TB challenges researchers

Before Andrew Speaker made his front page debut in 2007, most Americans had not heard of extensively drug-resistant tuberculosis (XDR-TB), but the ensuing media storm would soon guarantee almost everyone with a newspaper, radio, television, or Internet access was about to get pretty familiar with XDR-TB as well as Speaker's travel itinerary.

Speaker was the personal injury attorney who, at first, was diagnosed with multidrug-resistant tuberculosis (MDR-TB) and discouraged from traveling by the Centers for Disease Control. Nonetheless, Speaker traveled to Europe for his wedding and honeymoon, during which time further lab tests changed the diagnosis to XDR-TB, which is far more difficult to treat. Speaker was placed on a no-fly list. Upon his return to the United States, authorities put him in involuntary isolation and he was transferred to National Jewish Medical and Research Center in Denver where his diagnosis reverted back to MDR-TB, and he received the appropriate medical treatment.

While MDR-TB and XDR-TB were new acronyms to most, researchers at the Mycobacteria Research Laboratories (MRL) were well versed in the challenges presented by these particularly tenacious mycobacterial diseases and the concerns they were raising in the global health community. The fear awakened in the Speaker case simply brought to the public forum what researchers had been focusing on for years – new drugs were needed to treat drug-resistant bacterial diseases.

“If we just look at tuberculosis, there have been no new drugs developed for first-line treatment in probably 30 years,” said Dr. Richard Slayden, an Associate Professor in the Department of Microbiology, Immunology and Pathology, and member of the MRL team. “The appearance of drug-resistant tuberculosis, and other drug-resistant diseases, is driving new drug discovery forward as we seek to provide better drug options to doctors and their patients, as well as global health programs looking to prevent and treat publicly important diseases such as TB.”

Tuberculosis, caused by *Mycobacterium tuberculosis*, is an airborne infectious disease. The World Health Organization estimated 1.5 million people died from TB in 2006, mostly in developing countries with poor sanitation and restricted access to medical care. In addition, another 200,000 people with HIV died from HIV-associated TB. WHO is working to dramatically reduce the burden of TB, and halve TB deaths and prevalence by 2015, through its Stop TB Strategy and supporting the Global Plan to Stop TB. The development of new drug therapies is critical to that goal. Relatively new strategies in the drug discovery process to assist researchers in the development of novel chemotherapeutics are the use of computational biology and structural genomics.

Computational biology and structural genomics are interdisciplinary fields that apply the techniques of computer science, applied mathematics and statistics to address biological problems. Together, computational biology and structural genomics are used to identify new protein drug targets and to systematically produce accurate structural three-dimensional protein structures, which allows researchers to sort through seemingly endless possibilities of drug-protein combinations to find subsets of best candidates to progress further along the drug discovery process.

“We are examining the coding capacity of genomes of different pathogenic bacteria looking for unexploited targets, particularly unique metabolic processes in bacteria that allow them to survive, cause disease, tolerate treatment, and persist,” said Dr. Slayden. “The push is to develop novel broad-spectrum antibiotics with novel modes of action that will be effective against a diversity of bacterial illnesses.”

There are historical observations in TB drug discovery that potency of a drug does not always correlate well with treatment efficacy. How a bacterium interacts with its host affects drug performance so understanding host-pathogen interaction is an important aspect of developing the “next-generation” drugs. Researchers are studying how drug potency correlates with effectiveness in animal models of infection by molecular modeling and investigating, protein-drug residence time (how long a drug binds the target protein), drug-protein affinity, drug mode of action, and physiochemical characteristics.

MRL researchers work cooperatively with colleagues at Stony Brook University and with the

Rocky Mountain Regional Center of Excellence Genomics Proteomics Core and Animal Models Core, and the Center for BioInformatics at Colorado State University. Starting with genomic data from more than 1,000 pathogens, researchers first identify drug targets of interest. Then, via virtual screening, identify molecules that interact with the target (from a library of 8.5 million compounds) cutting off the data set at 1,000. Another analysis strategy is then applied, and the number of potential drug structures is reduced to between 15 and 20 representatives. A reiterative process of sensitivity testing and medicinal chemistry refines the compounds to a final candidate. Efficacy testing in animal models of infection is incorporated where appropriate. This comprehensive process optimizes the total number of compounds being investigated, thus streamlining the process and reducing the need for animals in early-stage development programs.

“With this collaboration, we are able to expand our capabilities, tap into more expertise, and split the responsibilities as well,” said Dr. Slayden. “Stony Brook has a team of 30 to 40 people, so we can build on each other’s experiences – both successes and failures – to move forward more quickly with drug development. We’ll be able to deliver the molecules we want that have a higher likelihood of effectiveness against the target diseases caused by bacteria.”

Pre-clinical evaluation of compounds is coordinated and contracted through the Rocky Mountain Regional Center of Excellence at Colorado State University, and through a grant from the National Institutes of Health.

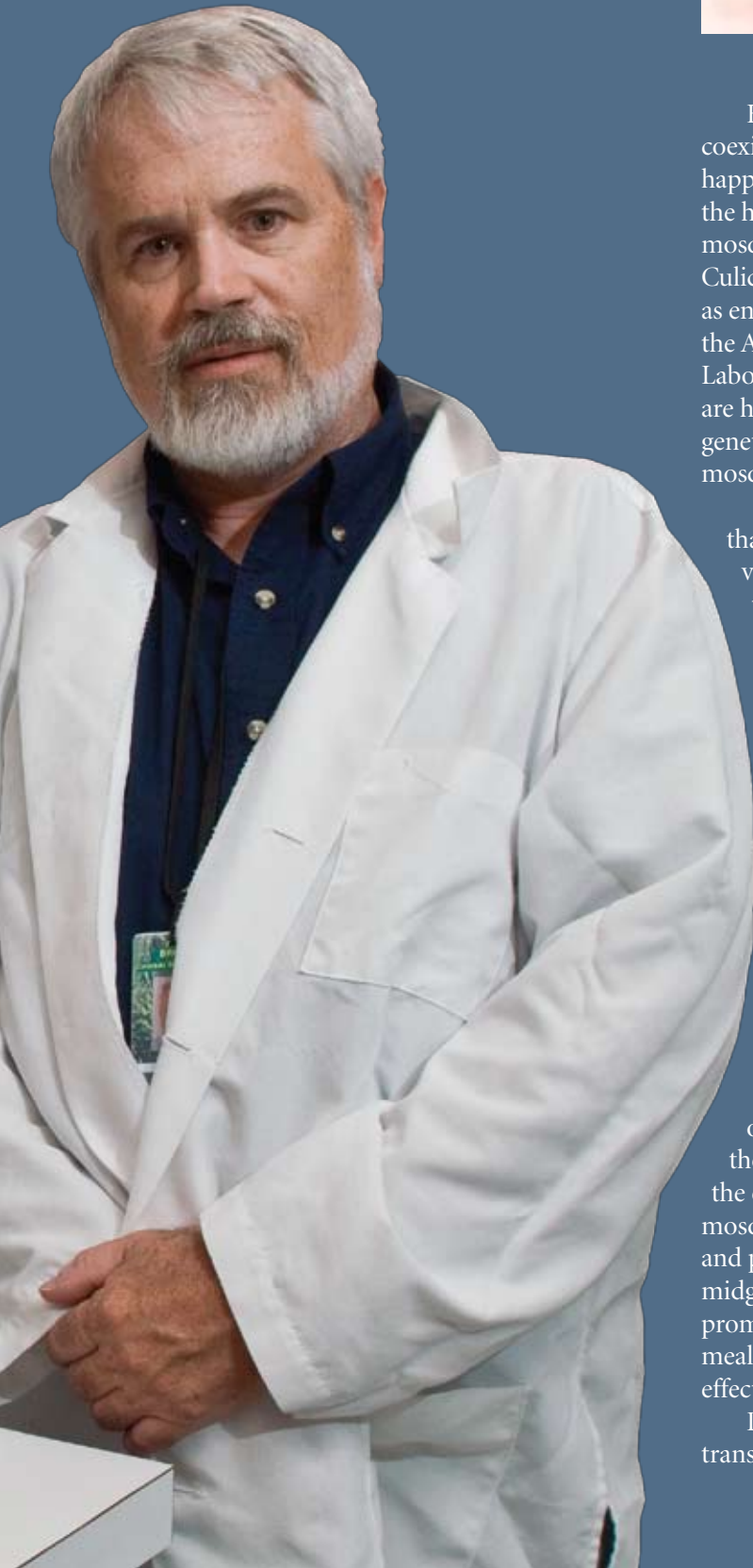
### *MDR-TB facts from the CDC*

- Multidrug-resistant tuberculosis is resistant to at least two of the best anti-TB drugs, isoniazid and rifampicin. These drugs are considered first-line drugs and are used to treat all persons with TB.
- Extensively drug-resistant tuberculosis is relatively rare and is resistant to isoniazid and rifampin, plus resistant to any fluoroquinolone and at least one of three injectable second-line drugs.
- While MDR-TB and TB are spread the same way, via droplets in the air, MDR-TB and XDR-TB strains are believed to be less fit and less transmissible.
- MDR-TB and XDR-TB have much higher mortality and morbidity rates than TB.
- Drug resistance is more common in people who do not take their TB medicine regularly or are prescribed the wrong medicine, do not take all their TB medicine, have a recurrent infection, come from areas with high incidence of drug-resistant TB, and/or have spent time with someone known to have drug-resistant TB.

# Disease-free mosquitoes?



From left: Dr. Alexander Franz, Dr. Corey Campbell, Dr. Irma Sanchez-Vargas, and Dr. Ken Olson.



Humans and mosquitoes have been coexisting on Earth for all of human history – happily for the mosquitoes, certainly not for the humans. Despite countless attempts at mosquito control, these members of the Culicidae family continue to plague humans as envoys of death and disease. Researchers at the Arthropod-borne and Infectious Diseases Laboratory (AIDL) at Colorado State University are hoping that their investigations into genetic modification will one day engineer a mosquito incapable of spreading disease.

“We are seeking to develop methods that control the transmission of dengue viruses using genetic techniques, including those that may block virus transmission by mosquitoes,” said Dr. Ken Olson, a Professor in the Department of Microbiology, Immunology and Pathology, and Director of AIDL. “Our research offers promising results for halting the spread of this disease by disarming the mosquito’s ability to contract and transmit the dengue type-2 virus, stopping the virus in its tracks. It demonstrates that it’s possible to develop a mosquito that won’t transmit disease to people by genetically triggering the RNA interference pathway.”

Researchers manipulated the DNA of mosquito embryos by introducing the DNA of a dengue-resistant gene into the embryo. The research team cuts the mosquito DNA in embryonic germ line cells and pastes in the effector gene linked to a midgut-specific promoter. Activity of the promoter is dependent on ingestion of a blood meal. Germ line transformation makes the effector gene heritable by future generations.

Dr. Olson’s research group is developing transgenic mosquitoes that are pathogen-

Researchers hope investigations into genetic modification will one day engineer a mosquito incapable of spreading disease.

resistant as well as new methods to identify components of the RNAi pathway, studying the molecular epidemiology of dengue viruses, and identifying and characterizing important

interactions between the vector and the virus. One challenge facing the researchers is that there are four distinct dengue virus serotypes, and they can make the mosquito resistant to one serotype. What they are searching for is a piece of genetic material common to all four serotypes that will still produce resistance to infection.

“We’ve started the transgenic work in that area and have a couple of lines that may be promising,” said Dr. Olson. “Once we have the transgenic material in our weakened mosquito line, we’ll take it into a number of wild lines, which are more fit. These tests should reflect more accurately what’s happening in the wild.”

As a part of the work, researchers are developing a site in Tapachula, Mexico, for conducting contained field trials. Efforts are

underway to obtain all necessary approvals and plans for cage construction are in development. Once constructed, researchers will be able to use the cage system to see how fit the mosquitoes are in the wild and how well they adapt to the environment, as well as how the environment adapts to the transgenic mosquitoes. The ultimate goal of the project is a population replacement strategy, moving the transgenic mosquito into the wild population, eliminating the mosquitoes’ ability to transmit dengue viruses. Drs. Alexander Franz, Imma Sanchez-Vargas, and Corey Campbell, all working with Dr. Olson at AIDL, have been critical to the population development strategies being developed at CSU.

Dengue fever is endemic to about 100 countries including the United States, Cuba, Africa, Columbia, Brazil, Puerto Rico, and the Caribbean Islands. Dengue fever infects 100 million people each year and has a case-fatality rate of about 5 percent if left untreated, primarily in children and young adults. Dengue hemorrhagic fever is the most severe form of dengue and can be fatal if not properly and promptly treated.

Dr. Olson’s work in dengue fever is part of the Bill and Melinda Gates Foundation Grand Challenges in Global Health, which funded







43 grants for \$436 million in 2005. Dr. Olson is part of global team led by Dr. Anthony James, the project's Principal Investigator, from the University of California at Irvine. Other research partners include North Carolina State University, Oxitec Ltd. in the United Kingdom, University of California at Davis, University of Notre Dame, Texas A&M, Fundacao Oswaldo Cruz in Brazil, Cornell University, California Institute of Technology, and Virginia Tech. At CSU, other collaborators include Dr. William C. Black and Dr. Jonathan O. Carlson, both in the Department of Microbiology, Immunology and Pathology. The stated goal of the grant is to control insect vectors by developing a genetic strategy to deplete or incapacitate a disease-transmitting insect population.

"The exciting thing about being a part of the Grand Challenges is the opportunity to work with others around the world to develop novel genetic strategies for stopping transmission of dengue viruses," said Dr. Olson, whose initial work in transgenics was funded by the National Institutes of Health. "By sharing our experiences and expertise, we are able to move the work forward to meet the goal of new approaches to controlling vector-borne diseases."

## Mosquitoes stop construction of Panama Canal

Carlos Finlay, a Cuban doctor and scientist, was the first to theorize a connection between mosquitoes and disease, specifically yellow fever, in 1881. In 1900, Army Major Walter Reed led a team of researchers that confirmed the theory through experiments in Cuba, including exposing human subjects to yellow fever. Dr. Reed also proved the vector-borne infectious route of malaria. The confirmation opened new fields in epidemiology and biosciences, and most immediately allowed the United States to resume work on the Panama Canal.

The concept of the Panama Canal dates back to the early 16th century, but the first attempt at construction wasn't begun until 1880 under the French. Yellow fever proved to be too formidable a foe to overcome so efforts were abandoned (nearly 22,000 workers died during this first attempt). Once the source of yellow fever was understood, the United States undertook the challenge of building the Panama Canal, starting in 1904 and completing the project in 1914. The building of the canal was still plagued by problems (including malaria, yellow fever, and landslides), but the numbers of workers who lost their lives during this time was greatly reduced (5,500). The mosquito species *Aedes aegypti* responsible for transmitting dengue virus in many parts of the world today is the same species spreading yellow fever back then.



# Advancing studies in avian influenza

“The good news is that we are making progress in bringing technology and training to a part of the world where avian influenza is not only a potential health risk, but a real health risk in terms of the local economies and food supply.”

As Dr. Kristy Pabilonia is finding out the hard way, establishing a veterinary research center in Indonesia comes with a lot of government red tape and bureaucracy – especially when the focus of the research center is avian influenza, a politically sensitive issue and constant public health threat. In a country

where poultry is one of the proteins of choice, avian influenza can destabilize a primary food source and cause major disruption to the small-market based sales of poultry, to say nothing of the fear of mutation and establishment of the virus in the human population.

The research project will be a collaborative effort between Colorado State University and two Indonesian institutions – the Center for Indonesian Veterinary Analytical Studies (CIVAS) and Institut Pertanian Bogor (Bogor Agricultural University or IPB). Colorado State University and IPB signed a memorandum of understanding in 2008 to

create an avian influenza research laboratory at the IPB veterinary college. This collaboration will help advance studies in avian influenza while helping Indonesia develop novel ways

to approach avian influenza. Dr. Pabilonia, an Assistant Professor in the Department of Microbiology, Immunology and Pathology, heads up the Colorado Avian Disease Surveillance Program for Colorado and is the lead investigator on the IPB project – including setting up and providing mentorship to CIVAS.

Highly pathogenic avian influenza is highly contagious among domesticated birds, causing sickness and death. Infection with avian influenza viruses in domestic poultry causes two main forms of disease that are distinguished by low and high pathogenicity. The highly pathogenic form spreads rapidly through flocks and has a mortality rate that can reach 90 to 100 percent within 48 hours. De-population of entire flocks (and neighboring flocks) is often the method of choice to control an outbreak.

“Starting in 2003, millions of bird deaths were attributed to outbreaks of avian influenza type H5N1 in Southeast Asia, both through disease and de-population to prevent spread of disease,” said Dr. Pabilonia. “In 2003, the first human cases were reported. Since that time, there have been 385 cases in humans with 243 deaths worldwide. Concerns began to rise that H5N1 would mutate, be able to spread from human to human, and be sustained in the human population leading to a catastrophic influenza outbreak. Because of those concerns, more resources have been put into the study of avian influenza and its epidemiology, as well as basic science studies to better understand avian influenza viruses. Our work in Indonesia is an expansion of those early studies, enabling us to study the disease where it is endemic.”

In 2006, researchers in the College of Veterinary Medicine and Biomedical Sciences were awarded \$2.6 million from the Centers for Disease Control to study how interactions between humans and birds may lead to more widespread transmission of avian influenza. The three-year study focuses on the western United States where H5N1 avian influenza has not been detected and in Indonesia where the virus has been detected in both birds and humans. Researchers study how infected humans interact with infected birds in Indonesia and study the impacts that suboptimal vaccinations given to birds in that area may contribute to elevated risk to humans. Dr. Richard A. Bowen, a Professor in the Department of Biomedical Sciences, is Principal Investigator on the grant. CSU also has a collaborative project with the University of Colorado Health Sciences Center in Denver which is doing human studies in Indonesia while CSU is focusing on animal studies.

“It is abundantly clear that we need to better understand avian flu in humans and animals, as well as the interactions between these two groups in order to devise and implement effective prevention and control strategies,” said Dr. Bowen, who is a member of the Animal Reproduction and Biotechnology Laboratory. “It is also critical to understand how the differences in ecological areas may affect these interactions.”

Dr. Pabilonia has traveled to Indonesia numerous times not only to set up research projects with IPB and CIVAS but also to help teach avian epidemiology training courses through a cooperative agreement with the United States Department of Agriculture. These courses are coordinated by Dr. Mo Salman, a Professor in the Department of Clinical Sciences. At the training courses, veterinarians, government officials, producers, diagnosticians, and public health officers learn about basic epidemiology as well as how to establish surveillance programs to track avian influenza and create awareness of potential outbreaks. Dr. Pabilonia’s first experiences in Indonesia were through these training courses, which helped her not only gain an understanding of the avian situation in Indonesia but also helped her set up projects with course participants.

A challenge was working through the laboratory system in Indonesia, so the

importance of establishing a dedicated laboratory as well as training laboratory staff and procuring equipment became part of Dr. Pabilonia’s outreach efforts. She is faculty advisor to the CIVAS laboratory.

She also regularly travels to villages and small markets throughout Indonesia to better understand the travel patterns of domestic fowl, and acquaint herself with the cultural aspects of domestic ducks and chickens. Dr. Pabilonia, in the name of research and cultural understanding, has eaten curried chicken lung, liver, and kidneys, deep-fried chicken intestines, and chicken feet. She draws the line at fish which, she says, mystifies her hosts who don’t understand the concept of not liking fish. It’s worth the effort, Dr. Pabilonia said, because the work in Indonesia will help researchers develop a greater understanding of the dynamics of avian influenza.

“The picture now in Indonesia is that avian influenza is still endemic, it is still spreading and traveling through markets and village flocks, and we still have concerns about mutation and global expansion,” said Dr. Pabilonia. “The good news is that we are making progress in bringing technology and training to a part of the world where avian influenza is not only a potential health risk, but a real health risk in terms of the local economies and food supply. With the laboratory, we will be able to do avian influenza diagnostics and establish good laboratory practices, which will help us obtain good, reliable results for our research projects.”



Dr. Kristi Pabilonia



# Bats provide clues to rabies outbreaks

The bat has long been a symbol of the night in Western culture, as well as the primary animal player associated with fictional characters of the night like vampires and superheroes. But it wasn't until the 1950s that bats became associated with something more real and a little bit scarier than vampires – rabies. Though rabies is readily prevented with a vaccination protocol, the disease continues to take tens of thousands of lives worldwide, and new outbreaks in the United States are resulting in calls for increased research into the natural reservoirs of rabies, including bats.

Researchers at the College of Veterinary Medicine and Biomedical Sciences, with funding from the National Institutes of Health and the National Science Foundation, are working to understand the prevalence of rabies in bat populations, and how human-bat interactions impact the incidence of rabies. CSU researchers are working with the United States Geological Survey and the Centers for Disease Control and Prevention to understand rabies transmission in urban ecosystems.

“We don't see big outbreaks of rabies in bat populations with big die-offs,” said Dr. Richard Bowen, a Professor in the Department of Biomedical Sciences and lead investigator on the four-year grant. “But we know that bats are a natural reservoir for a large number of zoonotic pathogens, including rabies. Our studies try to quantify the level of rabies exposure in bats and determine the impact of that exposure in the local bat population.”

Bats are natural reservoirs in part because of their high mobility, broad distribution, and social behavior. Many species also appear to have a high tolerance for harboring pathogens without developing disease. Dr. Bowen's research team, which involved many students in the Professional Veterinary Medical Program, would go out at night to catch bats in urban areas in and around Fort Collins, bring them back to the lab to collect blood samples and oral swabs, and implant PIT tags that would relay movement as the bats flew under readers placed above the entrance of their roosts.

“During the study, we collected tons of information on bat movement and biology,” said Dr. Bowen, of the approximately 2,500 bats tracked in Fort Collins. “As far as rabies was concerned, we thought of it as invariably fatal, but found that in some roosts, up to 25 percent of bats had antibodies to rabies virus, indicating that they had been exposed, but had not developed rabies disease. We did not, however, find evidence that bats can carry rabies for a long time as there is a sustained die-off, but not in what we see with an outbreak. It seems to be an endemic disease managed in the population.”

One interesting result of the work was the migration of bats out of Fort Collins each year in the early fall. No one knew precisely where they went, but radiotracking studies revealed that many of the bats migrate up Poudre Canyon each year to hibernate. One bat was located roughly 70 km from Fort Collins.

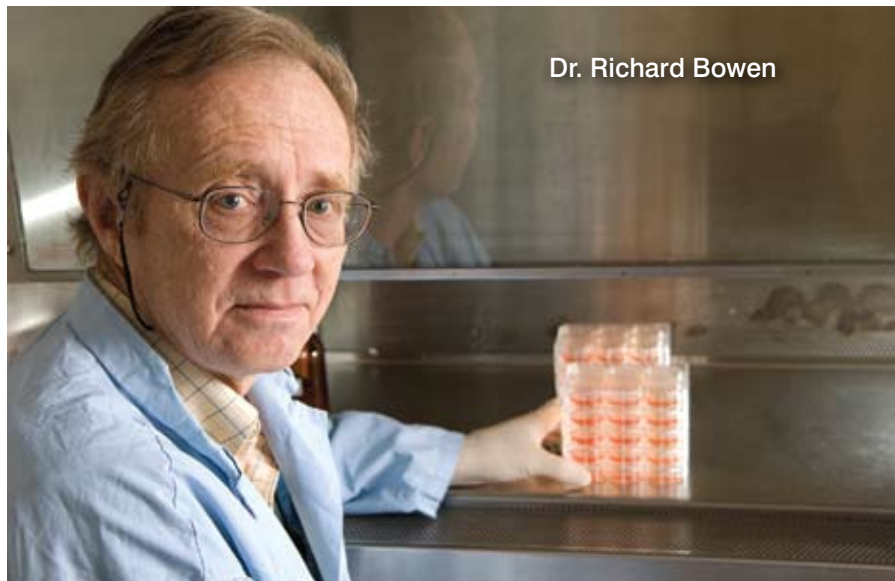
Dr. Bowen also is involved with a separate study looking at raccoon rabies which, once confined to the southeast United States, is now a significant problem in the Northeast and spreading to the Midwest. Working with the National Wildlife Research Center based in Fort Collins, the research team is testing vaccine-laden baits to determine how long immunity lasts in wild raccoon populations once the bait is ingested. The work is showing some protection at up to 18 months after the vaccine is administered.

“Raccoon rabies was pretty rare in the Northeast, but there has been an expanding epidemic since the mid-1970s,” said Dr. Bowen. “The virus variant was believed to have been brought north by hunters transplanting raccoons to help replenish the declining northern population. Unfortunately, some of the transplanted raccoons were apparently infected with rabies virus. Rabies is commonly diagnosed in raccoons from densely populated regions (including New York, Pennsylvania, and Virginia), so the interaction between humans and raccoons is of great concern.”

Of course, one advantage of being bit by a rabid raccoon is that you know you’ve been bit and can seek treatment (if you can consider that an advantage). Almost all rabies deaths (22 recorded deaths from 1980-1997) in the United States are attributed to bat bites and, in a number of cases, the victim did not know they had been bitten by an infected bat. Because rabies is ubiquitous in

bat populations, Dr. Bowen said, individuals need to assume that every bat is positive and react appropriately (avoiding contact, safe capture, reporting to health authorities, etc.)

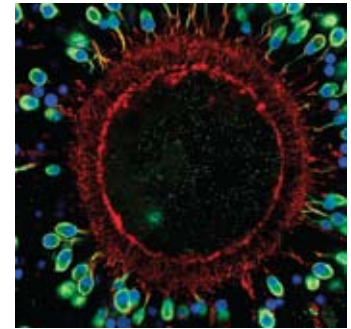
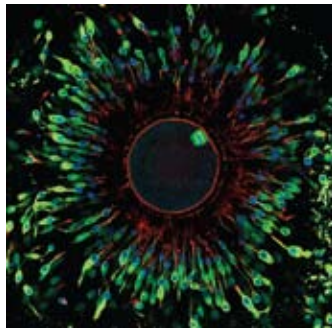
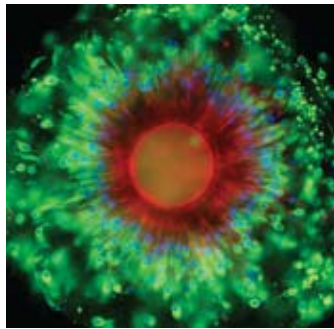
Thanks to Louis Pasteur and Emile Roux, who developed the first vaccine for rabies in 1885, most Americans and their companion animals remain relatively free from the threat of rabies. But this is not the case in other parts of the world. Almost all 55,000 annual human deaths caused by rabies are in Asia and Africa, with the largest number of cases reported in India. Dr. Bowen, who also is involved with bat studies in Mexico, said it continues to be an underappreciated but important health threat, with fundamental studies still needed on how rabies affects the brain, as well as continued studies on bat populations and their ability to harbor infectious diseases including not only rabies, but severe acute respiratory syndrome (SARS) virus, as well as the ebola and Nipah viruses.



Dr. Richard Bowen

“Bats are becoming a hot topic because they harbor some pretty scary pathogens,” said Dr. Bowen. “People are wondering what’s going on with bats. But it’s easy to see that with more than 600 species of bats, adaptation to a diversity of ecosystems, including urban ecosystems, we’re going to pick up some of their pathogens. Bats play an important and vital role in the environment as insectivores and pollinators, but we need to better understand their role as reservoirs of infection so that we can better protect ourselves from the diseases that are a part of their world and ours.”

# Aging mares shed light on infertility



Scanning confocal micrographs of horse oocytes

As mares grow older, their reproductive viability grows less robust. Researchers at the Animal Reproduction and Biotechnology Laboratory, in collaboration with the University of Kansas Medical Center, are studying interactions between follicles and oocytes, trying to determine how these mechanisms change with age and how that impacts fertility. They hope that what they find also can serve as a model for investigations into human infertility.

“The signaling mechanisms between the follicle and the oocyte change with age,” said Dr. Elaine Carnevale, an Assistant Professor in the Department of Biomedical Sciences and member of the Equine Reproduction Laboratory (ERL). “We know that the oocytes are not as viable, and signaling between the follicle and oocyte is not synchronized properly. The maturation signals in the old mares’ follicles are altered, and the egg appears to be maturing too fast for the follicle. Signals between the egg and the follicle assure that the follicle ovulates and releases the egg at the correct stage of maturity. The molecular signaling system is extraordinarily

complicated and, as the mare ages, is disrupted. The lack of synchronicity means an under-expression or over-expression of signals creating obstacles to normal maturation.”

Dr. Carnevale’s team is now working with Dr. David Albertini at the University of Kansas Medical Center to look at structural changes that are occurring in the egg and the surrounding cells during maturation.

The cells are harvested and fixed at the ERL, then sent to Dr. Albertini where they are further prepared and images captured throughout the target cells. With confocal microscopy, the researchers can examine cell-to-cell connections, chromosome alignment, cell polarity, and other areas of interest.

“The images give an incredible picture of what is occurring within the cells,” said Dr. Carnevale. “We can compare oocytes and their associated cells from mares of different ages and get an idea of the differences. Longer term, we hope to manipulate some of the signaling pathways and other important factors to improve viability and health of offspring.”

Dr. Albertini, who works in a human medical center, sees value in the model of older mares for human fertility concerns.

“There is a pressing need to identify appropriate animal models for reproductive aging in humans,” said Dr. Albertini. “Our reliance on the use of rodents as models for development of assisted reproductive techniques in humans has been unfortunate given the striking differences in reproductive physiology in these two organisms. This collaboration with Dr. Carnevale has not only allowed for detailed studies of reproductive aging in the horse but is providing insights into oocyte quality that will be directly translatable to the treatment of human infertility.”

Dr. Carnevale said the horse is an excellent model for the older woman because in many ways similar changes occur with reproductive aging. The horse is long lived, goes through similar cyclic changes, eventually stops cycling (similar to menopause in women), and has the advantage of being very easy to work with, given

the large size of the horse’s follicles and ease of harvesting cells from the follicle. People who want additional offspring from their valuable, older mares are facing similar challenges to women who want children later in life.

“The work with Dr. Albertini gives us another component to our research program,” said Dr. Carnevale. “We have a clinical program, and we know the age of a mare will affect our success in producing offspring. We have done molecular studies (working with PhD student Dr. Fernando Campos-Chillon) to further our understanding of what is happening at the cellular level. And now we want to examine how aging affects the structure and interaction between the oocyte and follicular cells, showing us what is or is not different. Dr. Albertini is an expert in this area of study, and we are fortunate to collaborate with his laboratory and expand our knowledge of maternal aging.”



Dr. Elaine Carnevale



Dr. Wayne McIlwraith

## Making race tracks safer

If you could recreate and graphically visualize the forces of a horse's hoof on a racetrack, would you be able to build or maintain a safer racetrack? With that safer racetrack, would you be able to reduce the rate of catastrophic injuries in the ultra-competitive world of Thoroughbred racing? Researchers at Colorado State University and the University of Maine are hoping to answer both those questions with a resounding yes, giving hope to those who want to help make the sport of horseracing safer for those doing the racing.

"When a catastrophic injury happens, it's common practice to blame the race track," said Dr. Wayne McIlwraith, Director of the Gail Holmes Equine Orthopaedic Research Center at the College of Veterinary Medicine and Biomedical Sciences. "We can look at statistics and see that different surfaces have different rates of injury but, until recently, we really couldn't understand what was happening as a

horse was running on a particular track, and how that might affect the incidence of injury."

Three types of tracks are used in horse racing: turf, dirt and synthetic. Turf tracks have the lowest rates of catastrophic injuries with studies in the United Kingdom showing an incidence of 0.38 deaths/1000 starts (29 out of 77,059). In the United States, recent studies reported 1.47 deaths/1,000 starts on synthetics and 2.07 deaths/1,000 on dirt tracks.

"We know that synthetic tracks can help reduce the rates of catastrophic injuries, but they are not reducing the rates as much as hoped," said Dr. McIlwraith. "Race track managers initially thought that synthetic tracks were a panacea – no maintenance, no problem in the rain, safe for training, and expected decreased injury rates. Overall, these rates have come down, but not as drastically as promised."

Part of the problem with synthetic tracks is that most were developed in England,



for the English climate. The systems are highly specialized, down to the size of the grains of sand, and attention to installation is critical. Making the move to the United States proved somewhat problematic for the synthetic tracks. Sand grains were variable in size, components degraded with UV exposure, and high temperatures melted the wax in the synthetic track creating instability and uncertainty regarding performance.

“In 2000, Dr. Mick Peterson from the University of Maine and I began working together to develop a system that would replicate the loading of a hoof on the track,” said Dr. McIlwraith. “Previous track measurement systems have used some type of light-weight drop to measure the vertical component of the horse’s hoof. A second and equally important element is the loading during the motion of the horse that is horizontal; this depends on the shear strength of the track surface. The machine sees the track the way a horse’s hoof does.”

Dr. Peterson, a Professor of Engineering, and Dr. McIlwraith developed tests that would reproduce the loads and speeds of a horse’s hoofs at a gallop and measure the response on a small surface area. The hoof-shaped impactor reproduces the hoof velocity in vertical and horizontal directions and the effect of mass at the moment of impact at a gallop. Sensors on the device record the loads and decelerations on impact with the ground. The system measures the effect of the deeper track layers on the impact load on the hoof. Dr. McIlwraith noted that race track managers want to keep their tracks safe and are eager for the data and training to help them do just that.

“Using this system and another designed to measure the base of the dirt or synthetic race track in terms of slope and irregularity (using Doppler radar), we hope to bring testing mechanisms to the track to evaluate track conditions objectively,” said Dr. McIlwraith. “With additional data, we will be able to characterize the ideal race track and hope to see a correlating decrease in the number of injuries from race track surface. Of course, there are other areas we are investigating at the Equine Orthopaedic Center, including genetic susceptibility to injury, early diagnosis of bone and joint disease, novel therapies, conformation, and rehabilitation after injury.”

Race track surface research at the Equine Orthopaedic Center is sponsored in part by

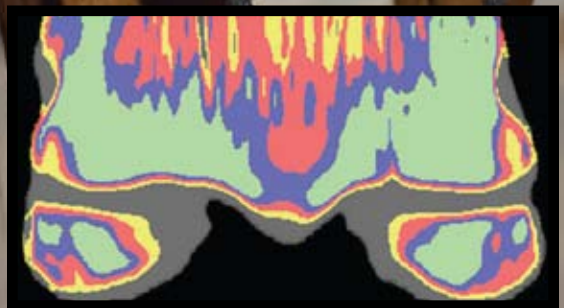
the Grayson-Jockey Club Research Foundation and the American Quarter Horse Foundation. In June, Drs. McIlwraith and Peterson were awarded the second annual Elastikon Equine Research Award, funded through a grant made by Johnson & Johnson Consumer Products Company to Grayson-Jockey Club Research Foundation, for their research designed to enhance the safety of race tracks. Dr. McIlwraith, who also holds the Barbara Cox Anthony University Endowed Chair in Orthopaedics at CSU, participated in the Grayson-Jockey Club Foundation sponsored Welfare Safety Summits in October 2006 and March 2008. He also serves as Chair of the Subcommittee on Race Track Surfaces. In June 2008, Dr. McIlwraith testified before Congress during a special hearing prompted by the death of Eight Belles at the Kentucky Derby.

“Race track surface performance is just one component of making horse racing safer for all horses,” said Dr. McIlwraith, who also has received funding from the American Quarter Horse Association for race track research. “More than anything, we want to see a significant decrease in the number of injuries and deaths, and we think proper investigations into and recommendations on track quality, along with establishing performance standards, will help to make that happen.”





Dr. Chris Kawcak  
Equine Surgery



Dr. Chris Kawcak; inset at right, 3-D model of bone indicating loss of density.

# Equine 3-D modeling

Three-dimensional modeling is common in the fields of engineering, architecture and human medicine, but now researchers at the Equine Orthopaedic Research Center (EORC) are using 3-D modeling with diagnostic imagery to better understand how joint shape in horses may impact the risk of injury.

The study has evolved from work originally funded by the Colorado Racing Commission. During the last 12 years, deceased Colorado racehorses have been brought to Colorado State University for necropsy and researchers have noticed a pattern correlating injury to joint shape. Horses with significant fetlock damage seemed to have abnormally shaped condyles and minimal support from the soft tissues, including tendons and ligaments, and a compromised supply of blood.

“Looking at pictures of the cannon bone, we can see that where the fetlock joint has a significant amount of damage there is usually an abnormal shape to the condyles,” said Dr. Chris Kawcak, an Associate Professor in the Department of Clinical Sciences and member of the EORC team. “We began to ask if we could develop parameters for using the shape of the joint to help determine the risk of injury. Having that knowledge ahead of time may help us prevent catastrophic injuries in racehorses by proactively treating those horses or taking them off the race track altogether.”

The condyle is the bulbous bottom of the cannon bone that fits into the fetlock joint. Condylar fractures can be repaired surgically, Dr. Kawcak noted, but the prognosis for survival and a return to racing soundness depends on the severity of injury. Asymmetry in the condyles seems to increase the risk of condylar fracture, compromising the fetlock joint, cannon bone and other structures in the front limbs. Last year, the EORC research team received a grant from the Grayson-Jockey Club Research Foundation to study a large group of racehorse specimens to further examine the pattern of abnormal geometry in the condyles. Working cooperatively with Dr. Kenton Morgan at the University of Liverpool, England, and Dr. Tim Parkin at the University of Glasgow, Scotland, Dr. Kawcak’s team is studying 150 fetlocks from

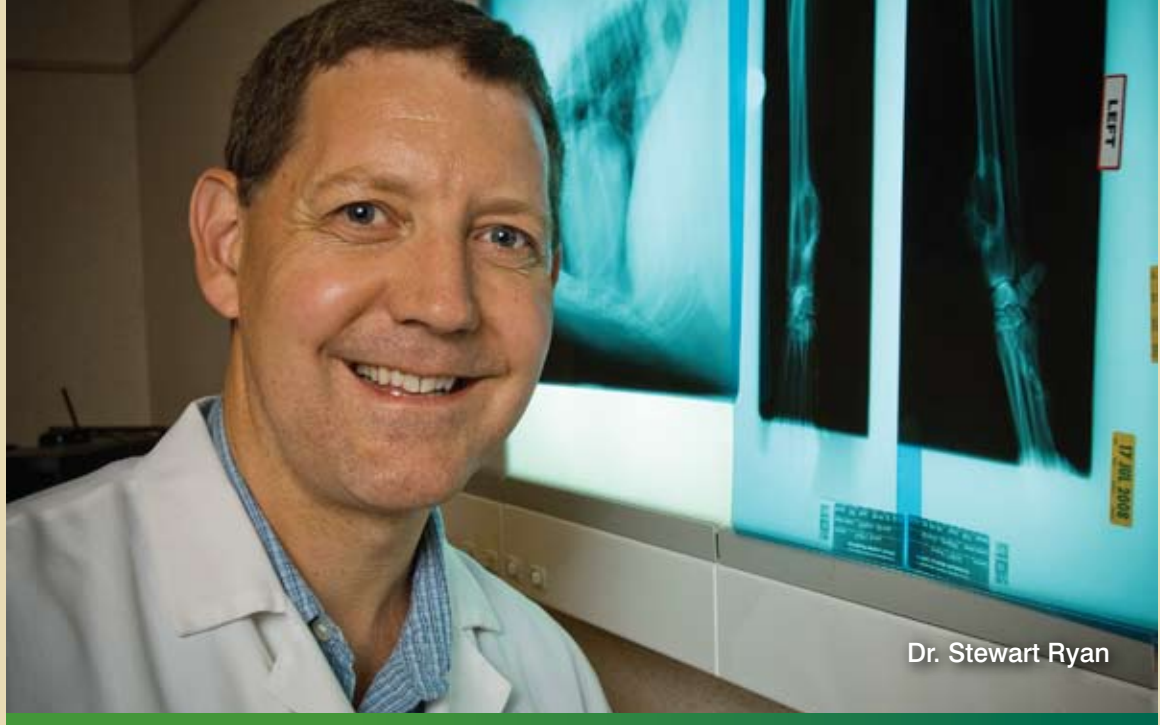
racehorses, 50 of which had condylar fractures, 50 with arthritis, and 50 normal specimens.

“The English have an amazing library of specimens and recordkeeping, along with epidemiological information, so using their samples and our studies we are able to put together a picture of the relationship between injury and joint shape,” said Dr. Kawcak. “They send us CAT scans of the fetlock joints, and we’ve built a software program that takes the scans and builds a three-dimensional model. We can apply special modeling to look at bone density, examine joint surfaces, look at the curvature of the back of the fetlock, and see how a number of different factors relate to bone abnormality.”

On the computer modeling forefront, Dr. Kawcak’s team is drawing from a number of experts in the field. Dr. Christian Puttlitz, an Associate Professor in the Department of Mechanical Engineering at CSU, is on the grant, as is Dr. Marcus Pandey from the University of Melbourne, Australia, who is a leader in musculoskeletal modeling in humans. Graduate Student Katrina Easton is working with the Biomedical Engineering Program at CSU to help build the model, working to calculate different stresses going through the joint and the muscle forces around the joint.

“A lot of this computer work is way outside of our (EORC) expertise,” said Dr. Kawcak. “We knew what we wanted, but didn’t have the knowledge or the technology to get there. Drs. Pandey and Puttlitz, and Katrina, are building us a very robust model that not only has applications in equine medicine, but also has promising applications in humans where abnormal joint shape can also lead to common problems in the knees and hips. This work simply wouldn’t be possible without them.”

In the short term, the gold standard for determining at-risk horses remains biomarkers and blood tests that reveal early indicators of injury. Dr. Kawcak hopes that eventually the joint shape model can be used clinically to give veterinarians the information they need to determine the chance of a fracture and make the best decisions for the health and well-being of the horse from there.



Dr. Stewart Ryan

# Enhancing safety of donated tissues

The Colorado State University Animal Cancer Center (ACC) has long been a leader in the field of limb-sparing surgery, where donor bone is used to replace diseased bone in canine patients with osteosarcoma (bone cancer). Through a research partnership with AlloSource, ACC researchers are helping to make similar tissue transplants safer while advancing the science of allograft transplantation for all species.

An allograft is cells, tissues, or organs that are transplanted from one person to another (or one dog to another at the ACC). The types of allografts routinely used in human patients include skin, corneal, heart and heart valves, liver, kidney, bone and cartilage, demineralized bone matrix, ligaments and tendons. The tissues come from deceased donors and are used to enhance or save other people's lives, from using skin grafts for burn victims to using donor bone to save the arm of a teenager, from repairing the knee of a young athlete to restoring vision when eyes are failing.

"During my research fellowship, I developed an excellent relationship with

AlloSource working on several projects" said Dr. Stewart Ryan, an Assistant Professor in the Department of Clinical Sciences and member of the ACC team. "AlloSource is one of the nation's largest tissue banks and a leader in the allograft recovery, processing and distribution field. We enjoy a free exchange of ideas that has erased the boundary between an external non-profit company and an academic institution. This has allowed us to work creatively to design research projects that help the clients of both AlloSource and the ACC."

AlloSource and CSU have a Master Research Agreement that provides a clear framework for the ongoing research relationship with a set of ground rules covering publication rights and intellectual property. Dr. Ryan noted that the partnership has led to numerous research projects including, most recently, a study to identify potential risk factors for bacterial contamination of donor allografts, another study focusing on the translocation of bacteria following death, and a third study looking at the development of an anti-adhesion membrane for spinal surgery.

Osteochondral allografts involve the transplantation of a piece of articular cartilage and attached subchondral bone from a cadaver donor to a damaged region of the articular surface of a joint to restore function. The goal is to provide viable chondrocytes and supporting bone that will be sufficient to maintain the cartilage matrix, relieve pain, and reduce further damage to the articular surface of the joint. These types of cartilage transplant procedures are being used with increasing frequency in the treatment of individuals with disabling cartilage injury or disease.

In the statistical risk factor study, Dr. Ryan works with Dr. Paul Morley, an Assistant Professor in the Department of Clinical Sciences and epidemiologist with the Animal Population Health Institute at CSU. They are identifying potential risk factors for allograft contamination of donor tissues used in joint restoration. In the study, 850 donors were analyzed for donor factors (such as age and gender) and tissue recovery factors (including autopsy procedure performed or not, seasonal effects, time of death to cool, and time of death to recovery) to determine parameters for joint restoration donors, helping to conserve time and resources by focusing on low-risk donors. The goal of the study is to establish statistically supported guidelines for donor suitability as joint restoration tissue donors and possibly identify and modify tissue recovery practices that will increase the number of allografts produced.

“Recently, osteochondral allograft procedures have become increasingly popular in the orthopaedic community,” said Dr. Ryan. “In an osteochondral allograft, where we are transplanting cartilage and bone, we can’t irradiate or sterilize the tissue as we can do with other allografts because these processes adversely affect the biomechanical properties of cartilage and the viability of the chondrocytes, so the risk of disease transmission is always a potential concern.

“Rigorous bacteriological testing of allograft tissues at recovery and during processing is performed to decrease this risk. Too many osteochondral allografts are rejected for transplantation because of bacterial contamination which is a poor use of the gift of donation, as well as time, financial and human resources. With this study, we hope to ensure a positive outcome for patients, as well as identify factors that will lead to better use of resources

while increasing the number of allografts available for transplantation in recipients.”

In another related study, researchers are investigating the phenomenon of bacterial translocation after death and how highly infectious bacteria move through the body. Using a rodent model, bacterial solutions are tagged with a marker, fluorescently labeling the bacteria. Following death, the bacteria are followed through the body using a special imaging camera at multiple time points. Dr. Ryan noted, for example, in humans a highly infectious bacteria can migrate from the respiratory or gastrointestinal tract to regions where these bacteria are not normally found, compromising suitability of those tissues for transplantation. Tissue collection and cooling are important factors in bacterial translocation.

“Other studies have shown that if someone dies of a chronic disease, the risk of bacterial translocation is higher,” said Dr. Ryan. “If they die of trauma and there is damage to the organs that may cause an increased risk due to shaking and impact on the internal organs. Right now, we are not able to track bacteria as well as we would like, but we are working on refining the model for application as a means to trial novel decontamination procedures.”

Dr. Simon Turner, a Professor in the Department of Clinical Sciences, is working on the third project, testing an anti-adhesion membrane for spinal surgery developed by AlloSource using sheep as a model. Postoperative fibrosis (the development of scar tissue) is a natural consequence of wound healing but can cause problems when the scarring adheres to nerve roots and the dura mater (a protective layer of the meninges surrounding the brain and spinal cord) following surgery on the spinal cord. Dr. Turner’s efforts will determine the effectiveness of reducing dural adhesions using this novel allograft tissue developed by AlloSource.

“These partnerships provide a new archetype for enhanced biomedical research, to quickly move academic research into the real world benefitting both humans and animals.”

*Dr. Stewart Ryan*



Dr. John Zimbrick with tanks of medaka

# Understanding radiation damage and repair

The damaging effects of radiation on the DNA of cells are well documented. What is less understood is why the cells of some individuals are more sensitive to radiation exposure while cells of others are more resistant to those effects, or how low doses of radiation over longer periods of time affect DNA damage and repair. A very small Japanese fish is helping researchers at Colorado State University to answer these very big questions, along with other mysteries of radiation exposure and DNA damage and repair.

The medaka is a small ricefish found in the rice paddies of Southeast Asia and is popular in aquariums (a school of medaka

also traveled into space in 1994 aboard the space shuttle Columbia). The medaka, *Oryzias latipes*, is a common model organism used in biological research because it is simple, tolerant of temperature changes, short-lived, reproductively prolific, hardy, and easy to rear in the laboratory. It can withstand cold and can be shipped easily, important to the research work in Dr. John Zimbrick's laboratory because his fish routinely have to make the trek from Georgia to Colorado. Working with the medaka fish has the added benefit of using a low-cost model system to conduct experiments that would be cost-prohibitive with a mammalian model.

“In our laboratory, we are using our fish to study the genetic effects of low doses of radiation, doses that would be similar to the doses radiation workers could be allowed to get over several years,” said Dr. Zimbrick, a Professor in the Department of Environmental and Radiological Health Sciences. “In addition, we are searching for so-called transgenerational effects in the form of mutations occurring in the offspring as a result of the exposures to the parents. For many years, the Department of Energy funded research into high-dose effects of radiation, but not too much attention was paid to low-dose effects. Legal limits for the doses workers could be exposed to were established at levels that will not produce detectable harmful effects, but few the studies have been done that show what the genetic effects of those doses might be over longer periods of time covering one or more generations of offspring.”

Dr. Zimbrick is working in collaboration with the University of Georgia’s Savannah River Ecology Laboratory (SREL) where the medaka are exposed to set levels of radiation. The collaboration with SREL gives researchers access to radiation facilities that they otherwise would not have, helping to expand and enhance their research program at a relatively low cost to the laboratory. SREL has a specially designed outdoor facility that consists of a series of large, circular fish tanks each with a radiation source at the end of a rod mounted above the center of the tank. Groups of fish are contained in screen buckets in the tanks and their location in the tanks determines the exposure for each group. Radiation exposure is carefully measured to determine accurate doses for each group.

Following exposure, the fish are shipped to CSU where researchers are conducting transgenerational studies, looking for DNA damage, changes in gene activity and mutations in first through sixth generations of the medaka. To date, they have data for the first three generations, and samples for the fourth and fifth.

“A major early finding is that if we irradiate parents and then examine the first, second and third generations, we see an increase in special mutations called micro-satellite DNA mutations in the offspring, which we can detect if there is a change in even one of the DNA bases,” said Dr. Zimbrick.

Dr. Zimbrick said it is possible that chronic radiation exposure of parents could result

in still higher mutation rates in even sixth and seventh generations, but the biological effects from these would have to be of such nature that the future generations survive in spite of the mutations. Over time these offspring may adapt to the radiation-induced mutations, becoming more genetically diverse and more resistant to radiation effects.

“The medaka fish is an excellent model for this type of work in that it is outbred, meaning that its genetic make-up is highly varied and diverse because it has intermingled and bred over many generations, similar to our global human population,” said Dr. Zimbrick.

A new facet of the study is to examine the inter-individual distribution of radiation-induced mutation frequencies which varies based on individual sensitivity to radiation. A study looking at 50 medaka families will measure this distribution of mutation frequencies among families and look at potential genetic markers that could indicate the relative degree of radiosensitivity. The researchers also will select specimens from the lower and upper ends of the sensitivity scale, breed them, profile the genes and look for patterns of gene activities associated with the genes involved in DNA repair. If they are successful and able to find a set of repair genes whose activities relate to individual radiosensitivity, those candidate genes may be elevated for additional study in a more complex model.





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