Oklahoma State University
Center for Veterinary Health Sciences

Summer Research Training Program

2016
The Summer Research Training Program at Oklahoma State University is designed to identify talented and highly motivated veterinary students interested in exploring a career in veterinary research and then provide those students with an outstanding biomedical research summer training experience. The overarching objective of this program is to persuade outstanding veterinary students to pursue biomedical research careers. The program is structured to achieve this objective both explicitly, through formal training in the process involved in becoming a research scientist, and implicitly, by providing professional support and encouragement through informal interactions with successful veterinary research scientists who are excited about the personal and professional satisfaction gained from their careers.

The 12-week program experience gives first and second year veterinary students the opportunity to conduct a mentored summer research project. Students are assigned to a basic or clinical research faculty mentor for the summer. The mentors guide the students through all aspects of a research project including experimental design, methodology, data collection and analysis, and drawing conclusions. Students also receive specific instruction on a number of research-related topics, tour specialized research facilities in the region, present the results of their research at our annual College Research Day, and have the opportunity to travel to present their research at a national research meeting.

The combination of professional research training and personal exposure to positive role models who can convey a sense of excitement and career satisfaction has the greatest likelihood of success in our efforts to recruit the next generation of veterinary research scientists. Students who have participated in the program in the past have given it high marks for being fun, exciting and beneficial to their development.
Oklahoma State University’s Veterinary Research Scholars Program supported by

Morris Animal Foundation

Office of Research and Graduate Education

Center for Veterinary Health Sciences
| May 9  | Orientation – Drs. Ranjan, Malayer, and Ross                  |
| May 17 | Research Ethics—Dr. Malayer                                   |
| May 23 | Literature search strategy – Dr. Taylor                       |
| May 31 | Student Research Project Presentations I                      |
| June 6 | Careers in Lab Animal Medicine: Why you should consider this option? – Dr. Curtis |
| June 13| Career in Pharmaceutical Industry—Dr. Bailey                  |
| June 20| Abstract and poster presentation – Dr. Lloyd                  |
| June 27| Journal Club – Dr. Maxwell                                     |
| July 5 | Experimental design and analyses – Dr. Taylor                 |
| July 8 | Tour of baboon research colony at El Reno and OUHSC – Dr. Ross |
| July 11| Student Research Project Presentations II                     |
| July 18| Career in Academics: Why you should consider this option? - Dr. Furr |
| July 20| Business of biomedical research – Dr. Hinsdale                |
| July 25| OSU Research Poster Presentations                              |
| Jul 28-31| National Veterinary Scholars Symposium – The Ohio State University |
Symposium
Monday, July 25, 2016

Students and Mentors

Kelsey Bentz.......................... Dr. Mason Reichard
Samantha Hancock...................... Dr. Todd Holbrook
Jenny Hardy............................. Dr. Susan Little &
                                        Dr. Brian Herrin
Casey Landis........................... Dr. Jerry Malayer &
                                        Dr. Ashish Ranjan
Cassandra Rodenbaugh............... Dr. Joao Brandao
Alexis Sirois........................... Dr. Jerry Ritchey
Susceptibility of *Amblyomma americanum*, *Dermacentor variabilis* and *Rhipicephalus sanguineus* to select acaricides

Kelsey L. Bentz, Jennifer E. Thomas, and Mason V. Reichard

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The purpose of this study was to evaluate the susceptibility of *Amblyomma americanum, Dermacentor variabilis*, and *Rhipicephalus sanguineus* to three common acaricides: fipronil, permethrin and diazinon. Acaricide resistance has been documented in multiple species of ticks across the world, and a recent study found resistance in *R. sanguineus* from the United States. Laboratory-reared ticks are used experimentally to test the efficacy of acaricides and therefore must also be evaluated for emerging resistance. We hypothesized that ticks from the Tick Rearing Facility at Oklahoma State University were susceptible to current acaricides. *Amblyomma americanum, Dermacentor variabilis*, and *Rhipicephalus sanguineus*, three of the most common species of ticks in the United States, were obtained from the Tick Rearing Facility at Oklahoma State University. Each tick species was tested against four different concentrations of fipronil, permethrin and diazinon using the larval packet test. Dose-response curves were evaluated to assess susceptibility of tick larvae to the differing doses of each acaricide. We found that laboratory-reared *A. americanum*, *D. variabilis* and *R. sanguineus* were susceptible to fipronil, permethrin and diazinon. The average mortality ± 95% confidence interval was 99.6% ± 0.3 when exposed to acaricides, while ticks not treated (i.e., controls) had an average mortality of 8.2% ± 3.0. Future research incorporating a wider variety of acaricides, susceptibility of lab reared ticks in comparison to field strains, and additional species of ticks will be discussed.

We acknowledge the support of the Oklahoma State University Center for Veterinary Health Sciences and the Summer Scholars Research Program.
Characterization of a novel tick-transmitted *Ehrlichia* spp. infection in horses

Samantha Hancock, Dr. Brian Herrin and Dr. Todd Holbrook

Tick infestations are a commonly recognized problem in horses, with *Amblyomma americanum* being the predominant tick on horses within its range. While there is no true equine-specific *Ehrlichia* spp., horses within the natural range of *Amblyomma americanum* have been shown to have antibodies to an ehrlichial agent. Therefore, to determine the vector potential of *Amblyomma americanum* as a source of ehrlichial infection in horses, describe which *Ehrlichia* spp. can infect horses, and document any clinical manifestations of infection, 5 naïve horses were infested with 100 (50F:50M) wild-caught *A. americanum* ticks per infestation on 5 separate days (500 ticks total). Daily physical exams and thrice-weekly blood draws were collected to document clinical manifestation and to be used for polymerase chain reaction (PCR), serology, and complete blood count/chemistry. Average tick attachment peaked on Day 21 at 80 ticks/horse, but no obvious signs of clinical disease were present in the horses at that time. Also, by day 21 all 5 horses had seroconverted by indirect immunofluorescence assay (IFA), while only one horse tested positive by enzyme-linked immunosorbent assay (ELISA). On day 19, one horse tested positive by PCR of whole blood, and the sequence of the product was confirmed as 99% identical to *Ehrlichia chaffeensis* [GenBank: KJ942243.1]. This study shows that horses exposed to *A. americanum* develop antibodies to *Ehrlichia* spp. and may potentially remain transiently infected, however, further studies are required to fully identify which ehrlichial agents can infect horses and the clinical significance associated with those infections.
A serological survey of horses in U.S. to *Ehrlichia* spp.

Jenny Hardy

Ticks and tick-borne pathogens are commonly recognized in many veterinary species, but there is currently limited serologic data describing equine infection with *Ehrlichia* spp. and no true *Ehrlichia* spp. is known to cause clinical disease in horses. Geographic analysis of the distribution of horses with antibodies to *Ehrlichia* spp. is key to describing the most likely tick vector or vectors responsible for transmitting these organisms, and therefore, can provide information about which *Ehrlichia* spp. may infect horses. To determine the prevalence of horses exposed to *Ehrlichia* spp., fifty serum samples were obtained from each of five geographically distinct diagnostic labs in the United States (GA, OK, PA, TX and WI) (n=250) and tested by indirect immunofluorescence antibody assay (IFA) to assess the presence of antibodies reactive to *Ehrlichia* spp. Of the horses sampled, 13.2% (33/250) had antibodies reactive to *E. chaffeensis* by IFA. There were significantly more seropositive horses in Oklahoma, 14/50 (28%), than Georgia or Wisconsin, 4/50 (8%) and 2/50 (4%) respectively. Antibodies were identified in at least one horse from each geographic region, but regions known to support higher populations of *Amblyomma americanum*, the known vector of several human and canine *Ehrlichia* spp., had more seropositive horses than regions where the tick is less common. These data show that there is a broad distribution of horses with antibodies to *Ehrlichia* spp., thus, further sampling is needed to accurately determine the true prevalence of horses exposed to *Ehrlichial* agents throughout the US and further describe the likely tick vectors responsible for transmitting the infections to horses.
Characterization of a co-culture model for observation of *Francisella tularensis* infection.

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*Francisella tularensis* is the etiologic agent of Tularemia, a potentially fatal disease of both animals and humans. As an intracellular bacterium, *F. tularensis* has evolved the means to evade processing by cells of the immune system, processes that have been studied in mouse models, but there is a lack of studies concerning the infection process in human cells. We hypothesize that a co-culture of macrophages and hepatocytes can provide an adequate model for infection. THP-1 monocytes, and HEP2 cell lines were used for a human co-culture. For comparison J774a.1 and AML-12 cell lines were used for a murine co-culture. The live vaccine strain (LVS) of *F. tularensis* expressing green fluorescent protein (GFP) was used to infect the co-cultures for observation. The co-cultures were characterized by confocal microscopy, flow cytometry and qPCR. For microscopy, cells were stained with DAPI and cytoID to visualize the genetic material and cell phagosomes, and the GFP *F. tularensis*. Flow cytometry was used to quantify cells at intervals over a 48 hour period to evaluate infection status and expression of MHC II, CD206, CD119, and CD89 cell surface markers. One-step qRT-PCR was used to identify and measure expression of cytokines and cell surface markers in response to infection. Preliminary results indicate clear differences between infected and non-infected host cells. Confocal microscopy visualized the process of infection, replication, and release. Flow cytometry revealed a decrease in the cell surface markers, supporting the efforts of *F. tularensis* to remain undetected by the immune system. Results to date confirm the co-culture model as an adequate representation for a living animal in observation of *F. tularensis* infection.

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Sonoclot® evaluation of whole blood coagulation in chickens

Cassandra Rodenbaugh, Andrew Hanzlicek, Ian Kanda, Mark Payton, Shane Lyon, Theresa Rizzi, and Joao Brandao

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Avian blood takes longer to clot than mammalian blood, due to differences in circulating cells and clotting factors. These differences make currently available mammalian coagulation assays of limited use in birds. In pursuit of a new, alternative methodology, we used a Sonoclot® Coagulation and Platelet Function Analyzer to measure the coagulation time of blood samples from 30 privately owned hens. Using a balanced incomplete block design, we compared the coagulation profile (fibrin formation and clotting rate) of fresh blood and sodium citrated blood, while stimulating coagulation with 1 of 3 activators: glass beads, tissue factor, or kaolin clay. Citrated samples were associated with an increased time until initial fibrin formation, with glass bead-stimulated samples taking four times longer than fresh samples, and tissue factor and kaolin samples taking twice as long as fresh samples. Citrated samples had decreased clotting rates of approximately 30% (kaolin), 40% (tissue factor), and 90% (glass beads) when compared to fresh blood with the same activator.

Results of this study suggest that sodium citrate leads to relative hypocoagulability of avian blood when compared with fresh blood. Although commonly used as an anticoagulant for mammalian coagulation assessment, the results of this study indicate that sodium citrate may not be optimal for chickens. Further research is necessary to determine whether the hypocoagulability associated with the citrated samples is a result of physiological or in vitro interactions between sodium citrate and avian blood, which are not observed with mammalian samples.

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Examination of genetic variation in the extracellular domain of the beta-2-integrin (CD18) in cattle as a marker for resistance/susceptibility to bovine respiratory disease.

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Bovine respiratory disease (BRD) is an important cause of morbidity and mortality in feedlot cattle. Although the pathogenesis of BRD is complex, Mannheimia haemolytica plays an important role. An important virulence factor for M. haemolytica is leukotoxin (Lkt), which binds and signals through the bovine beta-2-integrin CD18 on immune cells resulting in dose dependent effects ranging from activation and release of pro-inflammatory cytokines to cell lysis. Although Lkt can bind, it does not induce signal or effects through non-ruminant CD18 ostensibly because of differences in integrin structure related to interspecies genetic variation. Therefore, a hypothesis was proposed that individual genetic variation within cattle expressed as differences in the CD18 gene may result in less or more efficient binding to Lkt and thus relate clinically to resistance or susceptibility to BRD. To test this hypothesis, RNA was extracted from 137 blood samples collected from feedlot cattle in Oklahoma, Kansas and Texas. The cattle were clinically derived from two groups: Group A, those animals that remained healthy through the feeding period and, Group B, cattle that exhibited respiratory disease. A segment of the CD18 gene previously shown to be critical for Lkt signaling (corresponds to amino acids 541-581) was amplified by RT-PCR, the product isolated, purified and sequenced. Sequence results are currently pending and results will be presented.

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