

## **Is there a bias toward consideration of adaptationist explanations for behavioral phenomena?**

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Researchers often seek to understand biological traits in terms of adaptive functioning, assuming that a trait is selected for a specific function that increases fitness. However, over the years, several critics have pointed out potential issues with this assumption. Namely, Gould and Lewontin in their 1979 seminal paper entitled “The Spandrels of San Marco and the Panglossian Paradigm: A Critique of the Adaptationist Programme” demonstrated that alternative evolutionary explanations to adaptation often exist for the occurrence of a trait. They went on to call out followers of the “Adaptationist Programme” for failing to consider such alternative explanations for phenomena. In this study, we examined ten prominent behavioral research journals to determine if there is a bias toward consideration of adaptationist explanations over alternative explanations for the occurrence of animal behaviors and if the extent of such a possible bias has changed since 1979. Starting with the year 1980, articles every fifth year were examined manually for both adaptationist and alternative keywords, as well as for relevant context. Preliminary results suggest there is a bias in the behavioral sciences toward explaining traits as evolutionary adaptations even when equally or more plausible alternative evolutionary explanations could have been offered. We speculate that this bias may contribute to prejudiced beliefs and misleading conclusions about the origins of behavioral traits, slowing progress in uncovering the true nature of nature.

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## **Epigenetic control of tumorigenicity of testicular germ cell tumors**

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Testicular germ cell tumors (TGCTs) are aggressive yet can be extremely sensitive to cisplatin-based chemotherapy. Alternative therapies are needed for tumors that become refractory to cisplatin with the DNA methyltransferase inhibitor 5-aza deoxycytidine (5-Aza) providing one possibility. Two series of cell models were generated to better understand mechanisms of chemotherapy sensitivity and resistance: one was resistant to cisplatin and the other to 5-Aza. Interestingly, cisplatin resistance was associated with increased sensitivity to 5-Aza and 5-Aza resistance was associated with increased sensitivity to cisplatin. Preliminary data demonstrated reciprocal epigenetic alterations and a substantial decrease in tumorigenicity in cisplatin-resistant cells. The goal of the current study was to determine the tumorigenicity status of 5-Aza-resistant cells and to investigate reciprocal changes in gene expression that may account for tumorigenicity alterations. Transcriptomics revealed tumorigenicity genes (ZEB2, ID2, CCND1, CAPN2, COL12A1, and MMP2) upregulated in 5-Aza-resistant cells and downregulated in cisplatin-resistant cells. Inversely, tumor suppressor genes (PTMA, MEG3, ZNF208, KLF17, LRRC4B, and ARL10) were upregulated in cisplatin-resistant cells and downregulated in 5-Aza-resistant cells. Tumorigenicity assays were initiated using 5-Aza-resistant cells which are expected to exhibit increased tumorigenicity compared to cisplatin-resistant cells. This study provides insight into the mechanism of TGCT cell sensitivity to 5-Aza and cisplatin and suggests that epigenetics may reciprocally control cisplatin sensitivity and tumorigenicity of TGCTs.

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### **Development of a method for reducing the incidence of rotavirus transmission between farrowing groups**

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Porcine rotavirus is a pathogen of neonatal pigs that is increasing in frequency in commercial swine breeding herds. Porcine rotavirus is spread from carrier sows that shed the virus in feces. When piglets ingest the feces, the resulting small intestine enteritis and diarrhea cause reduced growth rate and high economic losses. The most-concerning source of rotavirus is an infected farrowing group. Without adequate cleaning, rotavirus remaining in the farrowing crate is spread to the next group of pigs. Conventional control

methods of vaccination and high-pressure washing with disinfection have been ineffective against this environmentally stable pathogen. We hypothesize that enhanced hygiene with surfactant, detergent, and/or physical removal (power washing) will reduce viral loads on flooring surfaces in swine farrowing facilities and reduce the incidence of virus transmission to new sows and piglets. Clean rubber mats were infected with a single dose of modified live porcine rotavirus vaccine mixed with 5 grams of feces and 5 ml of water spread over a 4-square-inch sample area. Mats were subjected to various cleaning methods involving combinations of pressure washing, surfactant, and disinfectant. Samples were collected from the mats using sterile cotton swabs, as well as a toothbrush to simulate pigs foraging and rooting. Cell culture will be used to quantify recovery of infectious virus from the mats. Results from this model system will be tested in a commercial swine operation experiencing clinical disease. Reduction of the transmission of rotavirus between groups of farrowing sows would provide a meaningful improvement to current swine production methods.

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## **The role of glycoprotein C in horizontal transmission of turkey alphaherpesvirus**

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Members of the *Alphaherpesvirinae* subfamily of the *Herpesviridae* infect mammalian, avian and reptilian species, and continuously cause problems affecting humans and food animals. Previous research showed that the alphaherpesvirus-conserved glycoprotein C (gC) is essential for horizontal transmission of Marek's disease alphaherpesvirus (MDV) in chickens. MDV causes a highly contagious infection in chickens that clinically presents with neoplasms and neurological signs. We hypothesized that gC homologs of other avian alphaherpesviruses are also necessary for horizontal transmission. MDV is controlled in the poultry industry by vaccination with modified-live vaccines (MLV) including homologous turkey alphaherpesvirus (HVT). Preliminary data in our laboratory showed that HVT does not spread from chicken to chicken during *in vivo* studies. This study aims to determine whether HVT gC is expressed during replication in cell culture and whether HVT gC replaced with MDV gC will facilitate horizontal transmission. To test

these hypotheses, two viruses were generated using the 2-step Red recombination method: one with an HA epitope in frame at the N-terminus of the HVT gC protein to facilitate its identification, and another in which MDV gC will be inserted into the previously generated HVT $\Delta$ gC to replace HVT gC. Viruses were characterized in cell culture for replication and then subsequently used in chicken-to-chicken transmission studies. The information provided in this study will allow us to understand more about the HVT vaccine strain commonly used in chickens and its future implications in gene modification research.

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## **Pharmacokinetics of Stokes compounding pharmacy extended-release theophylline formulation**

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Theophylline is used in veterinary medicine as a bronchodilator to treat canine bronchitis. However, immediate-release formulas require administration four times daily, leading to lower owner compliance and poor management of chronic disease. Additionally, only one commercially available extended-release formulation has been tested in dogs and showed poor bioavailability. These shortcomings led compounding pharmacies to create new formulations for which the pharmacokinetics need to be established. This study will use eight healthy dogs to establish the pharmacokinetics of oral extended-release theophylline compounded by Stokes Pharmacy. The design will be a randomized, two-way crossover with a 7-day washout period between intravenous aminophylline (IVA, 8.6 mg/kg theophylline equivalent), to determine absolute bioavailability, and oral Stokes theophylline formula (STF, 10 mg/kg) administration. Plasma theophylline will be quantified by liquid chromatography and tandem mass spectrometry. Non-compartmental pharmacokinetics analysis will be performed for both IVA and STF. The accumulation ratio and the superposition methods will be used to estimate whether steady state plasma concentrations fall within the therapeutic range established for adult humans (10 – 20 mg/mL), as there are currently no studies determining the canine therapeutic range. We predict that the STF will have similar bioavailability to

previously established theophylline products and produce adequate plasma concentrations and durations to be used at a twice-daily regimen. Validating STF will provide another product alternative for treating canine bronchitis, while improving client compliance and disease management.

Research Grant: Stokes Pharmacy

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### **Validating respiratory pathogen qPCR for use with feces as a non-invasive diagnostic tool for great apes**

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Human respiratory pathogens pose a serious threat to great ape populations, often causing high morbidities and occasional mortalities. Because research and tourism result in frequent contact between human and apes, quick and safe pathogen identification are essential for species health. Many respiratory pathogens are coughed up, swallowed, and passed in the feces, which are a non-invasive sample that is easy to collect for testing. Although previous studies screening for respiratory pathogens in wild great ape populations have used real-time PCR assays developed for humans, to our knowledge, these assays have not been validated for use with feces. To validate these assays, two different fecal DNA/RNA extraction methods were compared for identification of the respiratory pathogens *Streptococcus pneumoniae* and *human Metapneumovirus* (hMPV) in chimpanzee and gorilla feces. Varying concentrations of pathogens or their nucleic acids were added to fecal samples from clinically normal apes to evaluate assay performance compared to a standard curve. Results showed an effect of extraction method on target amplification and assay efficiency, demonstrating the need for assay validation. Newly validated assays will be used to test fecal samples collected from free-ranging chimpanzees in Gombe National Park, Tanzania during a respiratory disease outbreak of unknown etiology.

Research Grant: Zoological Pathology Program

Student Support: Office of the Director, NIH, T35 OD011145

## **Unmanned aerial vehicles (UAVs) for counting wildlife: a review of their efficacy compared to other methods**

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Population counts of free-roaming wildlife are essential for scientists to monitor dynamics of a species and provide information for improved management and conservation practices. Unmanned Aerial Vehicles (UAVs) are a novel technology for scientists to conduct research on free-roaming wildlife, including to evaluate population density, monitor health and behavior, collect remote images, and identify threats to their environments. The purpose of this literature review was to evaluate the existing literature that compares the efficacy of UAVs compared to other methods for counting free-roaming wildlife. We hypothesized that UAVs are more efficient and welfare-conscious for evaluating free-roaming wildlife compared to traditional methods. Eighty relevant publications were retrieved from Thomson Reuters Web of Science that utilized UAVs to evaluate free-roaming wildlife: 9 literature reviews, 43 studies that evaluated terrestrial wildlife, 22 studies that evaluated marine animals, 7 studies that evaluated aquatic animals, and 2 studies that evaluated livestock. Publications that discussed behavior offered important insights to the welfare implications of UAVs. Published studies from 2006-2020 indicated researchers are using UAVs to study wildlife with increased frequency. Preliminary analyses indicated that UAVs have a shorter flight distance than manned flights, and offer overall improved accuracy, increased safety, and reduced costs, compared to traditional wildlife counting methods. Important future frontiers in the use of this technology include standardization of methodology and development of artificial intelligence technologies for processing large volumes of wildlife imagery.

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## **The genetics of paternal behavior in unique stickleback fish populations**

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Parental care is crucial for offspring survival but can vary widely across populations of the same species. Stickleback fish (*Gasterosteus aculeatus*) provide a unique opportunity for study in this area. Stickleback parental care is male-exclusive: males build a nest, court a female, fertilize her eggs, then chase her away. However, different populations (called commons and whites) exhibit highly divergent parenting strategies. Commons care for eggs with frequent nest fanning and poking until they hatch, but whites immediately disperse eggs into surrounding algae before building a new nest. Behavior of pure common or white fish was compared to common/white F2 progeny. Assessing F2 survival, willingness to mate, and presentation of characterizable behaviors was a major goal of this study as they had never been produced. Nesting behaviors post-fertilization were recorded in 15-minute intervals twice daily for 4 days, at which time patterns were clearly identifiable. Parenting data was designated as time 0 (immediately post-fertilization) and long-term parenting (days 0.5-4). Data analysis, including principal component analysis, was performed using R. F2s exhibited high survival rates and readily mated with females. There was extensive variation in paternal behavior among F2 males in nesting time, fanning time, fanning bouts, and long-term nest attendance. Most F2 males provided care instead of dispersing eggs. These data provide a foundation for the long-term goal of this project, which is to use quantitative trait locus (QTL) mapping to identify genes that underlie differences in paternal care.

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Student Support: Office of the Director, NIH, T35 OD011145

### **Does misoprostol reduce inflammatory cytokine release caused by circulating endotoxin in equine patients?**

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Inflammation mediated by pro-inflammatory cytokines can have a profound negative effect on diseased equine patients. These cytokines are produced in response to circulating endotoxin and can lead to a severe inflammatory state known as Systemic Inflammatory Response Syndrome (SIRS) which can lead to further systemic, possibly fatal disease. NSAIDs are a common choice to treat inflammation but are often contraindicated. Thus, it is vital to identify other pharmaceuticals that can combat increased cytokine production. Misoprostol, a prostaglandin E1 analog, has anti-inflammatory effects in multiple species and may provide an alternative to conventional therapies. In vitro, misoprostol decreases the release of inflammatory cytokines by peripheral blood equine leukocytes. The aim of this study is to determine the effect of misoprostol on inflammatory cytokine release in vivo. Using a crossover design, six healthy horses were given a saline control or oral misoprostol and then challenged with lipopolysaccharide (LPS). Blood was then collected serially over 24 hours. Neutrophilia in all horses confirmed the expected inflammatory response to the LPS challenge and was not different between groups. RNA was extracted from frozen peripheral blood leukocyte pellets. Inflammatory cytokine production (TNF $\alpha$ , IL-1 $\beta$ , IL-10, and IL-6) will be measured using quantitative PCR and results compared between the saline control and misoprostol treatment samples. This study will hopefully provide clinicians with evidence to support this novel therapy for equine patients with endotoxemia and systemic inflammation.

Research Grant: American Quarter Horse Foundation, Auburn University  
Animal Health and Disease Research Program

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## **Genetic analysis of swine and human influenza A virus subtype H3N2 from 2014-2019 in the United States**

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Influenza A virus (IAV) surveillance is a vital aspect of both swine and human health industries because new IAV strains can arise by inter-species transmission, antigenic mutations (drift) and genetic reassortment. These characteristics create a genetically variable virus which can result in a pandemic with significant public health and economic implications. Previous research has shown that the H3N2 subtype has a high potential for cross-species transmission, yet little is known about IAV transmission between



humans and swine. H3N2 sequences of human and swine isolates from July 2014 to July 2019 were collected from NCBI's Influenza Virus Database. All RNA segment sequences from these strains were then aligned. The best-fitting models were selected using a Bayes factor with marginal likelihoods implemented in Beast. The resulting trees were then summarized in a maximum clade credibility tree after performing a 10% burn-in using TreeAnnotator. FigTree was then used to differentiate the human and swine strains. Swine strains found in human clades were considered human-to-swine spillover while human strains in swine clades were considered swine-to-human spillover. Results showed that the current influenza strains were more adapted to move from human hosts to swine hosts which was contrary to expectations from previous studies. Continued genetic surveillance and analysis of IAV is crucial to ensure the health and safety of swine and human populations.

Research Grant: Dr. Kenneth T. and Betty Wright Endowment, Lutz Family Endowment-IFAMS Award

Student Support: Foundation for Food and Agriculture Research

### **Characterizing the erythrocyte sedimentation rate in free-ranging Blanding's turtles (*Emydoidea blandingii*)**

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Erythrocyte sedimentation rate (ESR) is an indicator of inflammation across taxa but most commonly is measured in mammals. Our group is assessing the utility of ESR in evaluating the health of select chelonian species. The focus of this study is the Blanding's turtle (*Emydoidea blandingii*), which is endangered in Illinois. As an environmental indicator species, the health of the Blanding's turtle supports general ecosystem wellness which is vital for other free-ranging wildlife already being threatened by human impact. We hypothesized that ESR is elevated in adult Blanding's turtles compared to juveniles and in turtles with clinical signs of injury or disease. ESR will be measured in two free-ranging Blanding's turtle populations in Lake County, Illinois. ESR measurement would be a valuable addition to traditional chelonian hematological tests because it requires little blood, equipment, or expertise. Characterization of ESR will advance the knowledge and

conservation of Blanding's and other semi-aquatic and aquatic turtles, by increasing the number of validated methods to assess health.

Research Grant: Preservation Foundation of the Lake County Forest Preserve District and the Wildlife Epidemiology Lab

Student Support: Office of the Director, NIH, T35 OD011145

## **Ticks and tick-borne diseases: assessing knowledge, attitudes, and practices of medical professionals in southern Illinois**

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The prevention and control of tick-borne diseases in human populations is dependent on the expertise of the medical professionals who identify and treat them. Since their harmful effects were first discovered, tick-borne diseases have been an important part of epidemiological education for medical professionals. However, medical professionals need to keep pace with rapidly changing information about tick-borne diseases and their spread. The warming climate and altered migratory patterns of tick-carrying animals have distributed tick species and associated diseases to regions where they previously did not exist. Information on these spreading populations is available through a variety of health campaigns and educational programs, but the efficacy of communicating this information to medical professionals warrants analysis. To explore this topic, the Illinois tick survey team created an anonymous survey to assess the general tick knowledge of medical professionals, as well as the attitudes and practices that inform their patient differentials and treatments. Emergency and general practice physicians, registered nurses, and nurse practitioners in southern Illinois were invited to participate in the study, due to their diverse clientele and the rapidly changing tick-borne disease situation in this area. The results obtained from this survey will determine how well the information is being distributed across southern Illinois and highlight which aspects of tick and tick-borne disease education should be further emphasized in future campaigns. In time, this more-targeted education will hopefully reduce the rising incidence of tick-borne diseases in the southern Illinois populace.

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Student Support: Office of the Director, NIH, T35 OD011145

## **Biomechanical risk factors of pacing and trotting on the incidence of osteochondrosis in Standardbred foals**

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Osteochondrosis (OC) is a common developmental orthopedic disease that occurs when endochondral ossification is delayed. Both genetic and environmental risk factors contribute to OC. It was previously reported that pacers and trotters are susceptible to developing OC lesions at different locations in the tarsocrural joint (hock). This observation may be due to the biomechanical differences between pacing and trotting that could affect which OC lesions heal and which become permanent. The goal of this study was to quantify activity in Standardbred foals in their typical farm environment and correlate these data with the development and locations of OC lesions. We hypothesized that the amount of time foals spend pacing or trotting influences the distribution of OC lesions. Standardbred foals (n=137) on three farms were prospectively followed from 2-12 months of age. Radiographs were taken of their hocks every two months from 2-12 months of age to look for OC lesions. Foals were also video recorded in their normal paddock/pasture turnout for at least two consecutive hours per week. Based on the video recordings, activity was assessed in 30-second time intervals and categorized as nursing/eating, standing quietly, lying down, walking, trotting/pacing, and running. Foals spent ~82% of their time standing or eating with <18% of their time in active movement. Less than 1% of their time was spent pacing or trotting. Permanent OC lesions developed in 26% of foals; the majority of these were at the distal intermediate ridge of the tibia. Lesion distribution did not differ between pacers and trotters. More work is needed to elucidate the potential role of biomechanics on OC lesions.

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