Analysis of SorCS1 haplotypes associated with tame and aggressive behavior in selectively bred red foxes

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Since 1959, the Russian farm-fox experiment has used the red fox (Vulpes vulpes) as a model organism for the study of domestication. Through years of selective breeding, the experiment has created lineages of tame and aggressive foxes as well as maintained a strain of conventional farm foxes that were not under behavioral selection. Recently, genetic mapping and whole genome sequencing analyses identified the SorCS1 gene as a candidate gene associated with tame behavior in the fox. SorCS1 (sortilin related VPS10 domain containing receptor 1) encodes the main trafficking protein for AMPA glutamate receptors and neurexins, suggesting a role for synaptic plasticity in fox domestication. Subsequent analysis identified three common SorCS1 haplotypes in tame and aggressive populations with one haplotype strongly associated with tame behavior. Samples used for this analysis were approximately ten years old. The goal of the current study was to evaluate SorCS1 haplotypes in samples collected during 2018, which represent ten additional generations of selection beyond the initial work. Results showed that the frequency of the previously described tame or aggressive SorCS1 haplotypes remained constant in the tame and aggressive populations suggesting that these haplotypes were not strongly selected in recent years. Although most haplotypes present in the conventional population resembled those found in the aggressive population, a novel haplotype was identified. Results from this study will be used to develop breeding plans to further investigate the role of SorCS1 in fox behavior.

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The effect of formalin and paraffin storage time on RNAscope in situ hybridization signal amplification

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RNAscope (ACD Diagnostics) in situ hybridization (ISH) is a relatively new technique for detection of RNA targets and for studying gene expression in formalin-fixed, paraffin-embedded (FFPE) tissue samples. RNAscope protocols suggest that prolonged FFPE tissue storage and formalin fixation times may limit the ability to detect RNA in archived tissues. Yet, the technology has been successfully used in retrospective studies where tissue fixation time in formalin and block storage time were unknown and/or prolonged. To better understand the limits of storage and fixation time on the ability to detect RNA targets in archival FFPE tissues, we will compare RNAscope staining in FFPE tissues (n=15) known to be infected with Canine Distemper Virus (CDV) that have been stored for 6 months to 18 years at room temperature. Tissues were obtained from raccoons immunohistochemistry-positive for CDV and were fixed in formalin for up to 10 days. To determine the effect of prolonged exposure to formalin, staining of housekeeping gene mRNA expression will be compared in tissues fixed in formalin for 1 day, 2 days, 3 days, 5 days, 7 days, 10 days, 14 days, 21 days, and 28 days prior to embedding in paraffin. RNA target signal amplification will be scored using a semi-quantitative method described in the RNAscope Assay user manual. Results obtained from this study will determine the utility of RNAscope ISH for archival tissues with prolonged storage and/or fixation time and will guide protocol development for the use of RNAscope technology in future veterinary retrospective studies.

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**Effect of nerve growth factor-β in pregnancy outcomes in dairy cows**

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Early pregnancy loss due to luteal insufficiency in high-producing dairy cows compromises the sustainability of the dairy industry. Nerve Growth Factor-Beta (NGF) is a natural seminal plasma protein that has been shown to improve luteinizing hormone release, luteal development, and signs of maternal recognition of pregnancy in our previous studies. We are currently operating a large field study to test the hypothesis that NGF improves luteal function, which in turn translates into reduced pregnancy loss and improved fertility in dairy cows. The study includes 600 lactating Holstein dairy cows.
that will be blocked by parity. Cows within each block will be randomly allocated to either receive 1 ml of phosphate buffer solution (PBS) containing 300 µg of purified NGF intramuscularly (NGF, n = 300) or 1 ml of PBS (Control, n = 300). Cows will be synchronized for the first service and the treatment will be performed at day of artificial insemination. A subset of 120 cows will have blood samples collected and receive ultrasonography examination at day of insemination, and then 7, 14, and 19 days later to evaluate progesterone concentration and corpus luteum development. Blood samples collected on day 19 post insemination will also be used to extract RNA and measure expression of interferon stimulated genes (ISG 15, MX1, MX2, and RTP4) to assess markers for maternal recognition of pregnancy. Pregnancy outcomes will be evaluated at days 37 and 65 post insemination. The development of a novel tool to mitigate the negative impact of subfertility would improve milk production efficiency, environmental stewardship, and the global food supply.

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**Salmonella spp. prevalence and antibiotic resistance in free-ranging Eastern box turtles**

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Salmonellosis is an important zoonotic infection and exposure to pet reptiles has been implicated in several human outbreaks. Although several studies report a low prevalence of this pathogen in free-ranging chelonians, they may serve as a reservoir for human-pathogenic strains. In May-July 2019, free-ranging Eastern box turtles (*Terrapene carolina carolina*) from three populations in Illinois (rural) and Tennessee (urban) were collected through canine and visual search. We hypothesized that the prevalence of Salmonellae in free-ranging Eastern box turtles is less than 5%. Cloacal swab samples were collected from each turtle, selectively enriched with tetraionate broth, then plated on selective and differential media to isolate *Salmonella* spp. Genus was confirmed via MALDI-TOF and antibiotic sensitivities were performed. Of the 96 turtles sampled, three (3.1%; 95% CI: 0.6-8.9%) were detected with *Salmonella* spp. One isolate was serogroup PolyA and was resistant to penicillin, ampicillin, and doxycycline. The other
two isolates were serogroup C1 and were resistant to amikacin, erythromycin, and gentamicin. All confirmed Salmonellae will be sent to the National Veterinary Services Laboratory for serotyping. The serogroup PolyA contains multiple pathogens associated with foodborne outbreaks in humans, including serotypes Typhimurium and Enteritidis. Serogroup C1 contains the highly pathogenic serotype Choleraesuis. Documenting the prevalence of pathogenic Salmonella serotypes in animal indicators like the Eastern box turtle will further our understanding of the spread of these serotypes between humans, animal agriculture, and the environment.

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Effects of a CSF1R inhibitor on microglia and their interactions with glioblastoma

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Glioblastoma (GBM, WHO grade IV glioma) is the most common, malignant, and aggressive form of primary brain tumor in adults, accounting for approximately 50% of diagnosed gliomas each year. The transformed cells in GBM are star shaped glial cells of the central nervous system called astrocytes. Under normal physiologic conditions, astrocytes are responsible for providing energy for the neurons they support, maintaining the blood brain barrier, and modifying the transmission of an action potential across a neuronal synapse by maintaining extracellular ion concentrations and removing excess neurotransmitter. After transformation, astrocytes with activated oncogenes also gain the ability to assemble and control cells of the central nervous immune system, namely tumor associated microglia and macrophages (TAM). Intercellular cross talk between these two cell groups can be directly extrapolated to the clinical symptoms associated with tumor progression. Of these, the most concerning is the peritumoral edema and hemorrhage resulting from the proinflammatory state created by activated TAM. A theoretical remedy for this has been to diminish TAM proliferation and activation using a colony stimulating factor receptor (CSF1R) inhibitor to
compete with the factor released by glioblastoma cells to target the TAM. In vivo and in vitro studies are being conducted to examine the extent to which CSF1R inhibition suppresses tumor progression. If significant, the results of this study would provide further support for the progression of an approved CSF1R inhibitor to enter clinical trials and help people with glioblastoma.

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A non-invasive measurement of prostate tumor hypoxia: systemic utilization of hypoxia probe 1 (HyP-1)

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Hypoxia is a pathological hallmark of many cancers, and increased hypoxia has been associated with increased tumor aggressiveness and treatment resistance in prostate cancer. The highest standard hypoxia measurements are acquired through an invasive procedure that places oxygen sensitive electrodes directly into the tissue of interest. While less invasive, fluorescent probes cannot measure hypoxia in tissue deeper than 1mm. Hypoxia probe 1 (HyP-1) was developed to address the limitations of available hypoxia detection methods and successfully measures hypoxia when locally injected into tumor tissue. The aim of this study was to determine if HyP-1 is taken up by prostate tumors following systemic injection and whether probe reactivity is indicative of a low oxygen tumor environment. Transgenic mice (TRAMP; n=4) were injected with varying doses of HyP-1 retro-orbitally, and probe uptake and activity were measured via IVIS fluorescent imaging. Average HyP-1 activity was determined within a standardized region of interest (ROI) and normalized to tumor volume to assess changes over time. Tumor volume was assessed prior to IVIS imaging via VisualSonics 3D ultrasound measurement. Results confirmed intratumoral HyP-1 uptake and showed that as tumor volume increased, ratiometric fluorescence of HyP-1 within the ROI increased, suggesting increasing hypoxia of the tumor tissue. Results of HyP-1 reactivity will be compared against intratumoral protein expression of the low oxygen responsive transcription factor, hypoxia inducible factor-1α.
(HIF-1α). Taken together, these data will assess the feasibility of a systemic injection of HyP-1 as a reliable, non-invasive marker for tumor hypoxia.

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**Evaluation of subcutaneous estradiol benzoate capsule implantation to sterilize male hamsters**

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There are approximately 1.1 million pet hamsters in the U.S. Many hamster owners report unwanted, accidental litters creating a need for effective sterilization methods. Supraphysiologic levels of estradiol interfere with germ cell development in males. This study tests the hypothesis that sustained high levels of estradiol in neonatal hamsters inhibit male gonadal development. A silastic capsule containing 3 mg/kg estradiol benzoate (EB) or sesame oil (vehicle control) was implanted subcutaneously at postnatal day (PND) 21 in male hamsters (n=5). Blood estradiol levels were monitored on the day of capsule implantation and weekly afterward. Gonadal development was assessed by measuring serum levels of sex steroids and performing gonadal histology. These measurements will be repeated until the hamsters reach the age of sexual maturity when reproductive function will also be evaluated. Initial data from morphological examination of PND30 animals showed reduced testes size in the EB capsule group compared to the control. Inhibition of gonadal development is expected to induce sterility. EB-treated animals are expected to show no or significantly decreased sperm production and lower serum testosterone levels than the controls. EB-treated animals are expected to show low interest in mating and are not expected to produce pups. Development of an easily implantable, effective sterilization method for hamsters would provide an attractive alternative to costly and invasive surgical methods. The implantable capsule is expected to be adapted for use in other species, too.

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Suppression of superoxide sensitivity in *Salmonella Typhimurium*

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*Salmonella* Typhimurium is a gram-negative bacterium that survives the harsh conditions within macrophages during systemic infection. In this environment, *Salmonella* is exposed to reactive oxygen species such as superoxide and hydrogen peroxide. A previous genetic screen demonstrated that the ytfLMN locus is involved in resistance to oxidative stress in the macrophage. Mutations in these genes confer sensitivity to high concentrations of hydrogen peroxide in vitro. However, the mechanism of this sensitivity is unknown. To further characterize this phenotype, mutations that suppress the sensitivity to hydrogen peroxide in the ytfL mutant were sought. Exponentially growing ytfL mutants and wild-type cells were exposed to 1.5 mM, 1.75 mM, and 2 mM hydrogen peroxide for 3-4 hours. Dilutions of cells were plated, and the resulting colonies counted. Although results were highly variable, the ytfL mutant was generally several orders of magnitude more sensitive to hydrogen peroxide relative to the wild-type. Occasionally, a ytfL-derived culture showed resistance to hydrogen peroxide, presumably due to suppressor mutations. Seven putative suppressor strains were isolated; at least two of them exhibited increased resistance compared to their ytfL parent. Suppressor mutations will be identified by sequencing chromosomal DNA from these strains. The identity of these genes will provide a greater understanding of molecular mechanisms of *Salmonella* sensitivity to reactive oxygen species.

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Effects of oral tributyrin and g-cyclodextrin supplementation on swine ascending colon microbiota composition

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Some gastrointestinal bacteria produce butyrate, a short chain fatty acid (SCFA) recognized for its anti-inflammatory effects. Despite its ability to reduce inflammation, oral butyrate supplementation poses a challenge to administer due to its noxious odor and taste. Encapsulating butyrate as tributyrin (TB) with g-cyclodextrin (g-CD) not only improves palatability, but g-CD can also function as a prebiotic. The aim of this study was to assess the effect of two concentrations of TB/g-CD or g-CD on seven species of bacteria in the ascending colon (AC) of piglets. Piglets (2-day-old; n=40) were randomized into 5 treatment groups: control formula (CON); formula+4.15 g/L TB/g-CD (CDTBL); formula+8.3 g/L TB/g-CD (CDTBH); formula+3.69 g/L g-CD (CDL); formula+7.39 g/L g-CD (CDH). On Day 21 AC contents were collected and extracted for DNA. The abundance (Log_{10} number of 16s rRNA gene copies/gram of content) of total bacteria, Prevotella, Roseburia, Faecalibacterium prausnitzii, Clostridium perfringens, Lactobacillus, and Bifidobacterium were quantified via Quant Studio 6 and 7 Real-time qPCR using SYBR Green assays. Data were analyzed by one-way ANOVA via the MIXED procedure with Tukey-Kramer adjustments for diet effects. Data followed a normal distribution and no significant changes were seen among the bacterial populations in response to the treatments. Supplementation with TB and g-CD was well tolerated by the piglets, but did not affect the abundance of the specific bacterial taxa assessed. Ongoing investigations using next-generation sequencing of the V3-V4 region of the 16S rDNA gene will determine whether differences in diversity or the abundance of other bacterial taxa were changed in response to TB and g-CD.

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Impact of in vitro expansion on enrichment of osteoprogenitors from equine periosteum

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Mesenchymal stem cells (MSC) exist in low numbers in most body tissues. In vitro expansion is routinely used to enrich samples for MSCs, based on their capacity for prolonged replication. Recently, we found that in vivo osteogenic activity of periosteal osteoprogenitor cells (POC) in foals is far greater than in adult horses. However, this difference is lost after in vitro expansion. This study assessed the impact of in vitro expansion on the osteogenic phenotype.
of POCs and tested the hypothesis that in vitro expansion of isolated periosteal cells enriches for POCs. Periosteum was collected from five foals and five adult horses. A sample was frozen in liquid nitrogen and the remainder was digested in collagenase for 4 hours. Isolated periosteal cells were seeded in DMEM/10% FBS, expanded to near confluence and passaged twice. Samples were collected of the original periosteum, after initial isolation and at each passage for RNA isolation. Changes in Runx2 and Osterix (OSX) levels, both mandatory for the osteogenesis, were assessed by RT-qPCR. Both Runx 2 (3-fold) and OSX (6-fold) levels were higher in foal periosteum than in adult tissue. During in vitro expansion, Runx2 levels only modestly changed in both foal and adult cultures. In contrast, OSX expression fell significantly and dramatically in both age groups. These results emphatically disprove our hypothesis. In vitro expansion does not enrich for active POCs. Outer zone fibroblastic periosteal cells might dominate during in vitro proliferation, preventing POC enrichment. Alternatively, in vitro conditions might lack critical factors required for OSX expression in these cells. Further work is required to clarify this issue.

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**What the FOXA2?!—protein degradation causes mucus hypersecretion in canine and feline fungal pneumonia**

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Pulmonary mycoses are more difficult to treat than bacterial pneumonia and cause similar detrimental effects to the patient’s lungs. Microbial infections modulate lung immune response, inducing goblet cell hyperplasia and metaplasia (GCHM) and mucus hypersecretion in the airways. Mucus overproduction clogs small airways and reduces pulmonary function by decreasing oxygen exchange in the lung, leading to respiratory distress. Forkhead box protein A2 (FOXA2) is a transcription factor that regulates mucus homeostasis in the airways. Previous studies in bacteria-infected human and canine lungs showed that FOXA2 depletion in airway epithelial cells causes mucus hypersecretion. However, there are no published studies
investigating whether pulmonary mycosis modulates the expression of FOXA2 resulting in excessive mucus in either canine or feline lungs. In this study, we used a combination of immunohistochemical staining of clinical canine and feline fungal pneumonia cases from the University of Illinois Veterinary Diagnostic Lab and molecular analysis of Blastomyces dermatidis-infected immortalized canine bronchoalveolar cancer cell line (BACA) to study the relationships between fungal infection, FOXA2, and mucin expression. The results indicated that fungal-infected canine and feline lung downregulates FOXA2 expression, resulting in overexpression of MUC5AC and MUC5B mucins. Inhibition of FOXA2 was mediated through the activation of the Dectin-ROS-EGFR-AKT/ERK1/2 pathway. Further understanding the role of FOXA2 in mucus hypersecretion may lead to novel therapeutics to aid treatment of fungal pulmonary infections for human and veterinary patients.

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