Inhibition of NFκB by the MC159 Protein in A375 Human Melanoma Cells

Alice Bugman, Crystal Randall, and Joanna Shisler
College of Veterinary Medicine and Department of Microbiology, University of Illinois, Urbana, IL

Oncolytic viruses are viruses that selectively replicate in cancer cells and provide a promising alternative therapeutic treatment for proliferative cancers. Myxoma virus (MYXV), a poxvirus that solely infects rabbits, has shown great potential as an oncolytic virus in humans. Some cancers, such as melanoma, have increased cellular proliferation as a result of constitutive activation of Nuclear Factor-Kappa B (NFκB), a nuclear transcription factor involved in proinflammatory responses. Inhibition of NFκB induces cell death in A375 cells, a human melanoma cell line. Poxviruses produce immunomodulatory proteins capable of inhibiting NFκB activation. We are interested in the MC159 protein, a known viral inhibitor of NFκB activation produced by the molluscum contagiosum virus (MCV). Our goal is to characterize the effects of MC159 protein expression on NFκB activation and proliferation of A375 cells. We found that MC159 inhibits NFκB activation in A375 cells through electromobility shift assays (EMSA) and luciferase assays. The ability of MC159 to induce cell death in A375 cells is still being analyzed. MC159 has potential as both a solitary therapeutic or as part of an oncolytic viral system such as MYXV. This development could lead to further advances in the field of virotherapeutic treatments for numerous cancers affecting both humans and animals.

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Effects of Lighting Environment on Expression on Opsins, the Alteration of Ellipsosomes, and Genes Linked to Retinal Damage in the Bluefin Killifis

Kristin M. Claricoates and Rebecca C. Fuller
College of Veterinary Medicine and Department of Animal Biology, University of Illinois, Urbana, IL

Bluefin killifish live in various lighting habitats ranging from tannin-stained swamps to clear springs. Spring and swamp populations vary in the relative abundance of various cone cells, as well as in the expression of the opsins that determine the spectral absorbance. This project analyzes the effects of light on the expression of opsins (8 genes) to determine whether opsin expression...
is plastic and how quickly these changes occur. This project also examines the expression of 3 genes associated with ellipsosomes – a light filter at the base of cone cells, and 2 genes associated with retinal damage due to high light. Fish from three different natural Florida habitats (clear, tannin-stained or mixed water) were placed into a tank of either clear or tannin-stained water. Fish were euthanized at 7 different time points from day 0 to 28 and eyes saved for RNA extraction. We hypothesize that (1) spring fish in clear water will express high levels of UV and violet opsins and high levels of genes associated with ellipsosomes, (2) swamp fish in high light will show high expression of genes associated with retinal damage, and (3) fish from mixed water conditions will show rapid changes in gene expression between treatments. Primer sets for real-time reverse transcription polymerase chain reaction were designed from bluefin killifish gene sequences. This research will indicate the extent to which gene expression is readily plastic with respect to lighting environment and the extent to which this differs among populations. This work will also indicate whether the bluefin killifish can be used as a model organism for studying adaptation to stressful high light conditions.

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**Minimally Invasive Diagnostic Evaluation of Meniscal Disease in Dogs with Cranial Cruciate Ligament Rupture**

*Davye Dillon, Wanda Gordon-Evans, Dominique Griffon, Kim Knap, and Carrie Bubb*  
*College of Veterinary Medicine, University of Illinois, Urbana, IL*

Meniscal disease is common in dogs presenting with cranial cruciate ligament (CCL) injuries. The most definitive diagnostic tests for detecting meniscal disease are MRI and arthroscopy. However, both procedures are expensive, and can be stressful for the animal. We evaluated common, minimally invasive examination findings to see which factor(s) would be most accurate for diagnosing meniscal disease. Eighty dogs presenting with partial or complete CCL tears were evaluated preoperatively for factors that may increase the risk or aid in the diagnosis of meniscal disease. All dogs were evaluated arthroscopically to evaluate the menisci and cruciate ligaments. Our results showed that body condition score, weight, pain in the stifle upon extension, thigh circumference, cranial-drawer, thrust, duration of injury, and partial tears of the CCL had no significance in detecting or increasing the risk of meniscal disease. A positive meniscal “click” increased the likelihood that dogs with CCL damage would also have meniscal disease by a factor of 11.3
(confidence intervals: 2.2, 58.4). Dogs with pain upon flexion of the stifle were 4.3 times more likely to have meniscal disease and dogs with a complete CCL tear were 9.6 times more likely to have meniscal disease. The diagnostic accuracy using all three risk factors was 77% with 81% sensitivity and 60% specificity. Combining diagnostic factors including meniscal “click”, severity of CCL tear, and pain upon flexion of the stifle provide a novel approach to diagnosing meniscal disease.

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Research support: Morris Animal Foundation

**Correlation Between Body Size and Intestinal Size, Nutrient Processing Capacity, and Rate of Gain in Growing Piglets**

*Sarah Francik, Heather Mangian, and Kelly A. Tappenden*

*College of Veterinary Medicine and Department of Food Science and Human Nutrition, University of Illinois, Urbana, IL*

It is believed that intestinal size in growing pigs decreases as body size increases. The implications of this relationship are that nutrient processing capacity and hence growth also decreases as pigs gain more weight. We hypothesize the nutrient processing capacity and feed efficiency decrease as body size increases in growing piglets. The objective of this study is to assess the validity of a correlation between intestinal size and function and body size of growing barrows. Growing barrows (n=72) at 9 weeks of age will be divided into three replicates (n=24) with the pigs divided into two time points. Half of the pigs from each replicate will be fed a corn soybean meal diet for 7 days and the other half will be fed the same diet for 14 days. The pigs will be weighed at 0, 7, and 14 days, if applicable, to track growth, rate of gain and feed efficiency. At the end of a given time point pigs will be euthanized and intestinal samples will be collected for analysis of structure and function. The intestine will be divided into four segments: the duodenum, jejunum, ileum and colon. Each segment will be weighed and measured to assess size. Ussing chambers will be used to analyze mucosal ion and nutrient transport in the segments. The data will give further insight into the correlation between body size, intestinal size, and nutrient processing capacity which could lead to more efficient diet formulation in growing pigs.

Student support: National Center for Research Resources, NIH, T35 RR020292
Arachidonic Acid Specific Deficiency Results in Inflammation at the Ileocecal Junction

Caitlyn Getty, Manuel Roqueta-Rivera, Timothy Abbott, Kevin Hadley, and Manabu Nakamura
College of Veterinary Medicine, Department of Food Science and Human Nutrition, and Division of Nutritional Sciences, University of Illinois, Urbana, IL; Martek Biosciences, Columbia, MD

Arachidonic acid (20:4n6, AA) and docosahexaenoic acid (22:6n3, DHA) are important in gastrointestinal cell function, but the roles of each are not fully elucidated. AA and DHA are metabolites of the dietary essential polyunsaturated linoleic acid (18:2n6) and α-linolenic acid (18:3n3), and the rate-limiting enzyme is delta-6-desaturase (D6D). In order to study the gut requirement for AA, D6D knockout (-/-) mice were used as the experimental model of specific AA deficiency and wild-type (+/+) mice were used as a control. Three age groups were utilized: 1 month (+/+), 3 months (+/+, -/-), and 4 months (+/+, -/-); (n=3-4) for each group. Mice were fed a diet containing 0.1% DHA and no arachidonic acid. At each time point, fatty acid and histological analyses (3 and 4 months) were performed on the gut tissue. Tissue AA levels decreased in the (-/-) mice over the course of the study and were 92.1% depleted at 3 months and 96.6% at 4 months in the (-/-) mice compared to the controls. Histological analysis showed normal epithelium in the (+/+) mice and compromised epithelium at the ileocecal junction in the 3 and 4 month (-/-) mice. Increased lymphocyte infiltration and decreased goblet cell number were seen in all (-/-) mice before erosions or ulcers appeared. Erosions were seen in two 3-month mice, and more severe erosions or ulcers were seen in three 4-month mice. In conclusion, the study has shown essentiality of AA in gastrointestinal function; in particular, AA may play a critical role in goblet cell function.

Student support: National Center for Research Resources, NIH, T35 RR020292
Research support: Martek Biosciences

Meaningful Assessment of Behavior in the Study of Laying Hen Preference

Jennifer Carr, Angela Green, Patricia Paulausky, and Tatiana Sales
College of Veterinary Medicine and Department of Agricultural and Biological Engineering, University of Illinois, Urbana, IL

Laying hens (Gallus gallus) provide an ideal subject for preference research, as their welfare in agricultural environments is a subject of ongoing moral
and economic debate. In such preference studies, conclusions are often based on the pretext that a hen’s choices will reflect the pressures of its primal–or ethological–needs. The addition of behavioral analysis to these studies can provide further insight into the birds’ environmental or resource preferences. For example, the correlation of defecation with a presumably negative environment (such as one high in ammonia) offers a logical explanation for lack of aversion. To investigate the relationship between preference and innate behavior in the hen, a video analysis was performed concurrently with a preference study on passageways. Hens were trained to use three types of doors to move between two cages, one containing food and the other water. They were then allowed to choose between the three doors. Their choices were recorded via sensors in the connecting passages, and their behavior was monitored by video. The aim of this experiment is to explore the degree to which the hens’ choices are reflected in their general behavior. For behavioral analysis to be considered a meaningful tool in preference research, it should provide evidence of the birds’ experiential perceptions of the environment presented to them, and how these perceptions inform their choices. The results of this study will enhance the value of preference studies in the design of housing systems that reduce stress and optimize welfare for laying hens.

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Spatial Analysis of Human Behavior and its Relation to Exposure to West Nile Virus in Suburban Chicago

Alicia M. Hines and Marilyn O. Ruiz
College of Veterinary Medicine, University of Illinois, Urbana, IL

West Nile Virus (WNV) poses a significant public health threat, but the risk of infection for humans varies by location. Spatial analysis is useful to identify infection patterns in mosquitoes and hosts, but few studies have examined how to best measure the locations of people when assessing risk of infection. In this study, we examined a region south of Chicago, Illinois, where we selected 40 sites from a random stratified sampling of three environments (natural, residential, and other) and monitored human distribution and activity during evening mosquito feeding hours. Sex, age group (child, adult, senior) and activity were recorded for each observed human and additional subsets of humans were interviewed about their knowledge of WNV, use of mosquito repellent, place of residence, and frequency of visits to the
interview area. To determine how human behaviors affect WNV exposure risk, the demographic and behavioral data were analyzed in conjunction with mosquito WNV infection rates. Estimates of human location from survey data, U.S. Census data, and house locations were also used to compare estimated exposure risk given different data. Greater risk was expected in natural areas near residential spaces due to increased human use of the areas during mosquito feeding hours and a high abundance of mosquito hosts in which WNV is amplified. Pairing the knowledge of how the location of specific human behaviors influence WNV exposure with the ecology and epidemiology of the virus will aid in developing more accurate predictive models and improve protocols for controlling potential outbreaks.

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The Affect of TNF-α on Meiotic Maturation and Blastocyst Development

Jennifer Ida, Y. Yuan, E. Silva, and R.L. Krisher
College of Veterinary Medicine and Department of Animal Sciences, University of Illinois, Urbana, IL

Female infertility has been linked to elevated Tumor Necrosis Factor alpha (TNFα) in multiple tissues. TNFα is a cytokine released in response to inflammation and could potentially have an effect on gametes in vivo. Previous work in our laboratory has demonstrated elevated expression of TNFα in oocytes with reduced competence. The objective of this study was to determine the effects of TNFα on the meiotic maturation and developmental potential of immature porcine oocytes. Oocyte treatments were; 1) control with no TNFα, 2) 0.1 ng/mL TNFα, 3) 1 ng/mL TNFα and 4) 100 ug/mL anti-TNFα antibody. Porcine oocytes were aspirated from abattoir derived sow ovaries, and selected to assure good, homogeneous quality. Oocytes were placed into PPM maturation medium and matured in vitro for 42-44 hours. Then, a sample of oocytes was assessed for meiotic stage. The remaining oocytes were fertilized (mTBM medium) and cultured (NCSU23 medium) in vitro (IVF/C) to assess cleavage and development to the blastocyst stage. Preliminary data demonstrate there is no difference (p>0.05) in embryo cleavage or development after exposure of oocytes to TNFα during maturation, despite previous reports that TNFα reduced blastocyst formation. Compared to control oocytes, anti-TNFα tended to reduce development to the blastocyst stage after IVF/C, suggesting that some level of TNFα is necessary to support oocyte quality. Overall, these results suggest, however, that TNFα is not a critical factor during oocyte maturation in supporting
Oocyte development following fertilization. These results enhance our understanding of oocyte competence and factors affecting infertility.

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**The Use of Anti-Inflammatory Therapy as Adjunctive Treatment in Dogs with Blastomycosis**

*Melissa J. Munks, Julie K. Byron, Sarah K. Dowling, Nick Szigetvari, Marcella D. Ridgway, and Mark Mitchell*

*College of Veterinary Medicine, University of Illinois, Urbana, IL*

Medical records were reviewed for dogs diagnosed with blastomycosis at the University of Illinois Veterinary Teaching Hospital between 1992 and 2007 and dogs with a presenting PaO2 of ≤ 80 mmHg and clinical or radiographic signs of respiratory blastomycosis were included. All dogs were treated with either itraconazole, fluconazole, amphotericin B, or a combination of these. Group 1 (G1) dogs were treated with NSAIDs and Group 2 (G2) dogs were treated with anti-inflammatory doses of steroids as adjunctive therapy for blastomycosis treatment. The following comparisons were made: number of days of oxygen supplementation, number of days in hospital, survival to discharge, and long term survival of the infection. G1 consisted of 31 dogs and G2 consisted of 37 dogs. The two groups were found to be similar in weight, age, and sex distribution. There was no significant difference between the two groups with regard to duration of oxygen supplementation, duration of hospitalization, survival to discharge, and survival of the infection. There does not appear to be a difference in the efficacy of NSAIDs and anti-inflammatory steroids in dogs with severe pulmonary blastomycosis. Further studies need to be performed to fully evaluate the impact these adjunct treatments have on prevention of ARDs and additional respiratory complications.

Student support: Merial Veterinary Scholars Program

**Fecal Endocrine Monitoring in Snow Leopards (Uncia Uncia)**

*Jamie L. Reichert and Jason R. Herrick*

*College of Veterinary Medicine, University of Illinois, Urbana, IL*

Little is known about the complex endocrine interactions controlling ovarian function and conception in the endangered snow leopard. The goal of this work is to utilize an enzyme immunoassay (EIA) to analyze fecal concentrations of estrogen and progesterone metabolites to characterize
Evaluating ovarian activity throughout the estrous cycle, non-pregnant luteal phase (pseudopregnancy), and gestation in female snow leopards. Measuring fecal steroid metabolites to assess endocrine function is extremely useful in non-domestic species because it eliminates the need for anesthesia or restraint during sample collection, and greatly reduces stress on the animals. Fecal steroid metabolite measurements provide an accurate average concentration estimate because the hormone metabolites are excreted over a period of hours, compared to measurements from serum samples that represent a single point in time. For this study, fecal samples were acquired from 7 female snow leopards maintained at 5 different zoos accredited by the Association of Zoos and Aquariums. Zoo staff collected 3-4 fecal samples per week during routine daily cleaning beginning in January 2010 and continued until the end of the breeding season (May or June 2010) or gestation. The samples were lyophilized and a standard mass prepared for analysis (0.250 +/- 0.05g). Steroids will be extracted from these samples and EIA will be performed. The results of this study can be used to promote the success of snow leopard captive breeding programs by identifying the best time for mating and allowing early pregnancy detection and monitoring.

Student support: Morris Animal Foundation
Research support: Department of Veterinary Biosciences

**Effects of Population Density and Fecundity on Levels of Polyandry in the Least Killifish, Heterandria Formosa**

*Melissa Rohrs, Matthew Schrader, and Rebecca Fuller*

*College of Veterinary Medicine and Department of Animal Biology, University of Illinois, Urbana, IL*

The genetic mating system of a species will determine the strength of sexual selection, which can in turn influence the evolution of morphological and behavioral traits involved in mating. In species with post-fertilization parental care the genetic mating system will also determine the intensity of conflicts between parents and offspring over parentally supplied resources. These conflicts are expected to influence the evolution of traits involved in parent-offspring interactions (e.g. begging) as well as life-history traits, reproductive structures, and epigenetic phenomena such as genomic imprinting. In this study, we used microsatellites to estimate the level of multiple mating in two populations of the placental fish, Heterandria formosa, a species whose mating system involves polyandry and superfetation. We then combined these data with previously collected data from four other populations to determine if there is a consistent relationship between population density and levels of multiple mating in H. formosa and if a relationship exists.
between female reproductive success (i.e. fecundity) and mating success (i.e. the number of mates) in this species.

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Research support: National Science Foundation

Dendritic Morphology of Hippocampal CA1 Pyramidal Cell Neurons in the Newborn Domestic Pig (Sus scrofa domesticus)

Renee E. Seager, Matthew S. Conrad, Ryan N. Dilger, and Rodney W. Johnson
College of Veterinary Medicine, Department of Animal Sciences, and Division of Nutritional Sciences, University of Illinois, Urbana, IL

The domestic pig is emerging as a neurodevelopmental model. This study involves characterizing normal development of the hippocampus. The hippocampus is important in learning and memory, and is involved in many neurological and mental health disorders. The gross morphology of porcine hippocampal formation closely resembles that of the human, however there are no studies characterizing the complexity of pyramidal cell neurons within the hippocampus proper. In this study, dendritic spine density and apical and basal dendritic tree complexity of pyramidal neurons in the CA1 region were quantified using Golgi-Cox stained hippocampal tissues from 3 male and 3 female pigs at birth. The basal dendritic complexity including tree length, number of nodes, and spine density were not significantly different between the sexes. In both sexes, the number of intersections off of the basal tree decreased as the distance away from the soma increased. Female pigs had a significantly larger soma volume (P < 0.001), while the numbers of apical intersections were similar throughout the length of the dendritic tree, and the complexity was similar between the genders at this age. Thus, at birth, CA1 pyramidal cell complexity is very similar between males and females with significant differences only evident in the volume of the soma. Future studies will analyze dendritic complexity over time to fully characterize the normal pyramidal cell development in the pig. This work will allow comparisons of pyramidal cell complexity and development with humans in order to further evaluate use of the pig as a neurodevelopmental model.

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Mono-OH Methoxychlor (mono-OH) Inhibits Antral Follicle Growth through Oxidative Pathways

Julia Seitz, Zelieann Craig, and Jodi A. Flaws
College of Veterinary Medicine, University of Illinois, Urbana, IL

Female mammals are born with ovaries containing a finite number of follicles. Once these follicles mature from the primordial to the antral stage, they become capable of ovulation and synthesize the hormones that regulate and maintain normal estrous cycles. The organochlorine pesticide methoxychlor (MXC) has been shown to cause atresia of antral follicles through oxidative pathways. Although it was banned in the U.S. in 2002, it remains a concern because of its persistence in soil and insolubility in water. Cytochrome P450 enzymes metabolize MXC to two derivative compounds, mono-OH methoxychlor (mono-OH) and bis-OH methoxychlor, which are thought to be more toxic than MXC, though little is known of their effect on ovarian follicles. Thus, we tested the hypothesis that mono-OH inhibits growth of antral follicles via oxidative pathways. To test this hypothesis, antral follicles were mechanically isolated from CD-1 mice at 32-35 days of age. Follicles were placed in culture and treated with vehicle (dimethylsulfoxide [DMSO]) or a selected concentration of mono-OH (0.1, 1.0, 10.0 µg/ml). Every 24 hours follicle growth was measured in perpendicular axes for 96 hours. At the end of the culture, follicles were collected and subjected to RNA isolation and quantitative real time polymerase chain reaction for Cu/Zn superoxide dismutase (SOD1), catalase (CAT), and glutathione peroxidase (GPX). Results indicated that mono-OH significantly inhibited growth of antral follicles. Further, they showed that mono-OH may alter the expression of some antioxidant genes. Collectively, these data suggest that mono-OH may inhibit follicle growth via an oxidative pathway.

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A Retrospective Study of Seasonal and Circadian Presentations of Dogs with Congestive Heart Failure

Gary Steinberg Jr., Julie K. Byron, and Megan M. Mahoney
College of Veterinary Medicine, University of Illinois, Urbana, IL

Humans have circadian variations in cardiovascular events such as myocardial infarction and congestive heart failure (CHF), and other diseases such as asthma caused by bronchoconstriction. Previous studies of CHF in cats have shown daily (circadian) and seasonal variations in disease
presentation. This work tested the hypothesis that there is a daily or seasonal rhythm in the presentation of CHF in dogs. Medical records from the University of Illinois Veterinary Teaching Hospital were searched from 1997 to 2009 for acute cardiac problems (CHF). The effect of admission time, weekday, month, and season were analyzed separately using Chi-square tests. The results identified 163 presentations of 156 dogs with a clinical diagnosis of CHF. CHF presentations were largely confined to Monday and Tuesday (56%) and between 9:00-12:00 (56%). CHF was more common during September, October, and November (38%). Information from this study may assist veterinarians in the development of a preventative medicine plan, aid in educating clients about the timing of clinical signs, and enable veterinary hospitals to anticipate CHF cases during certain times of the day and year.

Student support: Abbott Laboratories

The Effect of Mate Choice on Immediate Early Genes in the Stickleback Brain

Sarah Turek, Molly Kent, Lauren Hostert, and Alison Bell
College of Veterinary Medicine and Department of Animal Biology, University of Illinois, Urbana, IL

Stickleback fish are widely used to study the evolution of behavior. During mating, females show a preference for certain males by exhibiting a “heads-up” posture. This “heads-up” posture is exemplified when a female lifts up the front half of her body facing a male that she has “chosen”. Three treatments were used to determine what areas of the stickleback brain were active during courtship: 1) females demonstrating heads up to a male, 2) females not interested in males (no heads up) and 3) a control group with no exposure to a male. After each treatment was completed, the females were placed into an isolation tank for ninety minutes before they were perfused and brains were dissected out then isolated and sectioned. Immunohistochemistry was used to detect proteins encoded by immediate early genes, specifically analyzing the optic tectum, hypothalamus and telencephalon. We hypothesized that changes would be visible in all three regions, but that changes in the telencephalon would be visible between females that adopted the “heads-up” posture and those that did not. Results from this study will provide new information about brain activity that accompanies animal courtship behavior and may be important for studies of human behavior because the human brain has analogous structures.

Student support: Merial Veterinary Scholars Program
Exercise-Induced Neurogenesis May Enhance Associative Fear Memory Formation in the Hippocampus

Christine E. Venghaus, Rachel A. Kohman, and Justin S. Rhodes
College of Veterinary Medicine and Department of Psychology, University of Illinois, Urbana, IL

Exercise enhances performance in a number of cognitive tasks, in part, through facilitating acquisition of new memories. However, whether exercise improves memory recall after a delay has yet to be investigated. Pro-cognitive effects of exercise may be related to increased production of new neurons within the hippocampus. The objective was to evaluate whether exercise training improves performance on a fear conditioning task when the time delay between training and testing is increased and whether number of new hippocampal neurons are associated with the performance differences. Four-month-old male C57BL/6J mice were housed in cages with or without running wheels. During the initial ten days, mice received BrdU injections (50 mg/kg) to label dividing cells. After 30 days of wheel access, mice were trained in a contextual fear conditioning task. Half of the mice were placed into a novel context and received seven footshocks at 1 min intervals. The remaining mice were placed into the context but no shocks were delivered (no-shock controls). Freezing to the context was assessed on day 1, day 7, and day 21 post-training. Mice were euthanized by transcardial perfusion 90 min after testing. Results show that exercise improved performance when testing occurred 1 day after training. No differences were observed between the exercised and sedentary mice 7 and 21 days after training. Furthermore, exercise is expected to increase the survival of new neurons in the hippocampus. These data will further our understanding of how exercise improves cognitive function and the potential contribution of new neurons to memory retrieval.

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Health Survey of the Free-Ranging Eastern Box Turtle (Terrapene carolina carolina)

Brittany Way and Matthew C. Allender
College of Veterinary Medicine, University of Illinois, Urbana, IL

Turtle populations across the world are declining, the Eastern box turtle included. They are the victims of increased vehicular traffic, urban sprawl, and
increased disease prevalence. For this reason it is important for us to study the health status of these populations to determine the effects these factors may have on them. During the summer of 2010, physical examinations, blood, feces, and ectoparasites were collected from Eastern Box Turtles (Terrapene carolina carolina) in Oak Ridge, Tennessee. The box turtles were collected through incidental encounters and canine searches. A total of 131 turtles were collected. Physical examinations were performed on 100 turtles. The most common physical exam findings were healthy animals (n=85), but rare cases of nasal discharge and conjunctivitis were encountered (n=5). Blood samples were analyzed for a complete blood count (n=125) and plasma biochemistries (n=100). The parameters measured included packed cell volume, white blood cell count, total solids, white cell differential, aspartate aminotransferase, bile acids, creatinine kinase, uric acid, glucose, phosphorus, calcium, total protein, albumin, globulin, potassium, and sodium. Reference ranges were established for all parameters. Fecal examinations were performed on four turtles and no parasites were present. Ectoparasites were observed on four of the turtles. The purpose of these samples is to serve as baseline health parameters for ongoing health assessments of this population and future investigations into similar turtle populations. With these parameters established, populations with a higher susceptibility to diseases and other stressors can be identified and the proper action to protect these reptiles can be taken.

Student support: Morris Animal Foundation

Assessment of Microbial Communities and Comparison of Culture-Based vs. PCR-Based Detection of Campylobacter or Salmonella from Laying Hen Facilities

Kathleen Whelan and Angela Kent
College of Veterinary Medicine and Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana, IL

Management of laying-hen facilities directly influences the health and production of the flock. Efficient and accurate detection of pathogenic bacteria is necessary for assessing the health status of these animals. Molecular methods based on amplification of specific genes using polymerase chain reaction (PCR) allow quick screening of environmental samples with high throughput technique. The objectives of this research were (1) determine the most informative sample types (e.g., hen feces) to test for bacterial community differences between hen facilities, (2) assess which microbial populations are responsible for the differences between the communities within the samples that test positive and negative for
Campylobacter or Salmonella samples, and (3) design PCR-based detection of Campylobacter and Salmonella in environmental samples collected from hen facilities under different management regimes. Surface and environmental samples (dust, feces, water) were collected in 2009 from hen facilities under different management strategies: caged-belt, caged-high rise, and caged-free. DNA was extracted from environmental samples and DNA fingerprint analysis was used to assess bacterial community composition. Culture-based techniques were used to determine presence/absence of Campylobacter or Salmonella in a subset of samples. From this subset of samples, PCR-based methods were used to screen the samples for Campylobacter or Salmonella. Fecal and surface samples (besides drinkers) were similar among different management schemes. PCR-based methods were compared to culture-based methods. If detection without enrichment from environmental samples is possible, then molecular-based screening of Campylobacter or Salmonella in hen facilities could provide another option for screening the health status of laying-hens and the microbial ecology of their housing facilities.

Student support: Merial Veterinary Scholars Program

**Pseudosubstrate Inhibition of Botulinum Neurotoxin A Through Modification of the SNAP-25 Cleavage Site**

Abigail Wolf, Jasmine Lee, Julianna Lui, Mengfei Ho, and Brenda A. Wilson

College of Veterinary Medicine, School of Molecular and Cellular Biology, Department of Microbiology, and Institute for Genomic Biology, University of Illinois, Urbana, IL

Botulinum neurotoxin A (BoNT/A), produced by the bacterium Clostridium botulinum, is the most potent bacterial toxin known to man. BoNT/A causes flaccid paralysis that can last for weeks to months by cleaving the SNAP-25 protein and inhibiting the release of acetylcholine from peripheral motor neurons. We hypothesize that modifying the cleavage site of the SNAP-25 protein will inhibit the catalytic activity of the BoNT/A light chain while still allowing the protein to be recognized as a substrate, thereby serving as a pseudosubstrate. Residues 141-206 of SNAP-25 (SNAP-25 peptide) are required for recognition by BoNT/A, with the toxin cleaving between Q197 and R198. We generated a series of DNA constructs that replaced the normal site of cleavage, ANQR, with 2-4 His residues followed by 1 or 2 Arg residues, which are thought to be necessary for recognition by the toxin’s catalytic site. The DNA constructs were created using PCR mutagenesis of the gene encoding the SNAP-25 protein, and the SNAP-25 peptide was cloned into an E. coli expression vector for heterologous production of the mutant proteins.
The resulting proteins will be purified by affinity and anion exchange chromatography and compared to the wild-type SNAP-25 peptide to determine their inhibitory activities using FRET-based assay analysis. Proteins that exhibit increased inhibitory activity compared to the wild-type peptide may be useful in post-exposure therapeutics for BoNT/A intoxication.

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The Role of Lactoferrin in Immunomodulation in Porcine Model of Human Infants

Krystal M. Woo, Sarah S. Comstock, Elizabeth A. Reznikov, and Sharon M. Donovan
College of Veterinary Medicine, and Department of Food Science and Human Nutrition, University of Illinois, Urbana, IL

Diet plays a critical role in the development of the newborn's immune system. Because they are omnivores that display similar intestinal physiology and function to the human infant, neonatal piglets are an outstanding model to study the impact of infant nutrition on development. Dendritic cells (DC) are an immune cell type that extend into the intestinal lumen, interact with intestinal tract contents and react to dietary proteins. One of these proteins is lactoferrin (Lf), an iron-binding milk glycoprotein with antimicrobial function, which may interact with DC to produce immunomodulatory effects. In addition, Lf is also released from neutrophils and apoptotic cells to prevent further migration of granulocytes to sites of inflammation. We hypothesized the addition of Lf to the diet of colostrum-deprived, formula-fed piglets would alter the phenotype and function of intestinal DC and decrease neutrophil numbers. Neonatal piglets (n=36) were randomized into 3 treatment groups: 1) Control (sow-reared); 2) FORM (colostrum-deprived/formula-fed); 3) Lf (colostrum-deprived/formula-fed+3.75 g/L bovine Lf). Tissue samples (duodenum, jejunum, ileum, ascending and descending colon) were collected on day 7 and 14. Tissue sections were analyzed via immunofluorescence, using antibodies previously demonstrated to identify porcine DC and neutrophils. The results of this study will improve our understanding of the influence of Lf on intestinal immune development in neonates.

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