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CRISPR/Cas9: A Novel Tool To Treat Disease
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The Veterinary Medical Experiment Station (VMES) was established as a budgetary entity by the state legislature in July 1976 following approval by the University of Georgia Board of Regents in 1973.

MISSION

The VMES mission is to coordinate research on animal disease problems of present and potential concern to Georgia’s livestock and poultry industries.

SPECIFIC VMES OBJECTIVES ARE:

- To improve the health and productivity of domestic livestock, poultry, fish, and other income-producing animals and wildlife through research;
- To assist in preventing disease epidemics by providing laboratory resources and highly skilled scientific personnel;
- To assist in protecting human health through the control of animal diseases transmissible to man;
- To improve the health of companion animals, which serve to enrich the lives of humankind;
- To train new scientists in animal health research in order to provide continuity and growth in this vital area of veterinary medicine.

The Veterinary Medical Experiment Station is committed to enhancing animal production, profitability, and well-being by improving animal health.

All programs and activities of the Veterinary Medical Experiment Station are conducted without regard to race, color, national origin, age, sex, or handicap.
Seventeen years ago, I gave a talk at the induction ceremony of our college’s local chapter of the Phi Zeta Veterinary Honor Society. The topic was veterinary medicine in the era of genomics, which had just started. The genome of the bacterial pathogen *Haemophilus influenzae* (with a genome size of 1.8 megabases) was the first to be sequenced in 1995 and was soon followed by *Mycoplasma genitalium*, *E. coli*, *Pasteurella multocida*, yeast (*Saccharomyces cerevisiae*), round worm (*Caenorhabditis elegans*) and fruitfly (*Drosophila melanogaster*). The human genome was completed in 2000, the year I gave my talk. I predicted that within 10 years the genomes of most laboratory animals used as models in biological research (e.g. mouse, rat, and zebrafish) would be available for study and comparison, and that the genomes of domestic animals and the microbial pathogens that affect them would also be largely completed. This all happened as predicted.

The gist of my talk on that spring day, however, was that this massive amount of new information in the form of genetic sequences was the starting point of a new era of scientific discovery and that veterinarians with their broad biology training were well positioned to use this new knowledge for the benefit of animal health. The ultimate goals of the post-genomic era have been to elucidate gene functions and how genes holistically interact. We are now on the verge of attaining those goals. As often happens in the progress of science, a completely unanticipated new discovery or technology comes along that is transformative: CRISPR/Cas9 is such a discovery. CRISPR allows scientists to easily manipulate genes with pin-point accuracy. In this year’s annual report Dr. Fred Quinn discusses CRISPR/Cas9 and its current and potential future impact on animal and human health research. It is a fascinating story and one that has far-reaching implications for our basic understanding of the physiology of health and disease. I anticipate that over the next 10 years it will have great translational impact on the science and practice of veterinary medicine.

We present the 41st Annual Report as part of the continuing documentation of the long and productive history of the Veterinary Medical Experiment Station. It provides an overview of peer-reviewed, competitive projects and new faculty start-up projects conducted during fiscal year 2017 (July 1, 2016 – June 30, 2017). Projects supported by the State of Georgia VMES funding, and projects funded with United States Department of Agriculture 1433 Formula funds are reviewed by veterinary scientists for quality of science and focus on relevant animal health issues or disease problems. The research must be innovative and applicable to the improvement of animal health. Further information on these projects is available by contacting the VMES office staff by phone, e-mail or website, or directly from the investigators themselves. A list of publications is provided. These peer-reviewed papers represent a selection of VMES-supported work and other scholarly research by the faculty of the UGA College of Veterinary Medicine. This has been another record year for research productivity in the college: over $29 million in extramural funding was attained. This is a testament to the quality of our faculty, staff, and students in the College of Veterinary Medicine.

As in previous years, we list in the VMES Annual Report the names of 34 individuals who received graduate degrees in 2017 after completing a comprehensive training program that includes original, hypothesis-driven research conducted under the mentorship of a College researcher. These students are attracted to our programs for the excellent research experiences and mentoring that they find here. The training of future researchers is of utmost importance to fulfilment of the mission of the Veterinary Medical Experiment Station and to meeting the future animal and public health needs of our state, nation and world.
A summary of the College’s research funding is provided in the charts above. During FY2017, approximately nine and a half research dollars were leveraged for each VMES dollar invested. Expenditures are from all sources including State Appropriations, Extramural Research Funding, and Donations. These expenditures include all budget categories including personnel costs.
In 2015, Dr. Tony Perry, a molecular embryologist from the University of Bath, UK generated mice that would have been brown-furred, but were white-furred instead. Modifying traits like fur color in mice is nothing new, as researchers have been disabling specific genes in “knock-out” or “transgenic” mice” for nearly 30 years. Generating knock-out mice is a long and cumbersome process that involves replacing parts of targeted genes in mouse embryonic stem cells with non-functional DNA and then breeding the new strain. What is most interesting about Dr. Perry’s study, published in December 2015 in Scientific Reports magazine, is that he used a newer gene-editing technology called CRISPR. This approach has been taking the scientific world by storm since it was first developed in bacteria in 2012, and then shown to work in insects, fish, mammalian (and human) cells in 2013.

What gave Dr. Perry’s work added importance is that he did his gene-editing not in a one-cell mouse embryo, which is how most animal CRISPR has been done to date.

Instead, he performed his gene-editing during fertilization, by injecting the CRISPR components and the mouse sperm into the mouse egg at the same time.

This is the same technique, namely intracytoplasmic sperm injection, that is widely used in in vitro fertilization (IVF). As stated in the Scientific Reports paper, “This or analogous approaches may one day enable human genome targeting or editing during very early development. If human germ-line editing were ever to be used clinically, incorporating CRISPR..."
into the intracytoplasmic sperm injection phase of IVF is how it might be.” This approach also raises the possibility of generating offspring that carry either no risk or a reduced risk of some genetic diseases. For example, it might one day be possible to correct a harmful mutation in the gene encoding BRCA1, to prevent inheriting the predisposition to breast cancer.

Since the 1970s when genetic engineering was in its infancy, there has been a consensus that human germ-line modification will not be performed. Germ-line genome editing is highly controversial, even for medical purposes. Peter Mills, assistant director of the UK Nuffield Council on Bioethics and the council’s lead on genome editing, expresses concerns about “playing God” and “designer babies”. According to UNESCO’s Universal Declaration on the Human Genome and Human Rights, germ-line interventions “could be contrary to human dignity”. However, human (and just about all other species) germ-line editing used to be science fiction. With the development of CRISPR, this capability may soon be a reality.

**What is CRISPR/Cas9?**

Google “CRISPR”, and what do you find? “NC State Professor using [CRISPR-modified] bacteria to treat diseases”, “CRISPR Therapeutics, Massachusetts General Hospital team up on new cancer treatments”, and “CRISPR gives sickle-cell patients hope for a cure”. In addition to eliminating some human genetic diseases, this method also could eliminate animal and plant disorders using the same generation-spanning gene-editing technology. It also has the potential to control many infectious diseases by making critical genetic modifications in bacteria and parasites that may yield avirulent super vaccines.

CRISPR, which is pronounced ‘crisper’, is an abbreviation for “Clustered Regularly Interspaced Short Palindromic Repeats”. It is a family of DNA sequences in bacteria that contain small bits of DNA from viruses that attacked but did not kill the bacteria many generations in the past. The earlier viral attackers left behind tiny bits of their own DNA inserted in the bacterial genome. The benefit to the host cell is that the current generation of bacterial cells now use these viral DNA inserts as targets for detecting and destroying similar viruses when they attack. It could be said that CRISPR is essentially a ‘single cell immune system’.

Researchers first uncovered the hallmarks of CRISPR in 2000, when a group of Spanish scientists noticed unusual sites in the genomes of bacteria, which contained multiple copies of a short repeated genetic sequence from viruses. Subsequently, investigators identified a crucial enzyme directly involved in the actual cutting of the target DNA region in the host genome. This enzyme, ‘Cas9’, is distinct from the CRISPR complex and uses specific RNA sequences that target the viral inserts. In 2012, Dr. Jennifer Doudna from the University of California, Berkeley, and Dr. Emmanuelle Charpentier of the Max Planck Institute for Infection Biology co-authored the key study that demonstrated the technical power of CRISPR/Cas9 to cut and splice genes with extreme efficiency.

To understand the molecular process, CRISPR/Cas9 can be though of as precisely-guided molecular scissors. These enzymatic scissors snap at a point in the cell’s DNA specified by a customized guide molecule, a single short piece of RNA. This precise piece of RNA is generated by the host cell using a previously-inserted viral DNA chunk as the template. Alternatively, the template can be added into the cell by a researcher wanting to target a specific area of the genome. The genome editing occurs as the cell rushes to naturally repair the break made by the scissors. In many cases, the cell’s repair process isn’t precise enough for the gene that has been cut to keep working and the gene is effectively knocked out or turned off. If a new piece of DNA is included along with the CRISPR machinery, defective genes can be corrected or whole new genes added during the cellular repair process.

As described above, CRISPR/Cas9 genome editing has many potential applications, including medicine and crop seed enhancement. Thus, scientists could, with the requisite knowledge, improve the vitality of crops in the face of drought and pests; eliminate viruses such as HIV from the genomes of infected individuals; or “silence” the genes that predispose humans and animals to a myriad of diseases, including cancer, diabetes, cardiovascular disorders and mental illnesses.

The use of CRISPR/Cas9 for genome editing was the American Association for the Advancement of Science’s choice for “Breakthrough of the Year” in 2015. John Travis, Science Magazine’s managing news editor noted its potential applications in medicine. “It’s only slightly hyperbolic to say that if scientists can dream of a genetic manipulation, CRISPR can now make that happen.”

**Use in the UGA CVM**

Dr. Ralph Tripp, in the Department of Infectious Diseases, has used CRISPR/Cas9 to develop enhanced vaccine-generating mammalian cell lines that could save many lives. To accomplish this, he screened >19,000 host cell genes to ultimately select a handful that when knocked out, could increase production of polio, rotavirus and other viral vaccines. The genes that were targeted had anti-virus properties and had naturally evolved in mammalian cells to fight viral infections. By knocking out these specific genes, the genetically modified cells were now able to produce larger amounts of vaccine virus compared to the standardly used production cell lines. Thus, the same vaccine manufacturing footprint can now generate larger amounts of vaccine, or the same amount of vaccine can be produced using less equipment and reagents, thus allowing for increased numbers of vaccines to be in production at one time. Dr. Tripp is now employing this enhancement technology on a number of additional vaccines for other viral diseases such as flaviruses (Dengue, Zika, and West Nile).
Drs. Pramod Giri and Russell Karls also in the Department of Infectious Diseases are using the CRISPR system in human and bovine macrophages to understand the mechanisms by which two key infectious agents, Mycobacterium tuberculosis in humans and M. bovis in ruminants, acquire iron and other essential metals from mammalian cells. These tuberculosis-causing bacteria have evolved mechanisms that disrupt iron regulation and transport systems in the host to steal the essential metals these bacteria need to grow. Drs. Giri and Karls are using the CRISPR/Cas9 system to selectively target and identify host cell genes and pathways subverted by these bacteria. Identification of host pathways utilized by the bacteria to gain iron and other trace elements is the first step toward developing therapies to thwart the ability of invading mycobacteria from acquiring sufficient amounts of such metals to grow inside its host.

Other UGA Investigators

James Hataway in the May 2016 issue of the University of Georgia Research Magazine does a great job of summarizing many of the terrific infectious diseases-related projects ongoing at UGA using CRISPR/Cas9. For example, Dr. Michael Terns’ laboratory in the Department of Biochemistry and Molecular Biology is using customized CRISPR RNAs to successfully disrupt expression of a protein responsible for resistance to commonly-prescribed antibiotics such as penicillin and amoxicillin. This discovery could help bolster treatments that, because of overuse and misuse, have become largely useless against infection-causing bacteria.

Dr. Rick Tarleton and colleagues in the Department of Cellular Biology and Center for Tropical and Emerging Global Diseases (CTEGD) are using CRISPR to edit the genome of the protozoan parasite, Trypanosoma cruzi, so that they may better understand how it interacts with host cells. This could ultimately help them identify potential weak points in the parasite’s life cycle, which researchers would then aim to exploit.

Similarly, Dr. Boris Striepen, formerly in the Department of Cellular Biology and the CTEGD, and co-workers are using CRISPR to genetically modify Cryptosporidium parvum, a parasite that causes the gastrointestinal disease cryptosporidiosis typically through the ingestion of tainted drinking or recreational water. Dr. Striepen, currently at the University of Pennsylvania, is continuing his work to develop a new vaccine for preventing this disease. By deploying CRISPR to knock out specific genes in the parasite, his laboratory is testing the genes’ importance for the parasite and assessing their potential value as drug targets.
Where to go from here

In October, 2016, a team led by Dr. Lu You at Sichuan University in Chengdu, China administered CRISPR/Cas9 modified cells into a patient with aggressive non-small-cell lung cancer as part of a clinical trial. Dr. Lu’s trial received ethics approvals from a hospital review board the previous July. The researchers removed immune cells from the recipient’s blood and then disabled a resident gene using CRISPR/Cas9. The disabled gene coded for the protein PD-1, which normally suppresses immune responses. Because cancers take advantage of impaired immune function to proliferate, it was their hope that without PD-1, the edited cells would attack and defeat the cancer.

Dr. Lu’s team then cultured the edited cells, increasing their number, and injected them back into the patient. This study is ongoing, and a number of other cancer patients have now been enrolled. Similarly, in June 2017, investigators at the University of Pennsylvania began the first trial in the US that uses CRISPR to target genes in participants’ cells, with the goal of treating various cancers.

Dozens of other proposed human trials all over the world are currently being assessed. We live in exciting times!

Now, the potential down side...

A National Public Radio story from May 27, 2017 demonstrates the power and potential danger of this technology. Middle-school students in Brooklyn, NY were taught how to edit genes. As Dr. Terns mentioned in the UGA Research Magazine article described above, “CRISPR technology has made gene editing practical for a much broader group of scientists. Almost anyone can do this. You don’t need a lab full of advanced equipment, and what used to cost thousands of dollars can now be done with just a few hundred.”

For example, some worry that the technology could be used to alter the DNA of commercially important plants and animals before the effects of these manipulations on ecosystems are fully understood. Others are troubled that CRISPR might trigger social havoc in a rush to create offspring whose genetic characteristics are customized prior to birth.

The efficiency and concerns surrounding CRISPR/Cas9 gene editing were well stated in a recent article in the journal Nature. The research paper describes success of Dr. Shoukhrat Mitalipov at the Oregon Health and Science University in targeting a mutation in gene MYBPC3. These mutations cause the heart muscle to thicken, and is the leading cause of sudden death in young athletes. The mutation is dominant, meaning that a child need inherit only one copy of the mutated gene to experience its effects. In addition to successfully replacing the faulty gene in 58 embryos, the team also tackled two lingering safety hurdles: the risk of making additional, unwanted genetic changes (called off-target mutations) and the risk of generating mosaics in which different cells in the embryo contain different genetic sequences. The researchers say that they have found no evidence of off-target genetic changes, and generated only a single mosaic among the 58 embryos. None of these embryos were destined for implantation.

In an accompanying Nature news article, stem-cell biologist Dr. George Daley of Boston Children’s Hospital in Massachusetts states “It puts a stake in the ground that this technology is likely to be operative,” he says. “But it’s still very premature.” Dr. Daley worries that the success reported in the paper could motivate clinicians to try the technique before it has been fully tested. He points to an experimental technique called mitochondrial replacement therapy, which aims to treat embryos for a disorder that disables the energy-generating parts of cells called mitochondria. Last September, news broke that a doctor had used the technique in a fertility clinic in Mexico, even though many experts believed it was not yet ready for clinical practice. Since then, reports have appeared documenting the use of the technique by other clinicians.

Developmental biologist Robin Lovell-Badge of the Francis Crick Institute in London shares the same concerns. But, he notes in the same Nature news article that worries about children who have been genetically enhanced, rather than merely correcting disease-causing mutations may be eased somewhat by the [Mitalipov Nature research] paper. In his experiments, Dr. Mitalipov provided a strand of DNA to serve as a template for rewriting the disease-causing mutation. Surprisingly, the embryos did not use this template. Instead, the embryos used the mother’s DNA as a guide to repair the MYBPC3 mutation carried by the father’s sperm. “This isn’t a clear step towards a designer baby,” says Dr. Lovell-Badge. “This suggests that you couldn’t add anything that wasn’t already there.”

Some scientists and review panels compare the current status of CRISPR/Cas9 genetic engineering tools to that of personal computers in the 1970s. It is a revolutionary technology, and the speed at which CRISPR is developing, combined with its potentially wide range of applications has spurred both concern and excitement; concern that it could cause harm, and excitement that with appropriate oversight and clarity of goal, it could change the world for the better.
Meningoencephalomyelitis of unknown origin (MUO) is a disease of dogs that results in inflammation in the brain and/or spinal cord. Affected dogs develop progressive neurological deficits that can include depression, seizures, vision loss, circling, loss of balance, difficulty walking, pain, and death. A presumptive diagnosis of MUO is based on concurrent evidence of inflammation on MRI and cerebrospinal fluid (CSF) analysis, and negative infectious disease tests. However, diagnosis cannot be confirmed without microscopic evaluation of brain tissue, which is often not possible antemortem. Treatment is with nonspecific immunosuppressive drugs. Unfortunately, the response to treatment is variable and often temporary. There is a critical need to better understand the etiopathogenesis of this condition to allow for development of accurate antemortem diagnostics as well as more reliable therapies.

The cause of inflammation in dogs with MUO is unknown, as numerous investigations have failed to reveal a single etiologic agent. Genetic risk factors have been identified in several toy breed dogs, but specific-disease causing mutations have not been identified. Currently, most specialists agree that the condition is likely an autoimmune disease based on lack of an identifiable infectious cause and clinical improvement after treatment with immunosuppression. Recently, two important pro-inflammatory cytokines, IFN-γ and IL-17, were reported to be elevated in brain tissue of MUO-affected dogs. These cytokines are involved in numerous autoimmune disorders. Predominately, they are thought to be produced by T helper cells as part of T helper cell 1 (Th1) and 17 (Th17) polarized adaptive immune responses. This research aims to determine if elevated levels of IFN-γ and IL-17 can be measured in routinely-obtained clinical samples, such as peripheral blood and CSF, in dogs with MUO. A secondary goal is to determine what cells are responsible for IFN-γ and IL-17 production affected dogs, to further our understanding of the aberrant immune response in these patients.

A pilot study was performed using ELISA to measure IFN-γ and IL-17 in CSF from affected dogs (n=8) as well as healthy controls (n=4) and dogs with other neurological illness (n=13). IFN-γ and IL-17 were not detected in the majority of samples (21/23). IFN-γ was identified in 1/8 samples from dogs with MUO, and IFN-γ and IL-17 were identified a single case of central nervous system bacterial infection. ELISA assays are currently being optimized to improve sensitivity for evaluation of additional samples of CSF as well as serum. Future studies will also include evaluation of CSF white blood cells by ELISpot to identify the number of cells that produce IFN-γ and IL-17 secondary to non-specific stimulation.

To evaluate MUO-affected dogs for Th1 and Th17 polarization, flow cytometry on peripheral blood has been optimized using healthy, control dogs. Normal canine peripheral blood was subjected to density gradient centrifugation with polycsucrose and sodium diatrizoate. The mononuclear cells were then incubated ex vivo with and without phorbol myristate acetate and calcium ionophore. 5-color polychromatic flow cytometry was performed including utilization of a fixable viability stain, surface immuno-staining for CD4 and CD8 antigens, and intracellular staining for accumulated IFN-γ and IL-17. The assay allows for the determination of: 1) the relative percentage of CD4+ T helper lymphocytes and CD8+ T cytotoxic lymphocytes to total lymphocytes, translation to absolute numbers of these as circulating cells, and a CD4/CD8 ratio; and 2) relative percentage of lymphocytes and absolute numbers of circulating T helper or T cytotoxic lymphocytes with the ability to secrete IFN-γ or IL-17. Results showed highly discernable and measurable discreet populations of IFN-γ and IL-17 expressing CD4 and CD8 after stimulation. Because MUO often occurs in small and toy breed dogs, this assay was evaluated for use with as little as 1ml of peripheral blood or a minimum of 4x10^6 total leukocytes as measured by CBC. The sample preparation workflow and instrumentation procedures are currently optimized for patient and control samples and collection of study data. Based on two recent publications that demonstrated production of IL-17 by CD3- mononuclear cells in dogs, this assay is also being expanded to include evaluation of this population of cells.

**Funding Agency**
New Faculty Startup/Companion Animal Funded Projects

**Principal Investigator**
Dr. Renee Barber

**Co-investigators**
Mr. James Barber, Dr. Marc Kent, and Dr. Simon Platt
Comparative Efficacies of Enrofloxacin and Tulathromycin for the Control of Bovine Respiratory Disease in Cattle at High-Risk of Disease

BOVINE DISEASE

Bovine respiratory disease (BRD) remains the most common cause of morbidity and mortality in the beef industry, and numerous management practices are used in an attempt to reduce its impact. As these approaches have been poorly effective in high-risk cattle, most producers routinely administer antimicrobials to entire groups of animals, a practice termed metaphylaxis. Strains of antimicrobial-resistant M. haemolytica are being isolated with increasing frequency from BRD-affected cattle. Although multi-drug resistant (MDR) strains are detected in <4% of cattle arriving at stocker facilities in Georgia, MDR strains are present in >72% of cattle at high risk of developing BRD after metaphylaxis, particularly in cattle administered long-acting macrolides, such as tulathromycin. Because these drugs persist in the respiratory tract at sub-therapeutic concentrations for days, sustained exposure of bacteria to these low concentrations may select for MDR strains. Consequently, there is a critical need to determine if short-acting drugs of other classes are as effective and less likely to induce MDR. We compared the efficacies of tulathromycin to the short-acting fluoroquinolone enrofloxacin in cattle at high-risk of developing BRD, and the prevalence of MDR strains of M. haemolytica before and after metaphylactic administration of each drug.

Stocker calves (n=341) were randomly assigned to 2 treatment groups: enrofloxacin (n=172) and tulathromycin (n=169). 33.7% of calves receiving enrofloxacin required treatment for BRD within 45 days after arrival, compared to 18.3% of calves receiving tulathromycin. The odds of being diagnosed with BRD were ~58% lower for calves receiving tulathromycin compared to those receiving enrofloxacin (P = 0.040). 10.5% of calves receiving enrofloxacin required more than one treatment, compared to 4.7% of calves receiving tulathromycin. The odds of requiring a second treatment were ~60% lower for calves receiving tulathromycin compared to those receiving enrofloxacin. (P = 0.107). 12.2% of calves receiving enrofloxacin died during the 45-day follow-up period, compared to 10.1% of calves receiving tulathromycin (P = 0.592).

In calves receiving enrofloxacin, M. haemolytica was cultured from 11.4% of calves sampled at arrival, and from 54.6% sampled at revaccination. In calves receiving tulathromycin, M. haemolytica was cultured from 10.8% of calves sampled at arrival, and from 48.7% sampled at revaccination. There was no significant effect of treatment (P = 0.737), and no significant interaction between treatment and sampling occasion (P = 0.845), but there was a significant effect of sampling occasion (P < 0.001). Both groups had higher prevalences of M. haemolytica at revaccination than at arrival. Only one calf in the group receiving enrofloxacin and none of the calves receiving tulathromycin were culture positive for MDR resistant M. haemolytica at arrival. In contrast, all calves in both groups had MDR strains at the time of revaccination, with the prevalence being significantly (P<0.000) higher than at arrival.

These results indicate that tulathromycin is superior to enrofloxacin for the control of BRD in calves at high risk of the disease. However, shedding M. haemolytica increased between arrival and revaccination, and the proportion of calves shedding MDR strains increased in both treatment groups. Research evaluating the impact of MDR on response to antimicrobial therapy and novel non-antimicrobial methods of BRD control should be performed.

Funding Agency
USDA Animal Health Capacity Grant

Funded Projects

Principal Investigator
Dr. Brent Credille

Co-investigators
Drs. Sydney Crosby, Roy Berghaus, and Steeve Giguere
Early embryonic mortality is a leading cause of economic loss for the cattle industry, and chromosome abnormalities are the single most common cause of pregnancy loss in mammals. Yet, the mechanisms leading to chromosome instability are not known. We have recently generated a novel transgenic mouse model demonstrating that the chromatin remodeling protein ATRX regulates the cellular response to chromosome instability in the early pre-implantation embryo. Importantly, ATRX is conserved in bovine oocytes. Here, we test the role of this critical epigenetic factor on genome integrity in the bovine embryo.

We aim to develop a physiologically relevant bovine embryo model to identify the mechanisms responsible for chromosome instability at the oocyte to embryo transition in vivo and in vitro. Using cell microinjection, we have developed a system for live cell imaging of chromosome segregation during bovine oocyte maturation (Figure 1) to detect both numerical and structural chromosome aberrations. Our system will prove invaluable to test new in vitro oocyte maturation protocols to reduce pregnancy loss in cattle.

Importantly, we have established methods for super resolution chromatin analysis in bovine oocytes and embryos. Using structured illumination super-resolution analysis and Stochastic Optical Reconstruction we provide the first analysis of bovine oocyte chromatin with nano-scale resolution (Figure 2). These strategies provide an unprecedented view of mammalian chromosomes that is changing our understanding of genome dynamics in mammals. Our results provide the first evidence of a conserved centromeric localization of ATRX in bovine oocytes and suggest that ATRX is a critical and conserved epigenetic marker of oocyte quality and developmental potential in bovine oocytes and embryos.

We also identified nucleolar ‘anchoring’ of kinetochore proteins a novel mechanism that may be important to coordinate chromosome-microtubule interactions and chromosome segregation during bovine oocyte maturation. These results have important implications for both human and veterinary medicine as recent studies indicate that kinetochore proteins may also be associated with the nucleolus in human cells.

Our studies focus on establishing the molecular mechanisms of chromosome instability and the biogenesis of translocations in the bovine pre-implantation embryo. Importantly, this research has provided preliminary data for a proposal to the NIH-USDA Dual purpose-dual benefit program for the use of agriculturally important domestic animals in biomedical research. Our studies address one of that funding opportunity’s priority areas: Identification of molecular mechanisms that regulate oocyte and embryo competency and validation of markers to determine oocyte-embryo quality and genome integrity. Elucidating the role of conserved chromatin remodeling proteins as epigenetic markers of oocyte quality and developmental potential is an issue of major significance for the design of procedures to prevent pregnancy loss in domestic animals.

**Figure 1.** Live cell imaging of bovine oocyte maturation A) Real-time analysis of chromosome segregation during in vitro maturation of bovine oocytes. B) Close-up of the oocyte shown above. Note the extrusion of the polar body (*), chromosome congression into a condense mitotic configuration and subsequent segregation. An abnormal segregation event is detected in real-time (arrowhead).

**Figure 2.** Super-resolution chromatin analysis in bovine oocytes A) Wide field analysis of the nucleus of a bovine oocyte. The position of the nucleolus is indicated by (*) and the edge of the nuclear membrane is indicated by an arrowhead. Kinetochore proteins are stained in green. Note the prominent nucleolar anchoring of kinetochore proteins forming a ring. Condensed heterochromatin is stained in red. B) 3D-intensity plot to quantify the levels of kinetochore proteins show a 5-fold enrichment near the nucleolus (yellow peaks).
Bolus materials, which are near water-equivalent materials, are commonly placed in direct contact with the patient’s skin in both human and veterinary radiation therapy. These materials are used to increase the dose of radiation to the skin and the underlying tissue to provide a uniform dose to the target tissue. When x-ray or electron beams enter the patient without a bolus, the skin is relatively under-dosed. This explains the lack of “dose-buildup” adjacent to the skin. Ideal bolus materials need 3 characteristics; (1) tissue-equivalent, (2) conformable, and (3) adjustable to a desired thickness. The radiation absorption and scattering characteristics of the bolus material also need to be known. None of the bolus materials available commercially is ideal.

The main problem with commercially available boluses, such as Superflab, is that small air gaps develop between the skin and bolus. This is particularly true for the irregular surfaces of a dog’s hock or paw. The resulting air gaps lead to loss of dose build-up and under-dosing of tumors in or near the skin. These air gaps can be minimized using lubricating gels that fill the gaps. The radiation therapy attenuating potentials of these gels have not been determined experimentally.

This study tested the hypothesis that McKesson lubricating jelly® has density and radiation dose-attenuating properties similar to water, and can be safely used with the tissue-equivalent bolus. We compared the x-ray (6 MV) and electron (6, 9, and 12 MeV) beam attenuating properties of water and McKesson lubricating jelly®.

Frames made of Sil-Tech®, a soft moldable material, were used to study radiation attenuation by the tissue control (water) and lubricant. Sil-Tech was mixed with a solidifying agent, and flattened with a rolling pin. “Frames” having a uniform 5mm thickness were created by putting Sil-Tech between two 5mm stacks of papers on a flat surface. A 13 cm x 13 cm x 5 mm square of solidified square of Sil-Tech was placed in the center of the frame. This material was eventually removed, giving the frame a 13 cm x 13 cm aperture. These “center pieces” were easy to remove from the surrounding Sil-Tech, because they were solidified at different times. Eleven frames were constructed and were stackable, allowing the central aperture to achieve a height of up to 55 mm (5 mm x 11 frames). This allowed the central hole to be filled with varying thicknesses of different substances to test their radiation attenuation. For liquid or gel-like substances, the central hole was designed to contain a small, open-top plastic bag.

The dose of radiation was measured in 5 mm increments for both x-ray and electron beams, comparing water (one time) and McKesson lubricating jelly® (three times; Figures).

These results indicate that the attenuating properties of McKesson lubricating jelly® are similar to that of water. Based on these findings, McKesson lubricating jelly® can be used as a bolus.

Funding Agency: New Faculty Startup/VMES
Funded Projects: Principal Investigator: Dr. Koichi Nagata
Co-investigator: Dr. Tim Pethel
Cytauxzoonosis is an emerging infectious disease of domestic cats (Felis catus) caused by the apicomplexan parasite Cytauxzoon felis. Without treatment, 97% of affected cats die and even with intensive and costly treatment, up to 40% of cats will not survive. The growing epidemic, with its high morbidity and mortality, points to the need for a protective vaccine against cytauxzoonosis. Cytauxzoon felis has a complex life cycle with three life stages in the mammalian host: sporozoites, schizonts, and merozoites. Of these, schizonts have been associated with a protective immune response, as solid immunity to C. felis occurs in cats that had previously survived the schizogenous phase of the disease. These cats survived challenge infection with no signs of illness while naïve control cats died of cytauxzoonosis.

In contrast, direct inoculation with C. felis merozoites alone fails to confer protective immunity. Collectively, these data suggest that antigens associated with schizonts are vaccine targets for C. felis. To date, attempts to isolate and assess the sporozoite life stage have not been successful. Furthermore, Cytauxzoon felis has yet to be cultured continuously in vitro, rendering traditional vaccine development approaches beyond reach.

In order to overcome experimental limitations and facilitate the rapid identification of vaccine candidate antigens, we sequenced the C. felis genome and identified ~4,300 protein-coding genes, each of which represents a potential protective antigen. Using genome-based vaccine design and high-throughput protein microarray screening, we identified 30 vaccine candidates that represent targets for humoral immunity. Our goal now is to identify which of these antigens satisfies additional criteria for a suitable vaccine candidate against C. felis. The criteria that are likely to be important for a vaccine candidate against C. felis include: 1) conservation of sequence similarity among C. felis isolates and 2) expression in the C. felis schizont life stage that is believed to be critical for the development of a protective immune response. We sequenced six C. felis vaccine candidates (VC3, VC10, VC11, VC13, VC19, and C. felis AMA1) from fifteen geographically diverse C. felis isolates across six states within the southcentral and southeastern U.S. and identified four vaccine candidates with a high degree (>98%) of sequence similarity. RNAScope® in situ hybridization was used to assess expression of four of these vaccine candidates in the schizont life stage of C. felis infected, formalin-fixed, paraffin-embedded splenic tissues. RNAScope® is a relatively new technology utilizing novel multiplex nucleic acid in situ hybridization that produces quantifiable signal amplification for detection of single mRNA transcripts in tissues. In situ hybridization revealed variable expression of three vaccine candidates within C. felis schizonts; slides will be scanned and HALO™ software will be utilized to generate a quantitative H-score to assess expression of each vaccine candidate. The results of this study will accelerate identification of suitable vaccine candidates for cytauxzoonosis.

Funding Agency
New Faculty Startup/Companion Animal Funded Projects

Principal Investigator
Dr. Jaime Tarigo

Co-investigators
Dr. Adam Birkenheuer and Mr. Daven Khana
Anderson, Lydia. Doctor of Philosophy – Infectious Diseases, Fall 2016
Bracken, Tara. Doctor of Philosophy – Infectious Diseases, Fall 2016
Burke, Emily. Master of Science – Comparative Biomedical Sciences, Spring 2017
Crum, Jo Anne. Master of Science – Veterinary & Biomedical Sciences, Spring 2017
Dickerson, Vanna. Master of Science – Veterinary & Biomedical Sciences, Summer 2016
Dixon-Jimenez, Amy. Master of Science – Veterinary & Biomedical Sciences, Summer 2016
Drouet, Victoria. Master of Avian Medicine, Fall 2016
Fogelson, Susan. Doctor of Philosophy – Veterinary Pathology, Fall 2016
Gamble, Tyler. Master of Avian Medicine, Fall 2016
Gresko, Anthony. Doctor of Philosophy – Infectious Diseases, Summer 2016
Hanson, Jared. Doctor of Philosophy – Infectious Diseases, Summer 2016
Lenz, Shannon. Doctor of Philosophy – Infectious Diseases, Fall 2016
Li, Rong. Doctor of Philosophy – Toxicology, Summer 2016
Li, Xin. Doctor of Philosophy – Infectious Diseases, Summer 2016
Liu, Liyun. Doctor of Philosophy – Toxicology, Summer 2016
Long, Kathleen. Master of Avian Health and Medicine, Fall 2016
Phan, Shannon. Doctor of Philosophy – Infectious Diseases, Summer 2016
Poulson, Rebecca. Doctor of Philosophy – Infectious Diseases, Fall 2016
Roquemore, Ashland. Doctor of Veterinary Medicine/Master of Public Health (DVM-MPH), Spring 2017
Rose, David. Doctor of Philosophy – Infectious Diseases, Fall 2016
Rui, Tang. Doctor of Philosophy – Integrative Physiology & Physiology, Fall 2016
Rushmore, Julie. Doctor of Veterinary Medicine/Doctor of Philosophy (DVM-PhD), Spring 2017
Sanches Madeira Afonso, Tiago. Doctor of Philosophy – Veterinary & Biomedical Sciences, Summer 2016
Sandu, Dulmelis. Master of Avian Medicine, Fall 2016
Schlegel, Benjamin. Master of Avian Health and Medicine, Fall 2016
Sil, Payel. Doctor of Philosophy – Infectious Diseases, Fall 2016
Snyder, Emily. Master of Food Animal Medicine, Fall 2016
Tien, Yung-tien. Master of Science – Comparative Biomedical Sciences, Spring 2017
Toulme, Natalie. Doctor of Veterinary Medicine/Master of Public Health (DVM-MPH), Spring 2017
Vahey, Grace. Doctor of Veterinary Medicine/Master of Public Health (DVM-MPH), Spring 2017
Willingham-Lane, Jennifer. Doctor of Philosophy – Infectious Diseases, Summer 2016
Worley, Rachel. Doctor of Philosophy – Toxicology, Fall 2016
Zhang, Xiwen. Master of Science – Veterinary & Biomedical Sciences, Summer 2016
Turkey Vaccine Study with Avian Influenza Highly Pathogenic H5N8 Virus. USDA ARS. $83,550

Peroni, John. Equine platelet lysate gel: a novel matrix for mesenchymal stem cell delivery. American College of Vet Surgeons. $12,879

Ex vivo modeling of exuberant granulation tissue (EGT) injury in horses. Morris Animal Foundation. $10,800

Platelet Lysate as a Novel Serum Free Media for the Culture of Equine Bone Marrow Derived Mesenchymal Stem Cells (resubmission). Morris Animal Foundation. $115,752

Blood Sterilization Pilot Study. Industry Sponsor. $11,681

Quinn, Frederick. Large-Scale Analysis of the Evolution of Organellar Social Networks. University of Pittsburgh as flow down from NSF. $150,802

Rada, Balazs. Anti-NET autoantibodies in CF. Cystic Fibrosis Foundation. $54,000

Anti-NET autoantibodies in cystic fibrosis. National Institutes of Health. $225,000

Cooperative Wildlife Disease Study. Various Other States. $150,000

Southeastern Cooperative Wildlife Disease Study. Various Other States. $841,000

Saba, Corey. Evaluation of the efficacy of Merial’s Canine Lymphoma Vaccine, DNA when administered to dogs concurrent with a CHOP-based chemotherapy protocol. Industry Sponsor. $30,000

Saliki, Jeremiah. Georgia Bioscience Fellowship Program, Naita Kartskia. CRDF Global. $14,922


Sanchez, Susan. One Health Epidemiology of Natural and Deliberate Contaminants (Infectious and Toxicties) In Pets and Pet Food. U. S. Department of Health and Human Services. $26,499

Schmiedt, Chad. Efficacy of ruboxistaurin (RBX) in a canine model of unilateral ischemia renal fibrosis. Elanco Animal Health. $18,038


Shollenberger, Lisa. For the Development of an innovative immunodiagnostic test for clinical forms of Schistosoma mansoni. American Association of Clinical Chemistry. $2,500


Trent, Michael. A High-Throughput Molecular Platform for Antimicrobial Discovery and Study. University of Texas at Austin as flow-down from National Institutes of Health. $50,000

Tripp, Ralph. EMORV-UGA CEIRS. Emory University as flow-down from NIH. $658,288

Emory-UGA CEIRS: Generation of recombinant influenza vaccines expressing exogenous miRNAs for increased in vitro vaccine production (Option 21A). Emory University as flow-down from NIH. $152,607

Emory-UGA CEIRS: Option 17B DIGS. Emory University as flow-down from NIH. $100,665

KPT-335 as novel therapeutic for respiratory syncytial virus. National Institutes of Health. $165,933

Pilot Project: KPT-335 to Reduce Influenza Infection in Balb/C MICE - Revision - 1. Karyopharm Therapeutics. $25,417


Verocai, Guilherme. Understanding the ecology of the emerging, zoonotic Onchocerca lupi and its black fly vectors in the endemic Los Angeles County, California. Oklahoma State University Foundation. $8,152


Wilkes, Rebecca. TVDIL Infrastructure for CVM Vet-LIRN Veterinary Diagnostic Laboratory Program. US Department of Health and Human Services. $26,499

Using Next Generation sequencing to characterize strains of Moraxella from the eyes of cattle with and without clinical signs of pinkeye. GA Commodity Commission for Beef. $19,400

Wolstenholme, Adrian. Genome-wide Association Studies of Macrocyclic Lactone Resistance in the Canine Heartworm, Dirofilaria immitis. American Heartworm Association. $20,000

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Trafficcking of parasitic nematode ion channels. National Institutes of Health. $187,500

Yabsley, Michael. Evaluation of a commercial coproantigen assay for detection of Baylisascaris in definitive hosts. The American Society of Parasitologists. $1,000

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Experimental studies on dogs as hosts for Guinea Worm. Carter Center, Inc. $199,852

Investigating the spatial and temporal drivers of Dracunculus medinensis epidemiology in Chad. Carter Center, Inc. $90,632

Spatial and temporal aspects of the distribution of Lyme disease. Companion Animal Parasite Council. $76,140
Dzimidzoi, Michael

Edwards, Gaylen

Eggleston, Randall

Epstein, Kira

Ezenwa, Vanessa


Fenton, Heath


Ferguson-Noel, Naola

Ferrer, Maria

Filipov, Nikolay

Findly, Robert

Fischer, John


Franca, Monique


Franklin, Samuel


Fu, Zhen


Garcia, Maricarmen

Garner, Bridget


Gogal, Robert

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immunogenic in cotton rats despite high attenuation. NATURE COMMUNICATIONS, 7, 12 pages.


Salik, Jeremiah


Sanchez, Susan


Schank, Jesse


Schmidt, Chad


Secrest, Scott


Sharma, Ajay


Sellers, Holly

Stanton, James


Sen, Tanusree


Stallknecht, David


Stanton, James


Storey, Bobby


Stringham, James


Tarigo, Jaime


Tompkins, Stephen


The key to improved animal well-being is animal health. The key to improved animal health is veterinary research.