

Under pressure: the neural and hormonal mechanisms underlying courtship and predator avoidance tradeoffs

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Reproductive success depends upon appropriate decision-making in the face of competing demands. For example, mating provides clear fitness benefits, yet courtship can involve conspicuous displays that make animals more vulnerable to predation. How do animals balance conflicting needs? Ecological evidence provides some insight into how animals balance needs such as courtship and predation risk, but the genomic and hormonal mechanisms underlying this tradeoff are not well understood. Threespined stickleback (*Gasterosteus aculeatus*) males were presented with a tradeoff between courtship and predator avoidance. Territorial males, identified based on breeding coloration and aggressive behavior, were placed in individual tanks with nesting material. Males with completed nests were then randomly assigned to one of four treatments: predation risk (chemical and visual cues of a common predator), courtship opportunity (exposure to a gravid female), both predation risk and courtship opportunity, and control. Courtship and vigilance behaviors were collected for 2 minutes before, during, and after treatment using Behavioral Observation Research Interactive Software (BORIS). After one hour, males were euthanized, brains were excised and divided into diencephalon, telencephalon, and cerebellum/brainstem for RNA sequencing, and bodies were collected for hormone analysis. We hypothesize that males balancing the risk of predation against the opportunity to mate will compromise courtship, prioritizing survival over reproduction. Further, comparing neural gene expression and hormone levels across treatments will reveal the mechanisms underlying this tradeoff, ultimately providing insight into how animals manage conflicting demands.

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Seeking intestinal inflammatory biomarkers in equine feces as a diagnostic modality for colitis

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There are few reliable diagnostic modalities for large colon inflammation in horses. Fecal biomarkers have been utilized to quantify intestinal inflammation in humans but not assessed in horses. The objective of this study was to validate commercially available enzyme-linked immunosorbent assay (ELISA) kits for the detection of the inflammatory biomarkers myeloperoxidase (MPO) and calprotectin (CP) in equine feces. ELISA kits validated for detection of MPO and CP in equine serum were used. Seventeen fecal samples were each processed to produce a supernatant that was then analyzed along with a paired serum sample. Assay validation steps included intra- and inter-assay variability, dilution linearity, spike recovery, and sample type correlation. Intra-assay coefficients of variation were 10.4 – 31.4% for CP and 0.8% – 34.7% for MPO. Inter-assay coefficients of variation were 54.8 – 62.5% for CP and 19.9 – 147.3% for MPO. Sample dilution resulted in linear measurements for MPO ($P = 0.001$) but not CP ($P = 0.27$). Spiking of fecal samples resulted in percent recovery of $64.2 \pm 66.8\%$ for CP and $360.5 \pm 107.8\%$ for MPO. There was a significant difference between serum and fecal samples for both CP ($P = 0.03$) and MPO ($P < 0.001$). There was no significant difference for fecal CP, fecal MPO, or serum CP for comparisons between sick and healthy horses, but sick horses had a higher serum MPO ($P < 0.001$). Limitations of the study include a small sample size and lack of a gold-standard test for comparison. However, these results demonstrate that the current commercially available ELISA kits for MPO and CP cannot be used reliably with equine feces and therefore this approach is not a valid diagnostic modality for equine colitis.

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Cytochrome P450 metabolism of keto-itraconazole to N-desalkyl-itraconazole in dogs

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Itraconazole (ITZ) is a prevalent antifungal agent often used for its efficacy and broad-spectrum coverage. Unfortunately, ITZ can cause hepatotoxicity in dogs. The specific mechanism of ITZ-associated hepatotoxicity has not been determined but may be caused by ITZ metabolites. ITZ is metabolized by the

cytochrome P450 (CYP) class of enzymes. Our laboratory previously showed that the CYP isoforms 2D15 and 3A12 are responsible for catalyzing the hydroxylation of ITZ to hydroxy-itraconazole (OH-ITZ) and the oxidation of OH-ITZ to keto-itraconazole (keto-ITZ). The CYPs responsible for the final metabolic step in this pathway in dogs, the dealkylation of keto-ITZ to N-desalkyl-itraconazole (ND-ITZ), are not known. This study aims to identify these CYP isoforms. First, the dealkylation reaction linearity was evaluated with a time-course experiment using dog liver microsomes (DLM), a sub-cellular fraction containing many drug-metabolizing enzymes present in canine liver. Then, concentrations of DLMs and the NADPH cofactor were optimized to maximize ND-ITZ production. These concentrations were then used to fully characterize the dealkylation reaction using DLMs. These data were then compared to reactions in recombinant canine CYPs to find which CYP is most important for catalyzing the reaction. Finally, these findings were confirmed in experiments using isoform-specific CYP enzyme inhibitors. We expect that the same CYP isoforms responsible for the first two steps of ITZ metabolism (2D15 and 3A12) also catalyze keto-ITZ dealkylation. This study improves understanding of ITZ metabolism which will guide future experiments to find treatments and prevention for ITZ-induced hepatotoxicity.

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OO-MY! Seeking novel anti-oomycete treatments to aid patients with life-threatening pythiosis

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Pythium insidiosum is the major cause of human and animal pythiosis, a historic disease with emerging importance. *P. insidiosum* is an oomycete that is found in the plants and soil associated with aquatic environments. It enters its host through ingestion of contaminated water or breaches in the skin. The primary clinical signs of *P. insidiosum* infection tend to be gastrointestinal or cutaneous depending on the route of zoospore entry. In animals, pythiosis can lead to severe clinical signs that result in amputation of an affected limb or euthanasia. Treatment options are limited because *P. insidiosum* is not a fungus and therefore generally unresponsive to clinically available antifungal drugs. Immunotherapy, which involves injection of the patient with an extract of *P. insidiosum*, has shown some success. Pythiosis cases have been

reported across the globe from Thailand to India, Brazil, Australia, and the United States. In the U.S., most cases occurred in animals in the southeastern states of Texas, Florida, Louisiana, Alabama, and Mississippi. However, pythiosis cases were documented in northern states during summer months, suggesting that *P. insidiosum* may be adapting to other climates. This potential for more-widespread emergence of *P. insidiosum* prompts further evaluation of current experimental resources to develop anti-oomycete treatments. Resources include genome sequences, libraries of chemical compounds to screen for anti-*Pythium* activity, and examination of approaches used to control oomycete diseases in agriculture. Leveraging these resources will aid in the development of new treatment approaches for pythiosis and ideally result in a better prognosis and longer life for infected humans and animals.

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Ticked-off plants: exploring the relationship between ticks and invasive plant species

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Nationally and throughout Illinois there has been an increase of tick-borne disease in humans and livestock. In recent literature, one risk factor for tick abundance may be the presence of invasive plant species and their effects on microclimate. Invasive plant species are non-native plants that disrupt an area's natural vegetative balance. These plants are typically unintentionally spread through foot traffic or the inclusion of non-native plant species in home gardens. This study sought to find the relationship between invasive plant species, microclimate, and tick abundance. Tick abundance was determined using a standard dragging procedure in which a one-square meter white cotton sheet was pulled across the detritus of three 10-ft transects within each experimental plot. Plots were classified based on abundance of invasive plant species. Tick drags were performed at Dixon Springs Agricultural Center from April to December 2021. Plots were classified as uninvaded (n=3) and invaded by *Alliaria petiolata* (Garlic Mustard, n=1),

Microstegium vimineum (Japanese Stilt Grass, n=3), and *Lonicera maackii* (Amur Honeysuckle, n=3). Collected ticks were identified for developmental stage, species, and will later be tested for pathogens. To collect microclimate data, dataloggers (HOBO), were placed at 0.3 and 1 meter above ground at each transect to record temperature and relative humidity at 30-min intervals from April to December. Descriptive and multivariable statistical analysis will be conducted to assess the relationship between tick population, invasive plant species, and microclimate. This study will lead to innovative approaches to tick-borne disease prevention through the control of invasive plants species.

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Getting to the meat about Salmonella: does antimicrobial susceptibility depend on intended use of the animal?

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Salmonella, a food-borne bacterium, causes the human enteric disease salmonellosis. Antimicrobial resistance (AMR) complicates the treatment and prevention of bacterial disease. The purpose of this project was to assess AMR trends in Salmonella isolates from meat products (ground beef, beef trim, and ground turkey) that went to the human food supply versus diseased or culled livestock diverted from harvest. Intended use of livestock affects the regulatory protocols that govern antimicrobial use in animals; the strictest regulations apply to livestock going to harvest. Salmonella samples from beef were recovered from beef products submitted to the Food Safety and Inspection Service by Illinois meat inspectors. Salmonella isolates from ground turkey were collected from a previous study. Salmonella isolates from cattle and poultry were retrieved retrospectively from cases submitted through University of Illinois Veterinary Diagnostic Laboratory. A broth micro-dilution technique (Sensititre) was used to measure antimicrobial susceptibility of Salmonella isolates. The sensitivity tests provided quantitative minimum inhibitory concentration (MIC) values based on host-specific antibiotics. The MIC values were used to build four separate MIC90 tables, two for cattle and two for poultry. We hypothesize that Salmonella serovars common to poultry or cattle that are going to harvest are more susceptible to antibiotics than serovars common to their diseased or culled counterparts. We also expect to find antibiotic susceptibility levels matching expected

antibiotic usage. These data will be used to understand how certain drug classes used in livestock may contribute to empirical treatment failures in human gastroenteric disease.

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Examining veterinarians' decision-making process surrounding antimicrobial use and resistance

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Antimicrobial resistance (AMR) is a growing problem that affects both human and veterinary healthcare professionals and their patients. AMR is spread among humans, animals, and the environment. According to the CDC, approximately 2 million people in the U.S. are affected by antimicrobial-resistant bacteria annually. Therefore, combatting AMR requires a complex approach that considers the prescribing practices in both veterinary and human medicine. Our goal is to examine the decision-making process and knowledge of veterinarians regarding antimicrobial medications. Veterinary clinics throughout central Illinois were identified using an online search. Veterinarians were recruited by cold-calling and emailing veterinary clinics, as well as announcements on the University of Illinois listserv for referring veterinarians. Participating veterinarians were then interviewed over video call about their experiences with AMR, prescribing antimicrobials, education on antimicrobials, and the scope of their practice. At the conclusion of our study we will identify the recurring themes in the decision-making process of veterinarians when prescribing antimicrobials. This effort is part of a larger One Health study that is conducting similar interviews with healthcare providers for humans, pet owners, and community members in Central Illinois. This information will be used to inform future studies and educational materials to understand and combat the growing issue of AMR.

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The impact of cooked broccoli on short chain fatty acids and related gene expression in lean and obese mice

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Dietary fiber is fermented in the gastrointestinal (GI) tract by the gut microbiome (GM) to produce short chain fatty acids (SCFAs). The major SCFAs produced by the GI tract are acetate, butyrate, and propionate. SCFAs act as ligands to various receptors that affect several physiological functions including cardiovascular and metabolic health. G-protein receptor 43 (GPR43) and GPR41 are SCFA receptors of interest because of their role in insulin sensitivity and gastric emptying. Broccoli is rich in dietary fiber and this fiber content can alter the GM with frequent consumption, potentially affecting SCFA production. It was hypothesized that daily broccoli consumption increases SCFA production and alters the expression of GPR43 and GPR41 in lean and obese mice. Twenty lean mice were randomized to consume a low-fat diet (LFD) or a LFD + 10% cooked broccoli (CB, w/w), and twenty obese mice were randomized to consume a high-fat diet (HFD) or a HFD + 14% CB (HFD + 14% CB, w/w, matched broccoli content with LFD + 10% CB per calorie). The mice were fed these diets for one week. Fecal samples were collected on day 0 and 7 and analyzed by gas chromatography/mass spectrometry to measure the change in acetate, butyrate, and propionate. Colon tissue (n=4/diet group) was collected for the analysis of GPR43 and GPR41 expression using RT-qPCR. There was no significant difference in the gene expression of GPR43 or GPR41 between lean mice fed LFD and LFD + 10% CB or obese mice fed HFD and HFD + 14% CB. The SCFAs are still being analyzed. Future studies will analyze cecal SCFA concentration and additional tissues for SCFA related gene expression.

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Detection of *Brucella* in histologic sections of cetacean tissues using RNAscope *in situ* hybridization

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In cetaceans, *Brucella* can cause diseases including meningoencephalitis, perinatal pneumonia, and abortions. Quantitative polymerase chain reaction (qPCR) is the most-widely utilized method for detecting *Brucella* in cetacean tissues, however qPCR provides no information on location or distribution of *Brucella in situ*. Although immunohistochemistry (IHC) has been utilized for some cetacean brucellosis cases, quality antibodies are not widely available for diagnostic use. RNAScope *in situ* hybridization (ISH) is a highly specific and sensitive technique that uses uniquely designed probes for optimal signal amplification with minimal background staining. This study sought to determine if RNAScope ISH can be used to detect *Brucella* in cetacean tissues. This study also aimed to determine if qPCR cycle threshold (CT) values are predictive of the limit of detection for *in situ* assays. Formalin-fixed paraffin-embedded tissues of known *Brucella* qPCR-positive cetaceans (n = 7) were used to test *Brucella* species-specific ISH probes. Control cases negative for *Brucella* but with *Brucella*-like lesions were also examined (n = 6). To determine sample quality, a positive probe specific to *Tursiops truncatus* was used, GAPDH. A negative probe (bacterial gene, *dapB*) was tested on all sections. Signal intensity using the *Brucella* probe was measured using image analysis and correlated to Ct values from *Brucella* qPCR-positive samples. Other variables including formalin fixation time and *Brucella* sequence types (STs) were also assessed in relation to quantity and location of positive signal. Histologic *in situ* visualization of *Brucella* in cetacean tissues via RNAScope will further pathogenesis research and infection control.

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Influence of prebiotics and probiotics on piglet intestinal CD4+ and CD8+ T cell abundance

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Human milk oligosaccharides (HMO), such as 2'-fucosyllactose (FL), influence intestinal development and microbial composition during the postnatal period. Additionally, HMO resist gastrointestinal digestion and provide energy for gut bacteria such as *Bifidobacterium longum* subsp. *infantis* (Bi). Bi

fermentation of HMO produces acetate, which cross-feeds other gut bacteria, promoting microbial proliferation and diversity. Administration of Bi to neonates has gained popularity due to these benefits. Less is known about the influence of FL and Bi on the infant immune system. We are evaluating the role of FL and Bi-26, a commercially available Bi product, on mucosal immune cellularity via quantification of CD4⁺ and CD8⁺ T cells. Two-day-old piglets ($n = 53$) were fed a formula without (CON) or with 1.0 g/L FL. Piglets within each diet were further randomized to receive either glycerol stock alone or Bi-26 (10^9 CFU) orally once daily. On postnatal day 34/35, animals were euthanized; sections of ileum (ILE) and ascending colon (AC) were collected. CD4⁺ and CD8⁺ T cells were detected by immunofluorescence and quantified using Image J software ($n=8$ /group). Data were expressed as the percentage of CD4⁺ or CD8⁺ positive area relative to total cells (DAPI⁺). A trend ($p=0.07$) was observed for interactive effects of FL and Bi-26 supplementation on CD4⁺ T cells in the ILE. In a posthoc analysis, the Bi-26 group had 26.5% fewer CD4⁺ T cells ($p=0.08$), while CD4⁺ T-cells in the FL group were slightly increased relative to CON ($p=0.08$). No difference was observed for CD4⁺ abundance of FL/Bi-26 relative to CON or CD4⁺ abundance in the AC. This work provides greater understanding of pre- and probiotic modulation of the neonatal immune system.

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