Platform Sessions

PS1 Optimizing Rat Handling Practices: What Do the Rats Want?

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In mice, there is clear evidence that tail handling is aversive and can negatively impact mouse welfare as evidenced by behavioral and physiological outcomes. However, there is little research on preferred handling methods in rats. The goal of this research was to compare behavioral and physiological responses to different handling methods in rats to determine which method is least aversive. Two studies were conducted. In Study A, 96 Crl:CD(SD) Sprague Dawley rats (48m, 48f; 42-48 days old at study start) received minimal handling once a week at cage change over 4 weeks and were randomly assigned to 1 of 4 handling methods (tail, tunnel, body, towel). Response to humans was assessed using a voluntary human approach test (latency to approach (s), and duration of time in contact (s) were measured). Anxiety-like behavior was assessed using an elevated plus maze (time spent in open arms (s)/total time in open and closed arms (s)) was used as a measure of less anxiety. Blood was collected and analyzed for glucose, corticosterone, and hematology parameters. In Study B, 132 Crl:CD(SD) Sprague Dawley rats of 2 age groups were used (1: 96 total, 46m, 46f, 61-133 d of age; 2: 36m, 11-12 mo of age). Rats received handling twice daily for 9 days and were randomly assigned to 1 of 3 handling methods (tail, tunnel, body). Response to handling was assessed using voluntary human approach test and blood glucose levels. Data were analyzed with linear mixed models with handling treatment and sex as fixed effects and animal nested within cage as a random effect. Neither study showed different behavioral or physiological responses to handling treatment (P>0.05). These results suggest that rats may not be as sensitive to handling method as mice or that the handling methods used in this study were not perceived as aversive. Future studies will use more frequent handling to try to understand preferential and aversive handling in rats.

PS2 Post-Mortem Study on the Effects of Routine Handling and Manipulation of Laboratory Mice

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Routine handling and manipulation of laboratory mice are necessary components of many research studies, but if performed incorrectly, have the potential to cause stress and physical harm to the mice. In addition to being a welfare issue, this can also lead to unintended consequences in terms of experimental outcomes. The pathological effects of these interventions...
are poorly documented and have been previously assumed to have a negligible effect on experimental variables. In that context, we provide a comprehensive post-mortem overview of the main pathological changes associated with routine interventions (i.e., restraint, blood drawing, and intraperitoneal injections) of laboratory mice, emphasizing presumed traumatic osteoarticular lesions. A total of 1000 mice from various studies were included, with 864 animals being heavily manipulated and 136 being handled for routine husbandry procedures only. The most common lesions observed were associated with blood collection or intraperitoneal injections and a series of traumatic osteoarticular lesions likely resulting from restraint. Osteoarticular lesions were found in 62 animals (61 heavily manipulated; 1 unmanipulated) with rib fractures and avulsion of the dens of the axis being over-represented. Histopathology and micro-CT confirmed the traumatic nature of the rib fractures. To reduce and ideally prevent these lesions from occurring, enhanced training of research personnel on gentle mouse handling, restraint, and phlebotomy techniques could help reduce the impact on animal well-being and enhance study reproducibility.

**PS3 Quantifying Blood Loss Volume of Submandibular Venipuncture in Mice Using Contrast-enhanced CT**

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Submandibular sampling is a method of blood collection in mice in which the plexus is punctured and blood is collected. Post-collection, pressure is applied to the sampling site to achieve hemostasis. Bleeding by this technique occurs rapidly and allows for volume loss in the periervascular tissues, fur, and collection tubes. This study quantifies the volume of blood loss to subdermal and extracorporeal spaces during submandibular bleeding. Five naïve, six-week-old BALB/c mice were assessed. CT images of the head were acquired using a 100um voxel size before and after submandibular sampling. A region of interest (ROI) was manually drawn around the submandibular region. Blood within and outside of the submandibular vasculature was quantified using connected thresholding based on the known radiodensity of the imaging contrast agent. Unaccounted blood loss in the punctured cheek was compared to the non-punctured cheek (internal control) using a one-sided student’s t-test. Preliminary results showed an increase of blood volume in the punctured cheek versus the non-punctured cheek post-bleed, indicating subdermal hemorrhaging. Contrast-enhanced CT is an effective method for measuring blood loss not previously accounted for following venipuncture technique that may have utility to refine IACUC blood collection standards and quantify individual skill and novel blood collection techniques.

**PS4 Nest Quality as an Early Indicator for Pregnancy in C57BL/6 Mice**

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Frequent handling and manipulation of pregnant mice and their cage can lead to stress and
reduced breeding performance. Nests, which can be assessed cage-side without manipulating the cage or dam, are an excellent indicator of pregnancy status and are often linked to maternal care of pups and positive breeding outcomes. Using a novel nest scoring system based on integration of nesting material, we were able to accurately assess pregnancy status for 28 matings (16 first- or second-time dams), without cage manipulation, as early as E6. Mating trios were housed together for 4 days, after which period female mice were housed singly in a clean cage and provided a pouch of nesting material (crinkled brown paper strips) and a shelter (a hut or tunnel). Nests were scored daily using a numerical scoring system from 1-4, based on nesting material manipulation and incorporation of enrichment, and nest scores were used as a basis for predicting pregnancy. Generally, pregnant females created high-scoring (3 or higher) nests earlier in the pregnancy than non-pregnant females, who often failed to make complex nests. One of the key indicators of pregnancy was the instinctual shredding of the shelter enrichment and thorough incorporation with other nesting materials. This behavior was more pronounced in cages provided with tunnels than in cages provided with huts, corresponding to a higher predictability at earlier timepoints. Using cage-side nest scoring, pregnancy was determined with 89% accuracy at E9 or earlier. This assessment reduced the need to manipulate the cage and handle the dam during gestation, potentially alleviated handling-associated stress on animals, and was an effective method for early pregnancy prediction.

PS5 Enhancing the Visual Environment of Indoor Housed Laboratory Nonhuman Primates to Reduce Stress and Maladaptive Behavior

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Nonhuman primates (NHPs) housed in indoor laboratory environments are generally exposed to nondescript visual stimuli. This environment is not ideal for a species whose primary sensory modality is visual. To provide a more naturalistic visual environment that would potentially improve their welfare, this study assessed a passive natural visual stimulus. A cohort of four (2 males, 2 females) 5–6-year-old cynomolgus macaques (Macaca fascicularis) and a cohort of eight (5 males, 3 females) 4–5-year-old African green monkeys (AGM, Chlorocebus aethiops) were exposed to 4 videos depicting scenes of natural landscapes projected onto a 10'L x 5'H screen placed on an unobstructed animal room wall. Scenes were projected from 7:00 – 18:00hr, 7 days/week (one nature scene/week). Stress indicators and behavior were assessed by comparing weekly urine cortisol:creatinine ratio levels and ethograms from video recordings (3x/week) of each NHP before (baseline; 4 weeks), during (4 weeks), and after removal (4 weeks) of the visual enrichment. The macaque cohort had a baseline urine cortisol:creatinine ratio of 0.00013 ± 0.00003 μmol/L (mean ± SE) which increased to 0.00017 ± 0.00003 μmol/L during nature scene projection, and a return to baseline levels of 0.00013 ± 0.00002 μmol/L post-scene projection. Similarly, the AGM cohort had a baseline of 0.00010 ± 0.00001 μmol/L, 0.00014 ± 0.00002 μmol/L during nature scene projection, and a return to 0.00010 ± 0.00001 μmol/L during post-scene projection. No significant differences were found between treatments or between females vs. males in either cohort. Although this finding may suggest no impact on this stress hormone, a clear trend where cortisol levels increased during scene projection and
returned to baseline post-projection was observed in both cohorts. The biological significance of this trend is unclear but other factors, e.g., projector management, experimental/clinical interventions, may have played a role. Conversely, preliminary behavior analysis showed an increase in positive behaviors such as allogrooming, playing, and cuddling, and a decrease in threatening behavior during the nature scene projection and the post-projection period.

**PS6 Evaluation of Alternative Communication Button Devices to Access Preferred Activities in Juvenile Swine**

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In the United States, raised slats or tenderfoot flooring are the most prevalent housing modalities used in swine agriculture and research. These traditional housing methods may limit the expression of species-typical behaviors and reduce access to agency, choices the animal can make within their environment. Wild counterparts, *Sus scrofa*, naturally engage with their environment through foraging behaviors such as rooting and grazing for more than 50% of their awake day, behaviors traditional housing setups do not easily accommodate. In a preference test evaluating straw versus no straw, research swine consistently chose the straw bedding condition. Therefore, straw bedding on a solid floor was chosen as the standard housing condition for swine at our facility. It was further hypothesized that allowing the animals to choose various types of foraging material throughout the day could increase the time spent engaging in natural behaviors such as foraging activities. To further evaluate this, five juvenile swine were taught to associate words with various foraging substrates (straw, shredded paper, beet pulp, and mushroom compost) utilizing an alternative communication button device. Videos were then collected and scanned in four different settings to measure frequencies and total durations of foraging behaviors (foraging, tail wagging, frenetic activity periods, and flehmen responses). Additionally, behaviors known to be incompatible with efficient and safe workflow by husbandry staff members were collected (pacing, biting, jumping, and freezing). When communication buttons were utilized, opportunities for the juvenile swine to engage with preferred materials increased, resulting in increased cumulative durations and frequencies of foraging behaviors. Furthermore, the frequency and duration of pacing and freezing were lower in button sessions than in other cognitive stimulating events and baseline behavior. Frequency of behaviors incompatible with efficient and safe workflow by husbandry staff members were also reduced in communication button sessions. Further research should be conducted to confirm the use of communication buttons as an enrichment device for swine and to validate the development of word associations with the button.

**PS7 Awake Electrocardiogram Recordings Using a Cage-side Commercial Device: A Medical and Welfare Refinement**

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Cardiac abnormalities are a common clinical morbidity in captive populations of rhesus macaques. Diagnosis and follow-up monitoring of these conditions with diagnostic equipment currently requires sedation. The need for sedation may persuade many clinicians to limit follow-up frequency to reduce the overall impact on the animals. In addition, the drugs used for sedation can both mask and induce cardiac abnormalities that can interfere with clinical interpretation and potentially result in misdiagnosis. At home medical devices have become increasingly popular for human patients dealing with similar diagnoses. One such product is a commercially available device for obtaining and recording a 30 second trace of a single lead electrocardiogram. We hypothesized that rhesus macaques could be operantly conditioned using positive reinforcement techniques to perform cage side awake electrocardiogram exams using the commercially available device, and this would result in diagnostic quality single lead recordings. We hypothesized that these recordings could be used to aid diagnosis of arrhythmia in awake macaques. In this study, 17 male rhesus macaques were operantly conditioned to place their fingers, 1-3 of each hand, on a mobile electrocardiogram device cage side utilizing positive reinforcement techniques in order to obtain accurate awake heart readings. Animals voluntarily performed this behavior on cue with a high degree of reliability for the 30 second recording interval. The recordings obtained were of sufficient quality to be able to determine normal cardiac electrical activity on a lead I recording trace. In addition, the device was also used on one awake animal that had previously been diagnosed with tachyarrhythmia of unknown origin under anesthesia. In this animal, we were able to determine that this arrhythmia was also present in the awake resting state. This was done without the need for more invasive monitoring such as jackets and Holter monitors and led to a change in our therapeutic strategy. Our work shows that animals can be trained to perform awake cage side electrocardiograms to voluntarily participate in their own improved veterinary care.

**PS8 Chlamydia muridarum Associated Pulmonary and Urogenital Disease and Pathology in a Colony of Enzootically-Infected IL12rb2 deficient and Stat1 knockout Mice**

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*Chlamydia muridarum* (Cm) has recently been reported to be unknowingly prevalent in laboratory mouse colonies, but clinical disease has only been associated with experimental infection of mice used to investigate human Chlamydial infections or following cohousing of NSG mice with Cm-shedding mice. Clinical disease and pathology were observed in 2 genetically engineered mouse (GEM) strains with impaired interferon-γ signaling and Th1 CD4+T cell responses in a colony of various GEM strains known to be colonized and shedding Cm. Experimentally naïve, underconditioned and hunched, B6;129S1-Il12rb2<sup>2<sup>ml1Jm</sup>/J</sup> mice (n=3) were submitted for necropsy. Mild-to-moderate multifocal peribronchiolar lymphoplasmacytic and histiocytic bronchopneumonia with bronchiolar epithelial cell degeneration and intralesional Cm inclusions were observed in 2 mice; the remaining mouse had focal peribronchiolar lymphocytic and histiocytic infiltrates. Immunohistochemistry (IHC) for Cm MOMP-1 antigen
demonstrated positive staining in bronchiolar epithelial cells correlating with inflammation as well as in small and large intestinal surface epithelial cells in all 3 mice. The other affected strain was a breeding pair of B6.129S(Cg)-Stat1tm1Dlv/J mice with poor fecundity. At necropsy, the male had severe unilateral hydronephrosis, moderate rhinitis/tracheitis/pneumonia, and minimal-to-mild gastroenteritis and typhlocolitis. The dam had severe unilateral metritis/salpingitis/periovian steatitis, mild-to-severe bilateral hydronephrosis, severe cystitis, moderate-to-severe arteritis, mild-to-moderate rhinitis/tracheitis/pneumonia, minimal-to-mild gastroenteritis and typhlocolitis, and a renal urothelial papilloma. IHC showed positive staining in epithelial cells at the gastric limiting ridge, small and large intestines, as well as in the urinary bladder, ureter, and papilloma of the dam. Given that Chlamydia is a known cause of infertility in humans, breeding issues were likely attributable to Cm. The robust inflammation associated with Cm inclusions in the papilloma indicates the possibility that Cm contributed to the lesion. Given we previously observed significant morbidity and mortality in Cm infected NSG mice, these cases further highlight the importance of excluding Cm from laboratory mice.

**PS9 Eradication of Chlamydia muridarum from Laboratory Mice: Aberrant and Not So Elementary**

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Chlamydia muridarum (Cm), a reemergent, moderately prevalent (academic colonies), Gram-negative, intracellular bacterium, that causes subclinical to severe clinical disease in laboratory mice, dependent on the host’s immune status. Subclinical infection, which is persistent, modulates both innate and adaptive immune responses, potentially impacting the animal’s research use. Eradication of Cm can be challenging as aberrant bodies can form in response to antibiotic therapy, resulting in treatment failure. A study was conducted to evaluate various antibiotics regimes with the aim of eradicating Cm from both immunodeficient and immunocompetent laboratory mice. NSG mice were cohoused with experimentally infected, Cm shedding BALB/c mice for 14 days modeling a natural route of exposure. Four groups of 8 infected NSG mice were treated for 7 days with either 0.08% sulfamethoxazole & 0.016% trimethoprim (TMS) in water, or feed impregnated with 0.0625% doxycycline, 0.124/0.025% TMS, or 0.12 % amoxicillin. The estimated ingested dose for a 23.5 g mouse was approximately 160/32 mg/kg, 119 mg/kg, 236/48 mg/kg, and 228 mg/kg, respectively. A control group was provided standard water and feed. All antibiotic treated NSG mice were euthanized with clinical disease consisting of dehydration, hunched posture, >20% weight loss, and dyspnea 21-40 days (32.6 ± 4.2 [mean +/-SD]) post treatment (DPTx) as compared to 14-33 days (23.75 ± 5.9) for the controls. At necropsy, all mice had multifocal histiocytic and neutrophilic bronchointerstitial pneumonia and/or bronchiolitis with bronchiolar and alveolar epithelial cell degeneration with prominent intralesional chlamydial inclusion bodies. Subsequently, groups of 8 C57BL/6J,
BALB/c and NSG mice infected with Cm, as described, were treated with 0.124/0.025% TMS feed for 7 (BALB/c and B6) or 21 days (NSG). Immunocompetent mice were negative, whereas all NSG mice were Cm positive by PCR 14 DPTx and euthanized. These results provide insight into the difficulties of eradicating Cm from immunodeficient mice, providing additional evidence that Cm should be an excluded agent.

**PS10 Chronic Wound Management in an Aged Female Macaque**

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A 20-year-old, 8.5 kg, female rhesus macaque (*Macaca mulatta*) with a recent history of reduced mobility presented with full thickness lesions of the ischial callosities and the plantar aspect of the hind feet bilaterally. Due to her clinical history and the presentation of the lesions, the wounds were suspected to have been traumatic in origin with progression and development of new lesions secondary to the changes in mobility causing pressure necrosis. Treatment was initiated with topical silver sulfadiazine cream and Manuka honey on all lesions during the inflammatory phase of wound healing as well as distal limb bandaging and tie over bandaging of the ischial callosities. Delays in wound healing to the proliferative phase prompted incorporation of calcium alginate as the primary dressing to encourage granulation tissue formation. The new dressing quickly helped create a healthy, even layer of granulation tissue in the wound beds. Despite the presence of healthy granulation tissue, delays in epithelialization of the wounds again prompted a new approach. A borate-based ointment containing copper and zinc was applied topically to each bandage change. Literature from rodent models suggests borate-based microfiber glass promotes angiogenesis and epithelialization, and in vitro studies show borate-based microfiber glass with the addition of copper and zinc has antimicrobial and anti-biofilm effects. The delays in wound healing during the various stages were suspected to be due to the locations of the lesions, secondary bacterial infections, and the age and mobility of the animal. With the introduction of wound dressings and topical agents selected to enhance the specific phase of wound healing and therapeutic interventions to increase mobility, progression to the next phase of wound healing occurred rapidly. After extended wound care management employing these targeted strategies, all wounds have fully resolved. This presentation will discuss the various phases of wound healing, unique considerations relevant to each phase, and techniques for management of chronic wounds in limited mobility nonhuman primate patients.

**PS11 The Use of Acupuncture for Treatment of a Lame Goat**

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Acupuncture has been introduced into laboratory animal medicine for over a decade. Its benefits include enhanced analgesia, improved wound healing, and as a treatment for various diseases. However, this technique has not gained wide acceptance or application in laboratory animal medicine for a variety of reasons including lack of training opportunities and peer reviewed
literature, resistance to incorporate complementary therapeutics, being a possible confounding study variable, and difficulty in applying the technique to some of the laboratory animal species. This presentation illustrates how acupuncture was successfully integrated into the clinical management of a research animal. A 3-year-old female Spanish Cross goat was used to investigate the efficacy of bone marrow aspirate concentrate (BMAC) on chondral injury in the setting of acetabular labral repair. After acetabular capsulotomy of the left hip joint, she demonstrated persistent lameness and pain. The differential diagnosis for the cause of chronic pain is the post-operative complication in a surgical procedure to create acetabular labral injury. The clinical signs were managed with chronic use of NSAIDs and opioids in conjunction with passive range of motion exercises for over a month with little to no improvement. The decision to add acupuncture treatment to her pain regime was made with the investigator since it is non-invasive and will have minimal to no impact on the study goal. Her clinical condition significantly improved through subjective observation after one dry needle and two series of electro-acupuncture (EAP) treatments. Opioid treatment was then discontinued until the study endpoint. A brief discussion on the fundamental principal of Traditional Chinese Veterinary Medicine diagnosis, treatment, selection of acupuncture points will be discussed through this case. The audience will gain a general picture of how to select potential clinical cases for acupuncture treatment in a laboratory animal setting and increasing the laboratory animal veterinarian’s awareness of the use of acupuncture for the management of pain.

PS12 Pappilomatous-like Lesions and Mortality Associated with Nannizziopsis arthrosporioides in Central American Boas and other Species within a Breeding Colony

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A shipment of Central American Boas (Boa constrictor imperator) (CAB) arrived infested with mites from a reptile vendor in the US. Within two weeks, proliferative lesions resembling papillomas began appearing on all surfaces on the majority of snakes including lips, eyes, body and head. Many began dying with clinical signs of dehydration and severe wasting. Molecular testing along with histopathology and necropsy demonstrated pathology due to Nannizziopsis arthrosporioides. This fungal species had not been isolated from snakes prior to this outbreak. The lesions differ in appearance from the typical Nannizziopsis sp. lesions in lizards. Lesions were keratinophilic with underlying ulcerations and necrosis in the epidermis and dermis. The fungus is highly contagious and was passed to additional snake species in different rooms within the entire breeding facility, prior to clinical signs appearing in the CABs. Spread was likely through fomites and personnel traffic patterns. Nannizziopsis sp. can be difficult to treat and eradicate. A program for treatment and prevention in other reptile species in the facility will be discussed. These cases illustrate why quarantine procedures are necessary in any facility receiving shipments from vendors where multiple imported species are housed. Nannizziopsis sp. as a pathogen in reptiles will be discussed.

PS13 Type 2 Diabetes Mellitus in Tupaia belangeri (Northern Tree Shrew)
Three older adult, singly housed, northern tree shrews presented for a range of clinical signs within a 1-year period, including lethargy, hind limb lameness, decreased visual acuity when performing tasks, polyuria, and polydipsia. Diagnostics included point of care blood glucose and ketone measurements, as well as urine dipstick readouts. These three shrews were found to have varying degrees of hyperglycemia, glucosuria, and ketonuria. The combination of these clinical signs and diagnostics led to a previously undescribed, presumptive diagnosis of diabetes mellitus type 2. Management of this disease process included modification of baseline diet to a feline diabetic management chow, as well as transitioning from their typical frugivorous diet to vegetable and protein sources such as peas, carrots, mealworms, and eggs. Additional medical intervention included metformin dosed at 10 mg/kg based on human and non-human primate literature. Bi-weekly blood glucose readings and urine dipstick measurements were used to track diabetic management and potential relapse. Using these techniques, animals were able to be clinically managed for 3-8 months from initial diagnosis, with demonstratable reduction in blood glucose values. Diagnosis of type 2 diabetes mellitus was confirmed through terminal blood collection measuring plasma insulin levels, as well as HbA1c measurements, CBC, and chemistry analysis. Post-mortem and histopathological examinations were performed, and main findings included pancreatic islet cell lipidosis, glycogen vacuolation of the pancreatic, biliary, and renal ductules or tubules, and glomerulosclerosis. Streptozotocin-induced type 1 diabetes has been previously described in tree shrews, which provided the literature characterizing their normal versus diabetic hematologic values; however, this is the first report of spontaneously occurring type 2 diabetes in this species and may present opportunities for future translational model development.

PS14 Animal Research: A Risky Business?

TJ Jameson*1, K Stepney2

Our desire to maintain the highest standards of animal welfare and scientific conduct can make us feel challenged by the need to control our complex and dynamic working environment. In response, we may find ourselves considering what could go wrong. However, this raises the questions of where do I start, how to identify these risks, who should I involve, how do I assess them, what should I do about them and how can I achieve this across multiple species, research projects, multiple facilities, and continents? This presentation details how a global-research organization decided to approach animal welfare and compliance risk management. It will explain how we developed partnerships across the organization to leverage existing knowledge of risk management principles and how we applied them to the complex world of animal research. During the presentation we will explain our journey so far, what we have achieved, the principles employed, how we approached approach cultural change, the methods, tools developed and key lessons learned.
PS15 Photobiomodulation Therapy Implementation in an Outdoor Nonhuman Primate Breeding Colony

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Photobiomodulation therapy, otherwise known as PBM therapy or laser therapy, has been shown in many species to be beneficial for adjunctive management of inflammatory conditions including acute and chronic pain. This adjunctive therapy can decrease time for wound healing and may reduce the need for additional therapeutics. These photochemical effects improve cellular metabolism at low level stimulation and provide analgesia with high level inhibition – a biphasic dose response. PBM therapy is used widely in human medicine and has recently been defined in veterinary medicine. The indications for use and expected outcomes are better described in companion animals than they are in traditional laboratory animals and no guidelines or recommendations have been described for nonhuman primates. Here we discuss the implementation of laser therapy in a large breeding colony of Rhesus macaques, focusing on the indications, frequency of therapy, and unique challenges. Species-specific considerations include sedation-only administration, a thorough evaluation of the social implications of hospitalization, and pregnancy status. Attention to the cost of equipment, application time, and disinfection between animals housed in pathogen-defined groups should also be considered. Management of these concerns is demonstrated using clinical cases that are presented in a large outdoor NHP breeding colony.

PS16 Examining the Relationship between Self-efficacy and the Implementation of Lean Methodology while Working in an Animal Care and Use Program

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Via our investigation since 2022, Animal Caretakers (ACTs) in our facility have expressed concerns regarding their ability to accurately assess mouse health while conducting daily cage-side observations. With the current nesting substrate, the mice are able to build large, robust nests that commonly decrease visibility of the cage occupants, resulting in lower than desired self-efficacy of the ACTs when performing this task. Lower than desired self-efficacy of key personnel in an animal care and use program (ACUP) can negatively influence workplace morale, quality of work, efficiency, and customer service. Although Lean management techniques are being used to improve the animal care processes (i.e., husbandry and animal health assessment) of lab animal programs, the use of these techniques on staff self-efficacy and problem-solving abilities have not been well documented. In this pilot study, we examined the relationship between self-efficacy and the implementation of Lean methodology while conducting cage-side observations by using a mixed method design involving quantitative and
Results from preliminary analyses of the final data sets suggest that most (n=3/5; 60%) participants experienced increased self-efficacy when performing the daily cage-side observation task and had a better understanding of Lean methodology after participating in the study (n=5/6; 83%). Qualitative responses aligned with quantitative data and revealed that implemented improvements resulted in increased ACT confidence levels, as well as positive feelings of advocacy due to the presence of a Lean coach. There appeared to be no change in problem-solving abilities in this short-term application of Lean. In summary, our study highlights several benefits to incorporating Lean methodologies in an animal care and use program. Future studies will focus on investigating potential barriers to incorporating Lean processes in this setting in the context of using Lean to address other program needs.

**PS17 Results from the ACLAM-ASLAP 2022 Workforce Demographic and Salary Survey of Laboratory Animal Veterinarians**

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The American College of Laboratory Animal Medicine (ACLAM) and the American Society of Laboratory Animal Practitioners (ASLAP) continue to perform periodic surveys of the workforce demographics, salary, and other employment parameters for the laboratory animal medicine specialty field of the veterinary profession. With ACLAM and ASLAP's continued interest in broadening the survey to include race, ethnicity, and other demographics, expanded employment information, and inclusion of veterinarians employed in this sector who are not members of ACLAM nor ASLAP, the questionnaire was modified in 2021 and again in 2023 to generate summaries and assess trends for each year prior, respectively. The 2023 Qualtrics web-based survey was distributed to all ACLAM, ASLAP, and American College of Animal Welfare (ACAWS) members and posted on related websites and social media sites in 2023, allowing for 38 days of response time. As of this abstract submission after 24 days of the survey being open, 173 completed responses had been received. Overall, 91% and 68% of these individuals were ACLAM and ASLAP members, respectively, and 6% of the respondents were neither ACLAM nor ASLAP members. Forthcoming results from this survey will include the total annual gross professional income of full-time laboratory animal veterinarians stratified by ACLAM diplomate status, the years of post-DVM laboratory animal medicine experience stratified by gender, demographic characteristics and trends, and other findings. Additional results will summarize race and ethnicity, geography (including possible state-based reporting), training program completion, employer and job role details, and cross-response analysis. New for 2023, inquiries into additional demographic data, remote work options, and career interests in remaining
employed in the field of laboratory animal medicine will also be explored. A salary calculator, developed from a multivariate analysis of the data, will be presented for use. The salary calculator and presented data will provide information for hiring and strategic planning for institutions, as well as individuals.

**PS18 Advocating for Aquatic Technician/Vivarium Employees to Upper Administration (Salary, Educational Experiences, Conferences, Job Levels, Retention, Work Life Balance, etc.)**

RA Malbrue*

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This platform session will review methods and strategies on how to effectively advocate for animal technical staff who work directly with aquatic species (i.e., zebrafish & xenopus). It is no secret that technical staff who work aquatic species face several unique challenges when compared to their counterparts. These positions also typically require very specialized training. Topics such as salary increases, professional development, promotion opportunities, and employee retention will be presented.

**PS19 Careers in Laboratory Science: Shareable Videos for the Promotion of Diversity in Laboratory Animal Science**

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Developing a diverse and inclusive laboratory animal science community can help with ongoing staffing and support concerns by ensuring a wide potential pool of potential individuals who would be interested in pursuing careers in laboratory animal science. Ensuring that the literature available to help promote career pathways in laboratory animal science is inclusive can help achieve this goal. The AALAS Foundation has created a series of videos that highlight Careers in Laboratory Science with laboratory animal professionals in a variety of career pathways with diverse backgrounds. In this session, the panel will demonstrate how they can be used to promote the diversity of career options in laboratory animal science and medicine. The targeted audience will be anyone who is interested in promoting careers in laboratory animal and science, to audiences of any age.

**PS20 Pharmacokinetics and efficacy of extended-release buprenorphine for post-operative pain management in the domestic ferret (Mustela putorius furo)**

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Buprenorphine hydrochloride (bup-HCl) is a common injectable opioid analgesic. In ferrets, bup-HCl must be administered every 8-12 hours to maintain therapeutic plasma levels. Extended-release analgesics offer multiple advantages, including reducing animal handling and injection frequency, improving compliance, and limiting the possibility of breakthrough pain. Although the efficacy of extended-release buprenorphine formulations has been demonstrated in other species, their use in the domestic ferret has not been investigated. In this study, we evaluated the pharmacokinetics of a compounded polymeric formulation of buprenorphine (bup-ER) with a duration of action between 48-72 hours in multiple common laboratory animal species, and a pharmaceutical-grade, FDA-indexed liposomal suspension (bup-XR) with a duration of action up to 72 hours in mice and rats. Two doses each of bup-ER (0.12 mg/kg and 0.2 mg/kg) and bup-XR (0.2 mg/kg and 0.6 mg/kg) were administered subcutaneously to 12 young adult female ferrets. All doses achieved therapeutic plasma levels in 30 minutes. Results revealed that high-dose bup-XR maintained therapeutic levels for 72 hours, followed by high-dose bup-ER (48 hours), low-dose bup-XR (<24 hours) and low-dose bup-ER (<12 hours). We also compared the analgesic efficacy of a single high-dose bup-XR (0.6 mg/kg SC) to bup-HCl (0.02 mg/kg SC every 10-12 hours for 3 days) by performing clinical assessment after routine ovari hysterectomy. Ferrets receiving bup-HCl had significantly higher respiratory rate and posture scores in the first 24 hours post-operatively than those that received high-dose bup-XR. Ferrets receiving bup-HCl were also more likely to react to surgical incision palpation overall. It is of note that sterile injection-site abscesses developed after administration of high-dose bup-ER (50%, 6/12) and high-dose bup-XR (10%, 2/20). This study demonstrates that a single dose of bup-XR (0.6 mg/kg SC) is a safe and effective analgesic option in ferrets, with a duration of action of up to 72 hours. The administration of bup-XR in pet and laboratory ferrets offers a refinement to analgesia in this species.

PS21 Pharmacokinetics of an Extended-release Buprenorphine in Female Yorkshire Swine (Sus scrofa domestica)

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Given their anatomical and physiological similarities to humans, swine are the most widely used large animal translational model in biomedical research. Despite the use of swine as a surgical model, precise dosing regimens for commonly used analgesics such as buprenorphine, are currently lacking in this species. A newly available extended-release formulation of buprenorphine (XRB) is FDA-indexed and approved for use in mice and rats; however, no studies have examined the efficacy and pharmacokinetic parameters of XRB in swine. The goal of this study was to determine the pharmacokinetics of the newly available XRB in swine. We hypothesized that after a single subcutaneous administration of XRB in adult swine, buprenorphine plasma concentrations would be above the therapeutic threshold of 0.1 ng/mL for up-to 96 h. XRB was administered once, subcutaneously to two separate cohorts of adult female
Yorkshire swine at low and high doses (0.2 and 0.4 mg/kg, respectively; n = 3 and 2). Blood was collected from an indwelling jugular catheter prior to and after XRB administration (13 total time points). Individual animal data indicated all animals reached therapeutic buprenorphine plasma concentrations by 8 h post administration. Average plasma buprenorphine levels for both the low- and high-dose cohorts reached therapeutic concentrations starting at 90 min after XRB administration and were maintained above therapeutic concentrations throughout the 96 h study period. In the low-dose cohort, the average half-life was 212.6 ± 107.1 h, while the half-lives in the high-dose cohort was 63.8 h and 48.9 h. These results support our hypothesis and indicate that all animals maintained therapeutic plasma buprenorphine levels beginning at 8 h and maintaining past 96 h. Thus, XRB at 0.2 or 0.4 mg/kg provide therapeutic levels of plasma buprenorphine and therefore its use should be further explored in swine.

**PS22 Transdermal Mirtazapine Pharmacokinetics in Rhesus Macaques (Macaca mulatta)**

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Decreased appetite is a common clinical problem in captive rhesus macaques (Macaca mulatta). Mirtazapine, a tetracyclic antidepressant originally developed for humans, has shown promise as a safe and effective promoter of weight gain and appetite in several veterinary species including rhesus and cynomolgus macaques. While mirtazapine is available in oral formulations, transdermal delivery in macaques with reduced appetite may improve patient compliance by allowing quick, painless, topical application. Here we report the pharmacokinetics of a single dose of a widely available transdermal mirtazapine veterinary formulation in six rhesus macaques. 0.5mg/kg of transdermal mirtazapine ointment, an efficacious dose in previous macaque work, was applied to the caudal pinnae of three female and three male young adult macaques, with 11 serum collections performed at 0, 0.5, 1, 3, 6, 8, 12, 24, 36, 48, and 72 hours post-dosing. Our data indicate transdermal mirtazapine is absorbed at a lower level in rhesus as compared with published values in domestic cats (rhesus peak serum concentration 1.2 ± 0.3 ng/mL), while drug half-life is longer than reported in cats (rhesus 33.3 ± 7.0 hours). Mirtazapine reaches peak plasma concentrations in rhesus 15.5 ± 9.75 hours after administration and may require up to seven days of serial dosing to reach steady state. While previous work indicates clinical efficacy of this dosage in macaques, further investigation is warranted to determine if rhesus may benefit from higher recommended doses than companion animal species.

**PS23 Alfaxalone as a Total Intravenous Anesthesia Protocol in New Zealand White Rabbits (Oryctolagus cuniculus) Improves Cardiovascular Stability Compared to Isoflurane**

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Isoflurane is commonly used for anesthetic maintenance in rabbits, but inhalant anesthetics can cause dose-dependent cardiovascular depression and significant hypotension, decreasing its anesthetic quality. Another alternative, alfaxalone, has been studied for induction in rabbits with rapid onset and a short duration of action but has not been evaluated as an option to replace isoflurane for anesthetic maintenance. This study compared Total Intravenous Anesthetic Maintenance protocols (TIVA) using alfaxalone against isoflurane. Twenty-four male New Zealand White rabbits were assigned to one of three treatment groups: isoflurane alone, alfaxalone with buprenorphine, or alfaxalone with midazolam. All rabbits were premedicated with buprenorphine SQ (0.02mg/kg) and induced with alfaxalone IM (6mg/kg). Following intubation, rabbits were maintained for 1 hour on either isoflurane (2.5%) or alfaxalone Constant Rate Infusion (CRI) (0.2mg/kg/min). For rabbits on the alfaxalone CRI, boluses of buprenorphine (0.01mg/kg IV or SQ) or midazolam (0.1-3mg/kg SQ) were given upon induction or adjunctively as needed dependent on positive tail-pinch responses that were conducted at t0, 15, 30, and 45. Heart rate, non-invasive and invasive blood pressure, respiration, end-tidal carbon dioxide (ETCO2), percent oxygen saturation (spO2), and temperature were recorded every 5 minutes. Blood gas was analyzed at t0, t30, and t60. Surgical plane or anesthesia was characterized by lack of positive response to a tail-pinch and was reached in all anesthetic groups. Results showed significant reduction in mean heart rate of the alfaxalone groups while mean invasive blood pressure was increased compared to the isoflurane groups. However, respiration in the alfaxalone groups was decreased with associated increases in ETCO2 levels. There were no significant differences noted between alfaxalone treatment groups. Our preliminary findings indicate alfaxalone given as a TIVA maintenance protocol could be considered as an anesthetic alternative to isoflurane with improved cardiovascular stability though respiratory monitoring or management would be warranted.

**PS24 Comparison of Two Different Formulations of Alfaxalone to Anesthetize Cynomolgus Macaques (*Macaca fascicularis*) for Plethysmography**

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Plethysmography is employed in nonhuman primates (NHPs) to calculate respiratory minute volume (MV) and determine the exposure time required to deliver an aerosol at the target dose. Anesthetic drugs can impact breathing parameters like steady state minute volume (SSMV) central to aerosol dosing. Alfaxalone-midazolam combinations (AM) provide superior parameters for plethysmography in cynomolgus macaques. An obstacle to the use of AM is the volume required to anesthetize via intramuscular (IM) injection. A more concentrated formulation of alfaxalone will reduce injection volumes and refine AM protocols. The purpose of this study was to compare AM using the Indexed 10 mg/mL (AM10) formulation versus an investigational 40 mg/mL (AM40) formulation for IM administration in cynomolgus macaques undergoing plethysmography. We hypothesized that AM10 and AM40 would show no difference in quality of anesthesia (QA), duration of anesthesia, SSMV, accumulated minute volume
(AMV), and side effects. We also hypothesized that female macaques would have a longer duration of anesthesia versus males using both formulations. The study used 15 cynomolgus macaques comprised of eight females and seven males. NHPs were compared between two separate and randomized anesthetic events no less than one week apart. Each animal served as its own control. Anesthetized NHPs were placed in a sealed plethysmography chamber and minute volume measurements were calculated every 10 seconds to determine SSMV. Once SSMV was achieved for 20 minutes, the trial ended. There were no statistically significant differences between AM10 and AM40 for duration of anesthesia, SSMV, AMV, side effects, or QA. AM40 had a significantly smaller injection volume. Females did not show a significantly longer median duration of anesthesia using either of the alfaxalone formulations. Overall, AM40 offers a more humane anesthetic than AM10 for plethysmography in cynomolgus macaques.

**PS25 Venipuncture Site Influences Blood Drop Volume in C57BL/6 Mice**

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Many experiments require the collection of serial blood samples from mice. However, the size of mice limits the volume of blood that may be safely collected as a survival procedure. In IACUC protocols, investigators may report the volume of blood they collect from mice as a number of drops. Many institutions, including ours, use an anecdotal conversion factor (e.g. 1 drop mouse blood = 25 µl) to ensure that these research protocols remain in compliance with institutional blood collection guidelines. To our knowledge, previous work has not experimentally determined the volume of a mouse blood drop and how this volume may be influenced by venipuncture site, needle size, animal user, or mouse sex and weight. In this 10-week experiment, two animal users bled 15 male and 15 female C57BL/6 mice from three sites (facial, saphenous, and tail vein). In our crossover design, mice were divided amongst 5 groups (n=6): left and right tail vein, left and right saphenous vein, and facial vein. Using institutional guidelines for gauge sizes, venipuncture was assigned so that either a 25 or 27 gauge needle was used for saphenous venipuncture and either a 20 or 23 gauge needle was used for tail venipuncture. Facial venipuncture was only performed with 20 gauge needles. A single blood drop produced from each site was weighed, and the volume of each drop was determined using the average blood density determined from 8 mice that were terminally bled at the end of the study. Only venipuncture site significantly influenced blood drop volume, with no significant effects of needle gauge, animal user, or mouse sex or weight on blood drop volume. Facial vein puncture produced the largest drop volume (mean: 21.7 µl), followed by the saphenous vein site (mean: 9.97 µl) and tail vein site (mean: 4.96 µl). Additionally, the facial vein was associated with more post-collection hemorrhage and morbidity, including seizures that required euthanasia of one mouse. The results of this study suggest that blood collection from saphenous and tail veins optimizes both serial collection of small-volume blood samples and animal welfare.

**PS26 Pharmacokinetics of Injectable Buprenorphine and Meloxicam in the Naked Mole-Rat**

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Unique characteristics of the naked mole-rat (NMR) have made it increasingly popular as a laboratory animal. They are used to study cancer, circadian rhythm, pain, longevity, aging, and many other fields of research. Currently, the analgesic dosing regimens used in the NMR mirror those used in other rodent species. However, there is no pharmacokinetic (PK) data supporting the use of injectable analgesics in the NMR. Therefore, we conducted two independent PK studies to evaluate two commonly used analgesics in the NMR; buprenorphine (0.1 mg/kg SC) and meloxicam (2 mg/kg SC). In each study, blood was collected at 8 timepoints after subcutaneous injection of buprenorphine or meloxicam (0 (pre-dose), 15min, 30min, 1hr, 2hr, 4hr, 8hr, and 24hrs). Three NMRs were used per timepoint for a total of 24 animals per PK study. We found plasma concentrations of buprenorphine were highest between 15 and 30 minutes post-injection. These levels remained above the human therapeutic threshold (1 ng/mL) for at least 8 hours. Plasma concentrations of meloxicam were highest between 30 minutes and 1 hour post-injection. These levels remained above the extrapolated dog and cat therapeutic threshold levels (390-911 ng/mL) for up to 24 hours. No skin reactions were seen in association with injection of either drug. In summary, this data supports the dosing of buprenorphine (0.1 mg/kg SC) and meloxicam (2mg/kg SC) in the NMR at a minimum frequency of once every 8 and 24 hours respectively. Further studies should be performed to evaluate the clinical efficacy of these drugs by correlating plasma concentrations with post-operative pain assessments.

**PS27 Assessment of Oral Albendazole and Fumagillin in the Treatment of Pseudoloma neurophilia in Adult Zebrafish**

GLAS: Yes
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*Pseudoloma neurophilia* (*Pn*), the causative agent of the most commonly-reported zebrafish disease, is a microsporidian parasite that confounds research by inducing behavioral and physiological changes in zebrafish. While there is no treatment for *Pn*, albendazole and fumagillin have been used to treat microsporidian infections of other species. To investigate the efficacy of oral albendazole and fumagillin in the treatment of *Pn*, we performed a pilot study that demonstrated the safety and palatability of novel gel-based diets containing fumagillin or albendazole in adult AB zebrafish. In a subsequent study, approximately 250 adult AB zebrafish (previously infected with *Pn*) were treated with these medicated diets for 4 weeks. Fish were randomly allocated to one of five experimental groups: (1) albendazole at 2 mg/kg, (2) fumagillin at 15 mg/kg, (3) combination treatment of both albendazole 2 mg/kg + fumagillin 15 mg/kg, (4) infected control, or (5) uninfected control groups. At four different timepoints (weeks 0, 5, 10, and 16 of the study), fish were euthanized via MS-222 immersion and whole-body qPCR performed to assess for *Pn* prevalence across treatment and control groups. There were no
statistically significant associations between treatment group and Pn prevalence at any timepoint, although potentially biologically significant reductions in Pn prevalence occurred in the combination therapy group at weeks 5 and 16 where Pn prevalence was reduced by 25% and 23%, respectively, compared to baseline prevalence for this group. Additionally, at week 5 in the albendazole group, the Pn prevalence was reduced by 25% compared to baseline prevalence results for this group. High-performance liquid chromatography (HPLC) analyses of the medicated feeds recovered less albendazole (approximately 46% of the expected concentration) and more fumagillin (approximately 235% of the expected concentration), highlighting the importance of validating medicated feed concentrations to ensure feed safety, efficacy, and consistency. While Pn remains challenging to eradicate and control, results from this study warrant further investigation into the utility of albendazole and fumagillin as potential treatments for this pathogen.

**PS28 Creation of a Swine Model of Oral Angioedema**

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Angioedema, a sudden swelling often caused by allergic reactions, commonly affects the face, particularly the lips and tongue. Swelling in this area may be life threatening, especially if breathing is obstructed. In addition, angioedema in the oral cavity may affect absorption of locally administered medication. Though swelling has been induced in the skin of miniature swine (MS) using histamine injections, there is no large animal model of angioedema in this target area (i.e., oral cavity). Thus, an animal model is needed to further investigate facial angioedema pathophysiology and treatments. It was hypothesized that histamine would induce swelling and erythema in the lips and tongue, similar to what is observed in skin irritation models. To test this hypothesis, 6 male Yucatan MS were injected in the mucosa of the inner lip (left and right) and into the tongue with 20 ul of histamine dihydrochloride at each site and at varying concentrations. The following concentrations were used (one animal dosed at each): 25, 125, 500, 1000, 5000, and 10,000 ug/ml. Animals were anesthetized with isoflurane to ensure accuracy of dose administration and measurements. Calipers were used to measure tissue swelling, and Draize scores were performed to monitor erythema and edema around each injection site. The animal dosed at 10,000 ug/ml displayed the desired reaction, and an additional 4 animals were dosed at this concentration to ensure reproducibility of results. For the total of 5 animals dosed at 10,000 ug/ml, the majority of swelling in both the lips and tongue occurred within the first 30 minutes (average change from baseline = 3.82 mm for the lips; 3.32 mm for the tongue). Lip thickness continued to mildly increase over 90 minutes, whereas swelling in the tongue decreased in 90 minutes, though not back to baseline. Draize scores showed increased erythema and edema starting at 10 minutes. Animals received diphenhydramine during anesthetic recovery to reduce any remaining swelling. All animals recovered uneventfully. Thus, histamine dihydrochloride serves as a suitable agent for inducing angioedema in the tongue and lips of MS and can serve as a valuable large animal model for assessing treatments of angioedema and investigating the impact of angioedema on drug absorption.

**PS29 A System to Monitor Health and Exploratory Behavior Reveals that Starved Gut-
Associated Bacteria Do not Induce Peritonitis in Myeloperoxidase-Deficient Mice

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Accurate and objective monitoring of laboratory animal behavior and health is a critical need in biomedical research, especially when mouse models manifest as subclinical signs of disease. Traditional methods, which rely on laborious/costly/observer-based evaluations, present challenges, such as potential biases/insensitivity to subtle changes. Herein, we developed and validated a novel real-time caging system to automatically monitor behavior and health using a mouse model of peritonitis with gut wall-associated bacteria (GWAB). To validate our monitoring system to quantify the disease severity in mice (n=20 different lines/arrangements), we set to assess the effect of bacterial starvation on the potential pathogenic induction of peritonitis by GWAB. First, we developed an automated system to provide real-time tracking of mice and their traffic to areas designated as ‘food’ and ‘water’ to infer health and exploratory behavior, activity levels, and social preferences. Then, to validate its relevance, we used GWAB isolated from cavitating microlesions in Crohn’s disease (i.e., E. coli, Parabacteroides distasonis) which we ‘starved’ in phosphate buffered saline, 24 hours prior to intraperitoneal injection in 20-week-old SPF-myeloperoxidase-deficient (knockout) mice. Then, a time-series analysis was employed to assess differences in mouse behavior over 3 days. Two human observers assessed animal health and scored the disease severity in each mouse over time. Of interest, none of the starved bacteria induced severe peritonitis in mice as expected based on the observers’ assessments. However, our system detected a significant temporary decrease in the mice activity and interest in food and water post-injection, which lasted approximately 6h (5-10h). The system also detected a gradual recovery in animal mobility which almost completely normalized by the end of the study when animals were euthanized to harvest peritoneal macrophages for polarization assays. Our findings highlight the significant benefits of automated monitoring systems over traditional observer-dependent evaluations and illustrate that starved GWAB may not be pathogenic.

PS30 Impact of Aspen versus Corn Cob Bedding on Reproductive Measures in a Transgenic Mouse Model of Cystic Fibrosis

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Corn cob is prevalently used as bedding for laboratory mice, Mus musculus. However, it has been associated with disrupted estrogen pathways in deer mice, which could have various downstream effects on reproduction and disease model survival. A popular, alternative bedding option is aspen wood, which comes in a variety of particle sizes. Laboratory mice prefer aspen bedding when given a choice between it and corn cob. However, it is unknown if aspen improves reproduction indices in inbred mice, or if it is better tolerated by disease models. Therefore, this study aimed to determine if aspen bedding (either large shavings or small chips) could improve reproduction in C57BL/6J mice, using a transgenic model of cystic fibrosis as an example. Trio
breeder cages were set up on either corn cob, aspen chips, or aspen shavings (n=6) when the mice were 6-8 weeks old. Cages were monitored until the dams reached 7 months of age for the following measures: latency to first surviving litter, number of litters, litter size, litter survival, individual pup survival, pup weight between 5 and 40 days old, and time of pup puberty onset. Measures were analyzed with general or generalized linear models, with bedding as a main effect. Breeder cageID was included in the models when repeated measures were taken. For models at the pup level, sex and genotype were also included, with any 2-way interactions. Most measures were not impacted by bedding. However, pups housed on aspen chips reached puberty sooner than those housed on other beddings (F2, 122= 7.56; P<0.001). Pups raised on aspen chips also weighed more than those raised on corn cob before reaching puberty (F2, 248=6.30; P=0.002). This data shows that C57BL/6J mice have similar reproductive indices across aspen and corn cob bedding. Aspen chips could be advantageous for increasing pup weight. However, all dams were raised on corn cob before the study began, which could have had unassessed effects on development.

PS31 Early Antibody Response and Viral Neutralization Correlate with Reduced SIV Infiltration in the CNS of Pigtail Macaques (M. nemestrina)

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Simian immunodeficiency virus (SIV) infection in pigtail macaques (Macaca nemestrina) is a valuable model for AIDS pathogenesis, especially in the central nervous system (CNS). This model closely resembles HIV infection in humans, exhibiting diverse immune responses and disease progression between individuals. We hypothesized that differences in the adaptive immune response to SIV drive inappropriate innate activation within the CNS, leading to poor clinical outcomes such as encephalitis. SIV-specific antibody titers and cytokine production in longitudinal plasma samples and cerebrospinal fluid (CSF) were measured in juvenile males (n=16) from baseline through 84 days post-infection using ELISA and an MSD U-PLEX assay, respectively. CNS involvement was evaluated through combined immunohistochemistry (IHC)/in situ hybridization (ISH) staining of basal ganglia for apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and SIV RNA. Viral load in basal ganglia and CSF was measured using qPCR. The functionality of plasma antibodies was examined using in vitro neutralization. Macaques that developed encephalitis showed pronounced CNS inflammatory pathology, including elevated CSF cytokines, increased ASC expression, and higher brain viral loads. Plasma antibody response to SIV was predictive of CNS outcome as early as 21 days post-infection, with differences between outcome groups becoming more apparent over time. Plasma immunoglobulin from macaques with encephalitis displayed limited neutralization capacity compared to those without encephalitis. These findings underscore the critical role of the early adaptive response in determining long-term clinical outcomes of SIV, particularly in relation to CNS involvement. Robust early antibody responses appear to protect the CNS from viral infiltration and nonspecific inflammatory activation. Understanding early B and T cell responses during acute SIV infection can provide insights into mitigating nonspecific inflammation and addressing chronic inflammatory changes observed in individuals with HIV. These findings contribute to our understanding of HIV CNS pathology and highlight potential
areas for further research.

**PS32 Immunogenicity of the mXCL1-PyCSP Fusion Protein Prime-and-Trap Malaria Vaccine**

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Malaria is a life-threatening parasitic disease caused by *Plasmodium* spp. and is transmitted by female *Anopheles* spp. mosquitoes. Annually, there are nearly 250 million cases worldwide causing over 600,000 deaths, primarily in children under 5 years of age in sub-Saharan Africa. Currently, no highly effective and efficacious (>80–90%) vaccine exists, hence the development of such a vaccine against human malaria infection is of paramount importance. The chemokine ligand XCL1, also known as lymphotactin, binds to its chemokine receptor XCR1. Recent studies have shown that XCL1-antigen fusion proteins efficiently induce CD8+ T cell responses by preferentially delivering antigens to cross-presenting dendritic cells expressing XCR1. In this study, we evaluated the immunogenicity of a fusion protein of the murine XCL1 chemokine and the *Plasmodium yoelii* circumsporozoite protein (mXCL1-PyCSP) in our “Prime-and-Trap” vaccine in a murine model of malaria. We hypothesized that this fusion protein vaccine would increase immunogenicity compared to the traditional unfused PyCSP vaccine. Forty male and forty female BALB/cJ mice (*n* = 80) aged 4-6 weeks old were immunized via DNA gene gun on Day 0. On Day 28, the mice were euthanized, and their spleens harvested and screened for interferon-γ-producing T cell responses using enzyme-linked immunospot (ELISPOT) assays. In both male and female cohorts, the mXCL1-PyCSP groups generated significantly higher numbers of spot forming units (SFU) per million splenocytes compared to the PyCSP groups (*p* < 0.0001). In summary, the mXCL1-PyCSP fusion protein vaccine significantly increases immunogenicity compared to the unfused PyCSP vaccine. Future research will evaluate protection induced by the mXCL1-PyCSP fusion protein vaccine compared to the traditional unfused PyCSP vaccine in our Prime-and-Trap immunization/challenge model to determine if incorporating XCL1 offers advantages to this vaccine approach.

**PS33 Analysis of Gross and Histopathologic Changes from Repeated Celiotomies in African Clawed Frogs (*Xenopus laevis*)**

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African Clawed Frog (*Xenopus laevis*) oocytes are used in biomedical research to study cellular biology, toxicology, and embryology. Repeated survival surgical oocyte collection is commonly performed to access high quality, viable oocytes for research procedures and to reduce the number of animals used in research. Despite this common practice, there is no evidence-based
limit for the total number of survival celiotomies that can be performed. To provide an improved reference a retired colony of frogs (n = 33), previously used for surgical oocyte collection, was euthanized for gross and histopathologic evaluation. Animals received anywhere from 4–11 celiotomies (average 6.3 ± 2.3) and were between 69–1646 (average 427± 251) days from their last surgery. Body weight, residual external skin sutures, and abdominal wall sutures were counted, and all skin and abdominal wall musculature that had been operated on was collected for histopathology. Tissues submitted were examined for evidence of abnormal anatomy or changes indicative of painful processes (ex: granulation tissue, histiocytic infiltrate, fibrosis). After a standardized scoring system was developed, histopathology lesions were scored according to severity and then compared to experimentally naïve, age-matched controls (n = 4). Results indicate that skin pathology score was significantly predicted by number of surgeries (P = 0.003), however it was not predicted by the number of skin sutures placed (P = 0.14). Abdominal pathology score was significantly predicted by number of abdominal sutures placed (P < 0.001) and by the number of surgeries (P < 0.001). Neither abdominal nor skin pathology was predicted by the number of days since the last surgery (P > 0.2). As expected, the results indicate that as the number of surgeries and abdominal sutures placed increases, there is a greater likelihood of developing histopathologic changes that correspond to painful processes in mammalian species. This study provides an improved reference for the development of institutional guidelines for *Xenopus* survival surgical oocyte collection.

**PS34 Effect of Gut Microbiota Transfer Methods on DSS-Induced Colitis Disease Severity**

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Differences in the gut microbiota (GM) of research mice can significantly impact model phenotypes. Previous studies in our lab demonstrated that methods of GM transfer also affect the severity of dextran sodium sulfate (DSS)-induced colitis in mice. Co-housing mice to transfer GM leads to greater disease severity compared to embryo transfer or cross-fostering methods. Additionally, transfer of low-richness GM into recipients with higher richness GM is associated with more severe colitis when compared to GM transfer in the opposite direction. However, it was unclear if disease was exacerbated by the stress of co-housing, or the later transfer of fecal material relative to other methods. To answer this question, we compared co-housing to fecal microbiota transfer (FMT) and bedding transfer. C57BL/6 mice from two suppliers with known differences in GM richness were used as recipients of two donor GMs differing in richness, via co-housing or a combination of FMT and bedding transfer. FMT groups were gavaged once per week for four weeks starting at weaning with twice weekly bedding transfer from donor cages. Each cohort contained 8 male and 8 female mice. At 7 weeks of age, all mice received 2.5% DSS in their drinking water for one week, followed by one week of regular water. Body weights (BW) were collected daily and mice losing 20% BW or greater were sacrificed. The remaining mice were sacrificed at 9 weeks of age and colon lengths measured. No differences in weight loss or mortality were detected between similar recipient groups receiving the donor GM via co-housing or FMT. Consistent with previous work, mice receiving low richness GM showed significantly greater weight loss and mortality (P<0.001) and shorter colon lengths (P<0.001) than those
receiving high richness GM, regardless of transfer method. We speculate that, during donor GM exposure prior to DSS, low richness GMs allow greater donor microbe colonization and development of tolerance, while high richness GMs resist colonization, resulting in lack of tolerance to novel microbes that is unveiled with DSS-associated intestinal damage. Further study is needed to determine if post-weaning exposure to new bacteria is associated with a lack of tolerance that worsens DSS-induced colitis.

**PS35 SARS-CoV-2 Doggybone DNA Vaccine Is Immunogenic and Protective in Immunosuppressed Hamsters (Mesocricetus auratus) following Viral Challenge**

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Vaccine platforms used to immunize for COVID-19 include nucleic acid, protein, and viral vector. DNA advantages include rapidity of design and production, thermostability, and adaptability for emerging viral strains. The novel doggybone DNA (dbDNA) platform consists of enzymatically produced DNA that is more rapidly manufactured and scalable than plasmid DNA (pDNA). We hypothesized that dbDNA vaccines delivered needle-free would be as immunogenic and protective against COVID-19 in Syrian hamsters (Mesocricetus auratus) as a previously tested pDNA vaccine. Animals (n=6/group) were vaccinated intramuscularly with spike-based nCoV-S(JET) pDNA, dbDNAS(JET), dbDNAS(ST-JET), or control DNA at two doses (0.2, 0.05 mg) using a needle-free jet injector at 0 and 3 weeks (week). Blood was drawn from the vena cava 3 and 5 weeks to measure serum neutralizing antibody (nAb) (PsVNA). Hamsters were challenged with 1000 PFU SARS-CoV-2 intranasally and immunosuppressed with cyclophosphamide at 1 (140 mg/kg), 3 (100 mg/kg), and 5 (100 mg/kg) days post-infection (dpi). Hamster body weights were measured daily for 9 dpi, followed by intracardiac terminal blood collection for nAb response and lung harvest for histopathology, viral RNA (RT-PCR) and infectious RNA (plaque assay) quantitation, and RNA labeling (ISH). All animals developed a nAb response with the exception of one hamster in the 0.05 mg dbDNAS(JET) group, with highest titers in the nCoV-S(JET) and dbDNAS(ST-JET) groups and a significantly increased titer in the nCoV-S(JET) group compared to controls after the second vaccination (p < 0.01). Statistically significant differences in body weights were observed in all hamsters vaccinated at the 0.02 mg dose. Lung viral and infectious RNA were decreased in vaccinated hamsters and the 0.05 mg group showed a decreased protective effect. Lung histopathology and ISH labeling similarly showed the 0.02 mg dose as more protective. Hamsters in the 0.02 mg groups mounted nAb against viral variants of concern, with the highest titers observed in the nCoV-S(JET) group. In conclusion, the dbDNA platform was effective and efficacious, performing similarly to pDNA in a hamster model of COVID-19.

**PS36 Developing Patient-derived Xenografts (PDX) Models to Evaluate Immunotherapies Targeting Human and Canine T Cells**

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Even though there have been many improvements in developing cancer therapeutics, only a small number make it through FDA approval. To combat the need for better preclinical models, we have developed PDX models of human non-small cell lung cancer (NSCLC) and canine osteosarcoma (OSA) by surgically implanting resected tumors subcutaneously in NOD scid gamma (NSG) mice (KN Suvilesh et al., Molecular Cancer (BMC), March 2022). To date, 26 NSCLC and 2 canine OSA tumors have been implanted. By using immunohistochemistry and quantifying mRNA levels, NSCLC PDXs retained expression of primary tumor-matched diagnostic pathological markers such as PD-L1, p40, TTF, and HLADR over 10 generations. NSCLC PDX tumors and spleens in the NSG mice have also retained passaged tumor associated CD3+, CD4+, and CD8+ human T cells over several generations without causing graft versus host disease. We then used both metastatic and non-metastatic NSCLC PDX tumors to test the potential therapeutic efficacy of a novel T cell targeting molecule, anti-human CD3e monovalent Fab fragment (Mono-OKT3-Fab) and compared tumor growth results with those of established anti-human PD-L1 mAb therapy. In our non-metastatic model, 10 mice received IgG-control and 10 received the mono-fab treatment. We observed that PDX-NSG mice treated with mono-fab had a significantly reduced tumor burden ($p<0.01$) and increased survival over control-IgG-treated mice ($p<0.05$). This experiment was repeated with a metastatic NSCLC model and our mono-fab was combined with anti-human PD-L1 for comparison. We observed that 10 PDX-NSG mice treated with the combination treatment showed significantly reduced tumor burden ($p<0.005$) and increased survival over 10 control-IgG-treated mice ($p<0.005$). For our dog osteosarcoma PDX model, the passaged tumors share the same histologic phenotype as the parental tumor over three generations so far. We have also generated growth curves using caliper measurements and produced gross images demonstrating growth of canine bone tumor in NSG mice after subcutaneous implantation. In summary, our PDX models are effective at representing parental tumor characteristics and serve as an in vivo model for studying new cancer treatments.

**PS37 Inappropriate Head Holding and Inappetence in a Rhesus Macaque (Macaca mulatta)**

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A 22-year-old 15.3 kg male, intact, singly housed Rhesus macaque (Macaca mulatta) was examined for a decade long history of intermittent head holding and inappetence. In 2006, the monkey underwent surgery for a headpost and recording cylinder implant that had complications with the skin surrounding the sites. A surgery to correct the skin defect was attempted later in
that year. Throughout 2009, multiple attempts were made to correct the issue, however unsuccessful, and the cylinder was removed. Shortly thereafter, he was frequently noticed to be holding the headpost with his feet, soft stool, decreased appetite, testing ability and quiet mentation. He was then sedated for a workup which also revealed a grade 3/6 heart murmur. An echocardiogram performed at that time was unremarkable. Multiple similar clinical signs between 2013 and 2019 were reported, and in January of 2020, the monkey's headpost was removed. Additional diagnostics were performed in 2022 after numerous reports of head-holding and inappetence. The laboratory reported visual deficits on behavioral testing. A CT with contrast was performed and considered unremarkable at the time. Following an episode of ataxia, trembling, inappetence and head holding, an MRI was performed and revealed a 1.2cm mass located within the pituitary, expanding the sella turcica and touching the optic chiasm. Revaluation of the CT scan identified a similar lesion. Based on these findings and poor prognosis, the macaque was euthanized. Gross pathology identified a highly vascularized and nodular pituitary macroadenoma expanding the sella turcica with mild asymmetric compression of the optic chiasm. Histology confirmed a pituitary macroadenoma. No other gross or histologic abnormalities were noted.

**PS38 Lethargy and Hyporexia in a 4-Month-Old Domestic Pig**

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Following a routine MRI, a 4-month-old, approximately 85 kg, male domestic pig was reported for lethargy and hyporexia. The pig had undergone a series of surgeries to occlude the renal arteries and create an arteriovenous fistula, with the most recent procedure occurring 15 days prior to onset of clinical signs. The physical exam was unremarkable, and the pig's demeanor improved with food rewards and socialization with caretakers. Over the next ten days, the pig remained intermittently lethargic and hyporexic. Carprofen (4 mg/kg PO) was administered to rule out occult pain or inflammation. The following day, the pig was reported for polyuria and polydipsia. The pig was anesthetized the next day for a previously scheduled MRI, and blood and urine were collected for point-of-care diagnostics. CBC revealed a marked leukocytosis characterized by lymphocytosis, as well as a mild anemia. A limited serum chemistry panel revealed hyperbilirubinemia and hypoglycemia. Urine specific gravity was 1.040. Based on the presence of marked inflammation, the primary differential diagnosis was infection, either a primary bacterial etiology or viral with secondary bacterial infection. Plans were made to begin antibiotic therapy upon return from MRI. However, approximately four hours after induction of anesthesia, the pig went into cardiac arrest during MRI and could not be resuscitated. Gross necropsy revealed generalized mild icterus and a severely enlarged, friable, fibrotic liver. Tissues were examined by histopathology which revealed liver lobules that were largely effaced and replaced by sheets of degenerate and largely lytic leukocytes composed primarily of lymphocytes. A diagnosis of hepatic lymphosarcoma with severe parenchymal replacement was made. From 2003 to 2023, and out of approximately 200,000 case submissions, the examining diagnostic laboratory diagnosed lymphosarcoma in 121 porcine submissions, mostly in animals younger than 6 months of age (range 2 weeks to 5 years). Although uncommon, lymphoid
neoplasia should be considered as a differential diagnosis for nonspecific clinical signs in young swine.

**PS39 Ocular Swelling in Two African Cichlids (Neolamprologus pulcher)**

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Two approximately 5-year-old female African cichlids (Neolamprologus pulcher) in a research breeding colony presented for unilateral ocular swelling of 1 week duration. They were housed with 1-2 other cichlids, and both were the dominant female in their respective tanks. The laboratory had attempted 30% water changes, replacement of the carbon filter, and a metronidazole-praziquantel water treatment with no improvement. On physical examination, both fish had severe exophthalmia of the right eye but were otherwise grossly normal with no lesions or buoyancy issues, normal swimming patterns, and appetite. Differential diagnoses included infection, trauma, or a mass. Given the severity of exophthalmia in cichlid #1, she was euthanized. A gross necropsy that included gill and fin clips, as well as a skin scrape, was performed and tissues submitted for histopathology. The remaining cichlid was treated with a continuous 0.1% salt bath which was increased to 0.2% 2 weeks later after a mild decrease in exophthalmia was noted. No appreciable improvement was noted after 3 weeks so treatment with a 3-day course of meloxicam (1 mg/kg, IM) and a 2-week course of enrofloxacin (5 mg/kg, IM, every other day) was initiated. Due to lack of response to treatment, the second cichlid was euthanized 7 weeks after presentation and submitted for gross necropsy and histopathology. Gross necropsy of cichlid #1 revealed an approximately 1cm soft brown-green mass in the coelom that was determined to be chronic lipogranulomatosis oophoritis on histopathology. Exophthalmia in both fish was found to be the result of an intraocular primitive neuroectodermal tumor (retinoblastoma), a relatively rare neoplasm in any species. While ocular swellings in fish are more commonly infectious in nature, tumors should be considered and ruled out. There are many possible treatments for infection, however prolonged salt baths or other treatments require considerable planning and coordination with animal caretakers.

**PS40 Multiple Subcutaneous Masses in an Aged Long Evans Rat**

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A 2.5-year-old female Long Evans rat presented with an ulcerated perineal mass. Upon a physical exam, the rat was mildly ataxic, but otherwise bright, alert, and responsive, and in good body condition. Additional subcutaneous masses were identified along her ventrum. The masses ranged in size from 1.5 to 5 cm and were lobulated, firm, or soft, and either fixed or freely moveable. This animal has a history of two previous subcutaneous masses presenting in the left inguinal region that were surgically resected 12 and 4 months prior. The initial masses were not submitted for histopathology and were presumed mammary fibroadenomas. Differentials for the
masses and ataxia include mammary fibroadenoma, abscess, cyst, pituitary adenoma, vestibular disease, radiculoneuropathy, and renal disease. Given the rat’s age, as well as the ulcerated and multifocal masses, euthanasia was elected. On necropsy, a large soft multi-lobulated subcutaneous mass was found in the right axillary region containing copious tan-white fluid. Cytologic examination of the fluid displayed proteinaceous fluid with aggregated of squamous epithelial cells. Two additional firm, fixed, and tan subcutaneous masses were present in the perineal space. A dark red ~ 1.5 cm mass was identified in the region of the pituitary gland. The remainder of the necropsy was unremarkable. The primary histopathology findings included a focal pituitary adenoma, multifocal fibroadenomas, a focal adenocarcinoma, and other age-related organ changes. While mammary tumors are common in aged rats (incidence of 30-90% in aged females), the majority are benign with ~ 10% being malignant. Pituitary tumors are benign tumors of aged rats with ~20-30% incidence. Though often subclinical and an incidental finding, pituitary adenomas can lead to neurological deficits and commonly produce prolactin, a hormone involved in mammary development and milk production. Together, these pathologic findings present an often-overlooked link between pituitary and mammary masses and remind us that pituitary adenomas can increase the incidence of mammary tumors via increased prolactin production. The initial masses were not submitted for histopathology and were presumed to be mammary fibroadenomas.

PS41 Bilateral Hind Limb Paresis in an Outbred Swiss Sentinel Mouse

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A 10-month-old female Tac:SW mouse presented with a 2-week history of ruffled fur coat and a 1-day history of unilateral periocular swelling. The next day, the mouse presented with acute bilateral hindlimb paresis and possible ataxia. This animal was housed with a conspecific in a cage receiving soiled bedding from 40 other cages weekly as part of a sentinel program. The cage mate was unaffected. Differential diagnoses for bilateral hind limb paresis and ataxia included degenerative arthritis or disc disease, autoimmune arthritis, traumatic injury, compressive nervous system neoplasia, infectious viral encephalomyelopathies, spontaneous brain infarction, and metabolic lysosomal storage disease. The mouse was euthanized and submitted for necropsy. It had a mildly ruffled fur coat and appropriate body condition (3/5). Gross findings were nonspecific and included mild thickening of the lower eyelid of the right eye with focal redness around the medial canthus and minimal clear ocular discharge, congested uterine serosal surface, and cystic enlargement of the left ovary with clear fluid. No other gross abnormalities were identified. Histologically, there was diffuse accumulation of foamy cells (within neurons in the central and peripheral nervous system, and macrophages in several other tissues). Central nervous system lesions consistent with neuroaxonal dystrophy, such as hyaline or granular axonal spheroids, neuronal chromatolysis, and gliosis, predominantly affecting the gracile, cuneate, and caudal part of the spinal trigeminal nuclei, as well as Purkinje cell loss within the cerebellum were also observed. The widespread accumulation of foamy cells is consistent with a lysosomal storage disease. This disease encompasses an array of metabolic disorders of lysosomal dysfunction involving a steady buildup of substrates inside of the
lysosomes, ultimately causing cell malfunction and death. These disorders are not widely reported in mice, making this a rare cause of neurological disease in mice. In this presentation, we will discuss the pathogenesis of several forms of spontaneous and induced lysosomal storage diseases in mice.

**PS42 Tail Mass in a Sugar Glider (Petaurus breviceps)**

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An approximately 7-year old breeder female sugar glider was anesthetized for a routine semi-annual physical exam. During the exam, an 8 mm x 11 mm firm mass was palpated about halfway down the tail. The mass was lanced, and thick opaque material was expressed. The mass decreased in size and had a sandy gritty texture. Other than mild calculus of the incisors, the rest of the physical exam had no significant findings. Radiographs of the tail showed no evidence of vertebrae pathology. There were pinpoint multifocal areas of increased opacity within the mass. A whole-body radiograph revealed no other significant findings. Impression smears of the lesion were submitted for cytologic analysis. The specimens were moderately to highly cellular and comprised a uniform population of polygonal to cuboidal cells. The cells had faint basophilic cytoplasm, mild anisokaryosis, and some rare binucleated or multinucleated cells. The differentials for the histopathologic diagnosis were possible chondroma or chordoma. Given both differentials have the potential to be locally invasive, a preemptive tail amputation was performed. At the time of amputation, an approximately 5 mm in diameter firm mass was palpated about 5 mm proximal to the original mass. The tail was amputated approximately 100 mm from the tail tip and proximal to the newly palpated mass. The tail was submitted for microscopic examination and two chordomas separated by approximately 2 mm were observed. There was no observation of local invasion. To the authors’ knowledge, this is the first report of a chordoma in a sugar glider. While common in ferrets, chordomas are rare in most other species and only a handful of reports have been published about this neoplasm in veterinary literature. Metastasis is not commonly observed in ferrets and surgical excision can be curative, but in humans, mink, and cats, chordomas are malignant, and considered highly malignant in rats. It’s uncertain how chordomas would behave in sugar gliders and so the prognosis is unknown.

**PS43 Menometorrhagia in a Cynomolgus Macaque (Macaca fascicularis)**

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A 7-year-old female cynomolgus macaque (Macaca fascicularis) with a history of
oligomenorrhea presented with a microcytic, normochromic, nonregenerative anemia and weight loss. Physical examination revealed a caudal oval-shaped abdominal mass roughly 13 cm long and 5 cm wide. Computed tomography and abdominal ultrasound showed a circumferentially and uniformly thickened uterine wall and hypoechoic nodules in the cervix and the right ovary measuring 1.2 and 0.6 cm in diameter, respectively. Differentials included endometriosis, adenomyosis, uterine polyps, endometrial hyperplasia, uterine neoplasia, and metritis. Treatment consisted of weekly 10 mg/kg iron dextran IM and 25 mcg vitamin B12 IM for 4 weeks, and daily 0.09 mg/kg meloxicam PO for 2 weeks until clinical improvement. 150 mg of medroxyprogesterone IM monthly for suspected endometriosis and/or adenomyosis was started. The non-human primate’s (NHP) clinical condition deteriorated 8 months later, when she presented with dyspnea, menorrhagia, and pale mucous membranes. Radiographs revealed the abdominal mass had enlarged and the NHP was euthanized due to a poor prognosis. Necropsy revealed a marked diffuse expansion of the endometrium by a diffusely congested and raised, multilobulated soft mural mass. On histology, the endometrium was markedly expanded by dilated and congested vasculature with the stroma infiltrated by numerous decidual cells, as determined by immunohistochemistry: SMA (smooth muscle actin) pos, CD10 (endometrial decidual cell marker) pos, Iba1 (histiocyte marker) neg, and pancytokeratin and CK8/18 (epithelial cell markers) neg. Based on the histomorphology and immunohistochemical features, the endometrial mass was diagnosed as endometrial decidualization (ED). Secondary suppurative endometritis and vaginitis were also appreciated. The initial presentation is suspected to be from endometrial hyperplasia with medroxyprogesterone treatment contributing to ED. Reports of ED in non-pregnant NHPs have been reported following synthetic progesterone therapy which has been associated with menometrorrhagia and dysmenorrhea in women. This case highlights ED as a differential for menometrorrhagia in NHPs. The clinical presentation, pathologic findings and the pathophysiology of ED will be discussed.

PS44 Vestibular Deficits in a Southern Giant Pouched Rat (Cricetomys ansorgei).

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Giant pouched rats, native to sub-Saharan Africa, are a muroid species with an exceptionally well-developed olfactory system. This feature, in addition to their small size and trainability, makes them a valuable species for applications in humanitarian work such as for odor detection of land mines, buried survivors of natural catastrophes, and diseases. The lack of literature on basic biology demands research on this valuable species to improve husbandry and clinical care. At our institution, a breeding colony of giant pouched rats is maintained to study their physiology and utility as scent-detectors. This report describes an aged (approximately 7.5-year-old) wild-caught female southern giant pouched rat (Cricetomys ansorgei) that presented acutely with vestibular deficits, including left-sided head tilt, ataxia, disorientation, and circling. Additional signs included poor body condition, dehydration, ocular discharge, and scaly dermatitis over the dorsum. Blood count, chemistry, thyroid panel, and urine analysis revealed
elevated AST, cholesterol, phosphate, and T3. Prophylactic enrofloxacin was administered to address a potential bacterial otitis as the cause of vestibular disease. Initial treatment resolved the ocular discharge, and the animal stabilized in current condition. Despite treatment and supportive therapy, vestibular signs persisted. An intracranial lesion was suspected, and an anesthetized MRI revealed a large, focal heterogenous mass arising from the pituitary fossa and extending into the suprasellar space causing foraminal herniation, venticulomegaly, and severe dorsal displacement and compression of the adjacent neuroparenchyma. Due to poor prognosis, humane euthanasia was elected. Necropsy revealed a pituitary prolactin expressing tumor, whose invasive behavior within the basisphenoid bone prompted a diagnosis of pituitary carcinoma. Pituitary tumors have previously been reported in various laboratory rats. Unlike reports of pituitary neoplasms in R.norvegicus, the pituitary mass in this pouched rat presented with uncommon malignancy. This case provides information important to our expanding clinical knowledge of a unique rodent species.

**PS45 Weight Loss and Unexpected Deaths in a Wild-Caught Meadow Vole (Microtus pennsylvanicus) Colony**

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A wild-caught meadow vole (Microtus pennsylvanicus) colony was established at our university to investigate tick burden differences between voles and white-footed mice (Peromyscus leucopus). Voles are housed in gang style ventilated cages, with CareFresh bedding, nesting material, a wood block, and a tunnel. Commercial rodent feed is provided on the cage floor and food enrichment items are provided by research staff weekly. Cage changes are performed weekly in a Rubbermaid bin using a tunnel. Several months after the colony was established, a series of unexpected vole deaths occurred. One of the animals necropsied displayed bilaterally symmetrical firm protrusions on the ventral aspect of the mandible associated with hair loss and dermal erythema. Around the same time, another vole in the colony presented with periocular alopecia of the left eye, 10% weight loss, and similar bilateral protrusions of the. Despite supportive care, the vole continued to lose weight and was euthanized. Histological examination of the jaws indicated molar apical elongation. Open-rooted, hypsodont incisor teeth are a defining feature of rodents. However, unlike many rodent species, voles of the Microtus genus have continuously growing molar teeth as well. Diagnostic imaging of the euthanized vole confirmed apical molar elongation of the mandibular teeth and of the maxillary teeth which resulted in intrusion of the tooth roots into the orbit, nasal cavity, and the vault of the skull. To address the welfare of these animals, the frequency of weight monitoring for this colony has been increased to every 2 weeks with a 10% body weight loss initiating veterinary assessment. Animals are anesthetized and ventral mandibles palpated for mandibular protrusions. Additionally, a handful of timothy hay or alfalfa pellets is provided weekly to encourage use and normal wear of the molar teeth. Hay and pellets are usually consumed within two days when voles are provided with other food enrichment. Careful attention and weight monitoring allows for the euthanasia of voles with this unique disease process before it becomes a severe animal welfare concern. It is too early to determine if this husbandry change is preventing the formation of apical molar elongation in this colony.
PS46 Creating a GLP Framework in an Academic Research Setting

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Biomedical researchers wishing to translate basic science results to human clinical trials have a need to conduct pre-clinical studies under federal regulation CFR Title 21 Part 58 Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies. These studies present many cultural and procedural challenges to an academic research laboratory and thus are often conducted at a commercial facility set up specifically for GLP work. However, an institutional animal research facility can leverage existing personnel expertise and capacities to deliver GLP services to faculty researchers, allowing them direct oversight of the studies, likely at a reduced cost. Responsibilities that fall outside regular operations may be contracted out to other university departments or to external companies. Assembling the study personnel team is the next step, which starts with identifying staff with direct GLP experience and/or technical skills. An administrator or facility manager who handles the Standard Operating Procedure (SOP) program is a good fit for Document Control Manager who will be responsible for creating study SOPs and forms. Veterinary technicians and research technicians will have the animal handling skills needed for daily health and room checks, body weights, test article administration and necropsy. Any team member who has previous GLP experience is an asset for training study personnel, and training personnel in footnoting and form completion is necessary before the start of the study. Pre-existing documentation practices can be incorporated and modified to GLP standards. Other tasks required such as space allocation and equipment certification are regularly handled by facility management and can be achieved more easily. Some GLP requirements may require the establishment of new operational procedures. For instance, if electronic signatures are to be used, a validation package documenting audit trails must be created and filed. An overall comparison of GLP requirements and existing facility capabilities can lead to establishing a framework in which pre-clinical studies can be successfully completed. Our program was able to begin a GLP study within six months, however times will vary depending on the assets already in place.

PS47 Ballin’ On a Budget: From Stockroom to Vivarium

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Small laboratory animal programs starting out with spatial and budgetary constraints should strategically utilize resources already available at the institution to meet regulatory and programmatic needs. In 2020, a university began converting a central receiving stockroom into a vivarium for ABSL-2 mouse studies. In early 2022, a Laboratory Animal Care Facility Manager (LACFM) was hired to develop and implement the program with an operational start date of September 2022. In addition, a veterinarian board certified in laboratory animal medicine was contracted as the attending veterinarian to assist in program development. To complete such a
large task in a relatively short amount of time, the LACFM formed a Vivarium Working Group committee which consisted of senior leadership within the Office of Research, Facilities Planning and Design, Environmental Health and Safety, Office of Laboratory Safety, and the Institutional Animal Care and Use Committee (IACUC). This working group collaborated to develop standard operating procedures and IACUC polices pertaining to pest control, occupational injuries and medical surveillance, animal emergency and disaster response, training and education requirements for vivarium personnel, vivarium security, post-approval monitoring, whistleblower policy, animal welfare concern reporting, vivarium staff, and more. The full group meets monthly to discuss overarching components and progress. Smaller breakout groups met biweekly to discuss specific needs and tasks relating to their departments. Within six months, driven by the leadership of the LACFM, the working group had developed 35 IACUC and facility SOPs addressing items for a fully functional laboratory animal program. Programs that were already in place were adapted to fit the needs of the vivarium, reducing the groundwork for startup. Work study programs for student assistants and pre-existing hazardous waste disposal procedures laid the groundwork for staffing and waste management within the vivarium, respectively. By collaborating with stakeholders and adapting existing resources to vivarium needs, implementation time and budgetary constraints can be reduced when starting a new laboratory animal program within a small university.

**PS48 New Method to Evaluate the Quality of Life of Laboratory Swine**

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There is a lack of quantitative research on the quality of life of animal models used in research studies. We evaluated if activity monitors can be used to measure the quality of life in swine cardiac models of atrial fibrillation and chronic heart failure. Yucatan mini swine were outfitted with activity monitors attached to nylon collars that were worn continuously throughout their time in the research lab (N=23). Multiple data downloads were conducted each week to evaluate the animals' activity levels. The raw data was processed using pivot tables to calculate an average hourly activity and generate area under the curve graphs. During one of our studies, we saw a significant change in the activity levels as the swine progressed through the disease model. To keep confidentiality, these phases will be referred to as phases 1 and 2, and the animal progressed in the specific disease model according to the study they were placed in. Sudden increases or decreases in activity were identified as potential indicators of changes in the animals' health and quality of life. A notable finding from our research is the correlation between decreased activity levels and lower health and quality of life in laboratory animals. For chronic heart failure swine, the average activity was significantly higher (p<0.05) in phase 1 (236.49 ± 8.9) compared to phase 2 (210.14 ± 7.7). By tracking hourly activity averages and analyzing 24-hour activity graphs, we were able to gain insights into the animals' activity patterns throughout the day. Furthermore, employing a trapezoidal area under the curve analysis enabled us to assess changes in activity levels across different study phases in terms of total activity and specific hours. This data show a novel and critical approach, as it contributes to ensuring the safety and well-being of laboratory animals. By utilizing activity monitors and employing quantitative analysis techniques, we can better understand and evaluate the quality of life experienced by...
PS49 Cooperative Care Techniques for Large Swine in a GLP Laboratory Setting

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Working with large breed swine presents multiple challenges in any environment. When large breed swine are participating in a GLP study at a contract research organization, finding a positive and safe method of interaction with those animals is not only a challenge but a necessity due to the frequent handling needed for study functions that are common on these studies. Voluntary cooperation with study staff on the part of the animals is required for data collection to be successful due to the exponential growth rate they experience and the safety concerns that creates. The research study discussed in this presentation included cage rotations, weekly body weights, ophthalmologic exams, multiple sedation events, and various veterinary procedures. For the safety of the technicians and the well-being of the animals, positive reinforcement training (PRT) was implemented to facilitate an environment of cooperative care on study. All animals were trained to follow a target via clicker bridge with food reinforcers. This basic targeting behavior was then applied to multiple required functions including mounting a body weight scale, moving between cages, moving onto transport carts, and stationing for various exams and procedures. Targeting was also utilized as enrichment and to encourage exercise for the animals. Study and husbandry technicians reported that training activities and resulting cooperative care opportunities created a low stress environment for the animals and the technical staff, an increase in animal welfare through enrichment, exercise, and positive interactions with handlers, and overall, a significant improvement in the human-animal bond. In conclusion, target training via PRT is an effective tool for implementing cooperative care for large swine. With planning, communication, commitment of resources, and coordination, PRT can be accomplished in a GLP environment, facilitating safe and accurate data collection. Inclusion of the study and husbandry team in training activities creates a low stress environment for both technicians and animals, allowing for successful data collection with a challenging species and significantly improved animal and technician welfare.

PS50 LED Light: An Extrinsic Environmental Factor that Enhances Laboratory Animal Health and Wellbeing

GLAS: Yes

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Light is an extrinsic environmental factor that profoundly influences circadian, neuroendocrine, and neurobehavioral regulation in laboratory animals. Responses to light are mediated via the
retinal photoreceptors, including the classical rods and cones involved in vision, as well as the recently discovered melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) of the non-visual system. Previous studies from our laboratory demonstrated that exposure of pigmented and non-pigmented rats to blue-enriched (465-485 nm) light-emitting diode (LED) light at daytime (bLAD), compared to broad-spectrum (300-700 nm) cool white fluorescent (CWF) light, amplifies nighttime circadian pineal melatonin production and positively influences metabolism and physiology. Here, in our GLAS-supported investigation, we tested the hypothesis in mice that exposure to bLAD, compared with CWF, positively enhances integrated visual and non-visual system photic responses, shown to promote the circadian regulation of neuroendocrine and neurobehavioral parameters that are associated with optimizing animal health and wellbeing. Three mouse strains commonly used in biomedical research (C3H, C57BL/6, and BALB/c; n = 120/group; male and female) were maintained under an IACUC-approved protocol in an AAALAC-accredited facility for 12 weeks on a common lighting regimen 12L (68.8 ± 5.2 lux, within cage); lights on 0600 h):12D (0 lux) on either CWF (control) or bLAD (experimental) lighting, and were assessed for retinal photon flux (cm²/s), radiometric (µW/cm²), photometric (lux), and photopigment illuminances (α-optic lux). Results (mean ± 1 SD) revealed in the 3 strains of mice that, although photon flux was similar between bLAD and CWF light exposure, stimulation of the non-visual melanopsin-containing ipRGCs and the visual S cones, rods, and M cones was 43.4 ± 0.8%, 45 ± 1.2%, 28 ± 0.7%, and 21 ± 0.1% higher (P < 0.001), respectively, in mice maintained under bLAD. These data show that daytime exposure of mice to bLAD, compared to CWF light, has a marked positive effect on mouse retinal photic responses regulating the circadian, neuroendocrine, and neurobehavioral parameters associated with the promotion of animal health and wellbeing.

PS51 Current Prevalence of Nonhuman Primate Pathogens

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Nonhuman primates (NHPs) remain a critical research model for clinical trials on new therapeutics. Understanding the prevalence of pathogens in NHP colonies is essential to ensuring reliable and reproducible research results. To this end, we have summarized the results generated by our commercial laboratory since 2006 for the most monitored pathogens from over 1.2 million NHP samples across numerous colonies around the world. Efficient analysis of this large dataset was made possible by employing Excel Power Pivot. The most recent pathogen prevalence levels comprise results from 2020 through 2022. During this period, the viruses most often monitored by serology were Herpesvirus simiae (B virus), simian T-cell leukemia virus (STLV), simian retrovirus (SRV), simian immunodeficiency virus (SIV), filovirus (FV), and measles virus (MV). Excluding MV, B virus was the most prevalent with just over 1% of samples testing seropositive, followed by STLV and SRV at 0.27% and 0.15%, respectively. It is noteworthy that since 2006, the seroprevalence of these three agents has trended downward. SIV and FV remain among the most tested agents but are not detected at any meaningful level. MV hovers between 50% and 70% of samples testing positive year over year, which is consistent with the push for vaccination of colonies to prevent infection. Of the bacteria for which NHPs have been screened most often since 2020, Campylobacter spp. was the most prevalent followed by Shigella spp., Salmonella spp., and Yersinia pseudotuberculosis, with prevalence levels by
cultural isolation of 11.93%, 2.05%, 1.46%, and 0.98%, respectively. Prevalence for these agents by PCR was also summarized. Based on serosurveillance, the prevalence of *Mycobacterium tuberculosi*s has experienced an uptick from just 0.12% in 2019 to 0.45% in 2022. Finally, recent parasite prevalence for both *Trypanosoma cruzi* and *Plasmodium* spp. was just over 5%. Prevalent agents should be included in surveillance of imported animals in quarantine and in routine monitoring of breeding and research colonies to avoid the confounding effects of adventitious infections and loss of valuable research resources.

**PS52 Corynebacterial Species Interaction in an Atypical Corynebacterium bovis Outbreak**

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*Corynebacterium bovis*, the cause of Corynebacterium-associated hyperkeratosis (CAH) in nude mice, is highly transmissible, persistent in the environment, can involve asymptomatic carriers, and currently has no effective treatment. In 2021, a *C. bovis* outbreak occurred in a sentinel colony of athymic nude mice that failed to manifest expected *C. bovis* growth kinetics and clinical disease, contributing to a 3-month diagnostic delay despite bimonthly testing. The purpose of this study was to identify factors associated with attenuated CAH, including in-vitro competitive growth dynamics of *C. bovis* and *C. amycolatum*, corynebacterial host origin, and virulence genes. We hypothesized that attenuated pathogenicity of *C. bovis* in this outbreak was due to competitive suppression by endemic microflora (*C. amycolatum*) and that corynebacterial host origin and differences in virulence genes are contributing factors. Routine health surveillance was performed via serology, PCR, culture, gross and histopathology and direct parasitologic examination for select agents. *C. bovis* was confirmed by culture, PCR, 16s rDNA sequencing, and Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry. Proximity and competition assays were performed via co-culture on blood agar inoculated from liquid monocultures. Whole genome sequencing (WGS) and a genome-wide association study (GWAS) were performed on isolates from the outbreak as well. Preliminary results demonstrate no definitive evidence of competitive suppression and that *C. bovis* and *C. amycolatum* isolates from the outbreak were genomically identical and genomically heterogeneous respectively, which suggests that the attenuated *C. bovis* pathogenicity observed was more likely due to microbial factors. Identification of contributing factors in this *C. bovis* outbreak and in attenuated CAH as well as determination of in vitro growth dynamics will facilitate a better understanding of bacterial skin colonization dynamics of *C. bovis* in mice, potentially informing new strategies to decrease *C. bovis* morbidity or even prevent infection altogether.

**PS53 Sensitivity of Polymerase Chain Reaction on In-cage Filter Paper for Helicobacter Species Exposure at Different Time Points**
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*Helicobacter* species infections in mice can have broad-ranging effects on gastrointestinal, reproductive, and immune systems. This can introduce significant confounding variables for research and may reduce scientific rigor. Screening mouse colonies for *Helicobacter* can be accomplished via non-invasive polymerase chain reaction testing on filter paper placed in animal-free dirty bedding sentinel cages. In our facility, 1 tablespoon of dirty bedding from each cage on a rack is added to a designated sentinel cage every 3 weeks at cage change and polymerase chain reaction testing is performed on in-cage filter paper quarterly. We hypothesized that cages that received *Helicobacter* positive bedding at later time points would have lower sensitivity of polymerase chain reaction testing compared to cages that receive positive bedding at earlier time points due to the filter paper becoming saturated. To determine if screening would be sufficiently sensitive to detect approximately 10% positivity on a rack, 9 tablespoons of *Helicobacter* positive bedding and 71 tablespoons of negative bedding were added at 3, 6, or 9-week time point to 14 empty sentinel cages per time point. Negative bedding was added every 3 weeks to cages not scheduled to receive positive bedding. Negative controls received 80 tablespoons negative bedding and positive controls received 80 tablespoons positive bedding at each time point. At each time point, cages were thoroughly mixed using a sanitized plastic knife after bedding was added. Cages were then agitated 3-5 times with the lid closed to ensure the in-cage filter media was completely exposed to bedding. Filters were tested via polymerase chain reaction for *Helicobacter* species at 12 weeks. All positive controls tested positive, and all negative controls tested negative. 2 3-week cages, 2 6-week cages, and 3 9-week cages were positive, indicating no difference between time points. This resulted in 13.5% sensitivity, which is low compared to previously reported 75% sensitivity. These results indicate that polymerase chain reaction on in-cage filter paper may not be reliable to detect low levels of *Helicobacter* shedding in dirty bedding.

**PS54 Particulate Collected from Shoes Contains Non-Infectious Nucleic Acid for Numerous Rodent Adventitious Agents**

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Urban rodent populations and associated zoonotic diseases increased during the height of the pandemic. The soles of staff shoes accessing vivaria may become contaminated while traversing urban streets and serve as a source of fomite-mediated transmission of adventitious agents to
laboratory rodents. While shoe covers may be employed to reduce this risk, their donning may lead to hand contamination. Shoe cleaners, i.e., motor-driven brushes that remove and collect particulates via vacuum from the top, sole, and sides of shoes, are utilized in our vivaria to reduce the potential of introducing excluded agents. Shoe cleaner debris and contact media (CM) mixed with the debris from shoe cleaners serving 5 distinct vivaria (A-E) utilized hundreds of times daily were analyzed by PCR for the presence of nucleic acid from 85 adventitious agents. To determine if the shoe debris was infectious, 3 NSG and 2 Swiss outbred female mice were exposed oronasally to a liquid suspension of debris and cohoused on debris-contaminated bedding for 7 days from each of the 5 vivaria. A control group was similarly exposed to autoclaved debris (A-E pooled). Shoe debris and contaminated CM from each vivaria were positive for up to 51 agents. Of those agents, 47% were zoonotic and 25.5% were frequently monitored for in rodent colonies. Noteworthy agents included: Orthopoxvirus (8%), Rodentibacter heylii (8%), Mouse Hepatitis virus (8%), Sarbecovirus (33%), Ornithonyssus bacoti (33%), Spironucleus muris (50%), Mouse Norovirus (58%), Entamoeba (58%), Mouse Parvovirus (67%), Chlamydia muridarum (100%), Helicobacter spp (100%), and Tritrichomonas spp (100%). There was a statistical difference in the odds of direct debris examination detecting more pathogens than CM in the control and 1 vivarium group, whereas there was no difference in all others. All NSG and Swiss mice remained clinically healthy, and PCR (fecal, buccal & skin samples) and sera were negative (SW) for all agents in the test panels. Preliminary histological evaluation did not reveal pathologic abnormalities. These results provide insight into the adventitious pathogens present on shoes in NYC and suggest that the debris is not infectious to laboratory mice.

**PS55 Seroprevalence of Adeno Associated Viruses (AAVs) in Nonhuman Primates (NHPs)**

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Adeno-associated virus (AAV) has become the preferred vector for gene therapy studies but can be limited by the presence of neutralizing antibodies (NAb), from prior exposure to the virus, in patients or research animals. Therefore, cell based AAV NAb prescreening of subjects prior to enrollment in studies has become commonplace. Researchers routinely screen 8X-10X the number of NHPs enrolled in studies to find enough animals that are AAV seronegative for the AAV vector serotype utilized. However, given today’s NHP shortages, it’s difficult to find enough animals to accommodate testing these large numbers leading to increased costs and delays in studies. Using data collected by our lab over the past 2 years, we sought to identify variables that better predict the likelihood of AAV seropositivity in NHPs to minimize the number of animals required for prescreening to achieve the desired total of seronegative NHPs. To this end, data for four AAV serotypes including AAV2, AAV8, AAV9 and AAVrh74 from nearly 2500 cynomolgus macaque sera was collated and analyzed. Results showed that seroprevalence ranged between 50% and 80% for all serotypes with AAV2 being the most prevalent and AAV9 the least. Interestingly, the seroprevalence dropped substantially to between 30% and 60% at a serum dilution of 1/40. Sex did not influence the percentage of AAV NAb positive NHPs for any of the tested serotypes. Similarly, no correlation was observed between the age of animals (2-3 years old) and the seroprevalence of AAV NAbs, despite suggestions that
older animals are more likely to be positive for AAV NAb. Small variations in seroprevalence were found in NHPs from different countries of origin and even different colonies from the same country. For example, between two Asian countries, one showed ~15% higher prevalence of AAV9 NAbs but ~10% lower prevalence of AAV2 NAbs. Additionally, in some cases, seroconversion was observed between prescreening and the study start date, so it is advisable to screen NHPs as close to the start of a study as possible. In summary, when screening NHPs to select candidates for study, it’s important to consult the NHP supplier for suggestions about optimal screening timing and numbers based on specific seroprevalence observed in their colonies.

**PS56 Prevalence of Mouse Kidney Parvovirus in Sentinel Swiss Webster Mice**

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Mouse kidney parvovirus (MKPV), also known as murine chapparvovirus (MuCPV), induces inclusion body nephropathy (IBN) and kidney fibrosis in aged immunodeficient mice and, to lesser extent, in immunocompetent mice. The presence of MKPV in academic and biomedical animal facilities can be an important confounding factor complicating interpretation of in vivo experimental findings. We surveyed prevalence of MKPV using feces, kidneys and livers collected from 212 sentinel Swiss Webster (SW) mice[(Crl:CD1(ICR)] from 8/2019 to 12/2020. SW mice were cohoused, as sentinel mice using a dirty bedding protocol, on racks with colony mice used in research located in MIT and Whitehead vivaria. The MKPV genome copies in tissues and feces were determined via qPCR, and selected kidney and livers were evaluated for histopathology and MKPV RNA expression via RNA scope. Rates of MKPV positivity were 16.1%, 14.7% and 10.2% for feces, kidney, and liver respectively; in aggregate, prevalence of MKPV was 23.6% (50 out of 212 mice). Thirty-three out of 103 rooms (32%) were MKPV positive; however, MKPV infection did not induce overt IBN or liver lesions in infected sentinel SW mice. MKPV RNA was sporadically detected in MKPV-positive kidneys but not in MKPV-positive livers. Our data indicate that sentinel SW mice can be infected with MKPV, and this viral pathogen was modestly distributed in sentinel mice housed in our animal facilities. In addition, MKPV infection can cause sporadic inclusion bodies, but given the age (6mos) IBN was not observed. Furthermore, fecal DNA qPCR for monitoring MKPV status in animal facilities is less invasive and more sensitive compared to targeted murine tissues. Our results emphasize the importance of monitoring MKPV distribution using qPCR in sentinel mice housed in vivaria.

**PS57 Evaluation of Sentinel-Free Soiled Bedding PCR Sampling as a Quarantine Method**

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Soiled bedding sentinels (SBS) have historically been relied on to screen rodents in quarantine for excluded infectious agents prior to their release into a research vivarium. However, due to
growing reports that many infectious agents are not effectively detected by SBS, direct animal sampling (DAS) for PCR-based quarantine has become a common alternative. Sentinel-free soiled bedding (SFSB) PCR sampling, which relies on dust collected from pooled soiled bedding, has been reported as a successful method for routine screening of research colonies. As an alternative to DAS, we investigated the efficacy of SFSB as a quarantine method. Pet shop quality mice supplied soiled bedding: one cage of (n=5) >6-month-old, one cage of (n=5) 6-10 week-old, and two cages each housing a dam and litter. DAS were collected from mice on arrival (D0); fecal pellets were collected from the dams and pups, while feces, body swabs, and oral swabs were collected from all older mice and pooled by cage. Soiled bedding from the shipping crate for each age group was divided into three equal quantities and used to expose contact media via manual agitation (D0). Shipping crate bedding was then placed with the mice of the appropriate age group in cages in a cube isolator for one week. At D7, DAS for PCR and traditional diagnostic methods were used to screen mice for infectious agents. As described for D0, soiled bedding for each age group at the end of study (D7) was divided into three replicates to expose new contact media via manual agitation. Both SFSB and DAS had more positive replicates at D7 vs. D0 for bacteria, parasites, and protozoa. Positive replicates for some viruses diminished by D7, likely due to normal clearance by the immune system. Overall, 12 different bacteria, 10 parasite/protozoa, and 17 viruses were detected in the mice by DAS. SFSB detected all infectious agents present in the survival samples. In general, estimated PCR copy numbers were equivalent or higher for all samples and agents at the D7 timepoint. This data supports that SFSB has equivalent sensitivity to direct animal sampling and may serve as a viable option for a sentinel-free, PCR-based quarantine program.

**PS58 Mouse Coronavirus MHV-1 Disease in A/J and NOD Mice is Accompanied by a Hyperinflammatory State and Fewer Tissue Repair Macrophages.**

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Mouse hepatitis virus (MHV) is a betacoronavirus of mice. To understand the similarities of MHV disease with COVID-19, the immune response caused by MHV-1 was characterized in four mouse strains. NZO/Hil LtJ, NOD/ShiLtJ, C57BL/6J, A/J male and female mice (18 per strain) were infected with 1.5 x 10^5 pfu of MHV-1 (or media) and euthanized at 3- and 8-days post infection (DPI). Blood (plasma) was collected for cytokine analysis or flow cytometry of peripheral blood mononuclear cells (PBMC). The cytokine results for MHV-1 at 8 DPI showed no significant differences in PBMCs, recognizing the severe clinical phenotype in A/J mice precluded assessments beyond 3 DPI. Significant differences (p <0.05) were detected at 3 DPI in PBMCs including: 1) B6 mice had significant increases in interferon gamma and tumor necrosis factor alpha (IFNγ and TNF-α); 2) AJ mice had significant increases in IFN-α IFN-γ and TNF-α; 3) NOD mice has significant increases in IL-4, IFN-γ and TNF-α; and 4) NZO had significant increases in IL-6, IL-18, IFN-γ, and TNF-α. The most striking feature of the cytokine data was the “cytokine storm” that occurred in A/J mice post infection. Flow cytometry results indicate neutrophils, inflammatory monocytes (Ly6C+/CX3CR1 hi) and patrolling monocytes increased after infection, except in NZO mice, which have almost no patrolling monocytes but the transition to tissue repair macrophages [CD124+(IL4Ra)] of low inflammatory status [CD119-
applies rapid in NZO mice. A/J mice produced more CX3CR1 inflammatory macrophages and fewer tissue repair macrophages. Both B6 and NZO mice had mild clinical disease and had higher levels of tissue repair (CD124+) macrophages compared to the more clinically affected A/J and NOD mice. The cytokine and flow cytometry results show A/J mice have a pre-existing inflammatory state and develop hyperinflammatory after MHV infection. NOD mice also have a pre-existing inflammatory state exacerbated by MHV infection. Both NOD and A/J have pathologic and clinical disease (A/J more severe), defects in their innate immune systems, and a C5 deficiency. The combination of a pre-existing inflammatory state and defects in innate and adaptive immunity set the stage for more severe MHV disease.

**PS59 Chlamydia muridarum Causes Persistent Infection in C57BL/6, BALB/c and Swiss Mice Following the Presumptive Route of Natural Infection**

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Recently, we described the moderate prevalence of *Chlamydia muridarum* (Cm) infection in global academic mouse colonies. To better understand the natural biology and significance of Cm infection in commonly used inbred strains and an outbred stock, 2.7 × 10^3 IFU of a Cm field isolate was administered to a cohort (n=27) of 8-week-old female BALB/cJ (C) mice via orogastric gavage. After confirmation of Cm shedding via fecal PCR through 95 days post-inoculation (DPI), these mice were utilized to investigate the transmissibility, shedding kinetics, and tissue tropism of Cm following cohousing with C57BL/6J (B6) and C mice (Th1 and Th2 skewed, respectively), and Swiss (J:ARC[S]) mice (n=30 each strain). A Cm infected C mouse was cohoused with 4 naïve mice of each strain for 30 days and exposed naïve mice were followed for 6 months. Exposed (n=3/strain) and control (n=1/strain) mice were evaluated 7, 14, 21, 60, 120, and 180 DPI for CBC/chemistry, fecal qPCR, and pathology with Cm-immunohistochemistry performed on select tissues. All B6 mice were fecal qPCR positive by 3 through 180 DPI. While only 1 of 3 C mice was positive at 3 DPI, all mice were positive by 7 through 180 DPI. S mice remained fecal qPCR negative at 3 and 7 DPI with only 1 of 6 mice positive at 14 DPI. All remaining S mice were positive by 14 through 180 DPI. No significant histologic lesions were appreciated in the lung, gastrointestinal tract, or reproductive tracts in any strain. Cm antigen was only detected in surface intestinal epithelial cells, primarily in the cecum and colon, often associated with gastrointestinal associated lymphoid tissue (GALT).

Importantly, these findings indicate that 3 commonly utilized strains of laboratory mice are susceptible to chronic enteric infections with Cm, despite differing genetic background and immune response profiles. Cm’s colonization of mucosal epithelial cells in the large intestine suggests that Cm may induce at least a local immune response, warranting further investigation.

**PS60 Chlamydia muridarum Modulates Splenic Monocyte and T-cell Response and Induces Sustained Intestinal T-cell and ILC3 Responses in Inbred and Outbred Mice**
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*Chlamydia muridarum* (Cm) was recently shown to be prevalent in academic mouse colonies. As Cm causes persistent infection in immunocompetent strains, we assessed the immunologic impact of Cm infection on commonly utilized C57BL/6J (B6), BALB/c (C), and J:ARC(S) (Swiss) female mice to determine its potential to confound research. A cohort of Cm infected C mice was cohoused with naïve mice (n=12 each B6/C/Swiss) for 30 days. On days 14 and 63 after cohousing, infected and uninfected control mice (n=6 each/strain/timepoint) were necropsied, and their spleens processed for immunophenotyping via flow cytometry. On day 14, infected (compared to uninfected control) mice demonstrated significant differences (p<0.05, Wilcoxon rank sum). In B6, CD4+ T cells preferentially differentiated into effector memory cells and CD8+ T cells were activated. In C, the frequencies of macrophages decreased and CD4+ T cells were reduced among the T cell population. In Swiss, effector CD8+ T cells were reduced among the total CD8+ T cell population. On day 63 in B6 mice, Cm infection led to a reduction in B cells, an increase in monocytes, and preferential differentiation of both CD4+ and CD8+ T cells into effector cells. In C mice, CD8+ T cells preferentially differentiated into memory T cells. In Swiss mice, CD4+ T cells preferentially differentiated into central memory and CD8+ T cells to effector memory cells. Additionally, the gastrointestinal tract was removed from the B6 cohort on the same days and the intestinal immune response was assessed. Sustained increases in the total number of CD45+ cells, including neutrophils, Th1, and Th17 CD4+ T cells, were observed indicating a prolonged inflammatory response. There was also sustained elevated cytokine expression from type 3 innate lymphoid (ILC3) and effector T cells in the large intestinal lamina propria in Cm-infected B6 mice compared to the controls. Collectively, these results demonstrate that while no clinical disease or histopathology were appreciated, there is potential for induction of monocytes and activation of T-cell subsets at different stages of Cm infection. Considering the widespread use of mice to study GI disease, an assessment needs to be made as to whether Cm-infected mice should be used as models.

**PS61 Improving the Design of Cranial Implants for Sensory Neuroscience in Ferrets**

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Ferrets are an important model species in sensory neuroscience due to their well-developed visual and auditory systems and trainability on behavioral tasks. For these studies, cranial
implants are often necessary for restraint during awake behavioral tasks or for stability during imaging and electrophysiology. As in other species, it can be difficult to maintain these implants for long term use. Here we describe improvements in implant design and surgical implantation methods for chronic use in ferrets. Implants described are metal posts for head-fixation with enhanced stability from specially designed t-bolts and low profiles allowing skin to be tightly opposed to the posts. We observe that these implants are well-tolerated both by implanted individuals and their cage-mates and can last for over 4 years. Further these implants require minimal maintenance with no instances of infections or need to revise margins. The stability of the implants also makes them useful as a base for expanded implants for imaging chambers and electrodes in chronic recording experiments. We conclude that our modifications to previous methods offer refinements by increasing survival time of the implants and decreasing maintenance requirements without reducing functionality for ferret studies. The lab is currently adapting this implant style for chronic recording devices. Refinements to implant methods mean reduced animals lost to complications and fewer sedations or surgeries for invasive maintenance procedures for each animal, thus improving animal welfare based on the 3Rs.

**PS62 Evaluation of Enrichment Preference of Tree Shrew (Tupaia belangeri)**

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Although the tree shrew (Tupaia belangeri) is an increasingly popular animal model used in ophthalmology and infectious disease research, the environmental enrichment preferences of this species are largely unexplored. We sought to determine the preference of 2 male and 6 female (N=8) naïve adult tree shrews aged 1-3 years (mean 2 years.) by offering them 5 novel forms of enrichment. A single novel object was placed, and criteria evaluated included species-specific behaviors such as scent marking, anxiety like behaviors such as stereotypic circling, time until first interaction with novel object, and time spent engaging with each form of enrichment. Subjects in the home enclosure were observed by video recording for up to 8 hours. All events were evaluated and included a baseline video without novel enrichment present. Novel objects were removed at the end of each recording with a 24–48-hour washout period before a new item was placed. Mirrors, external forage feeders, artificial plant spheres, suet feeders containing crinkle paper, and plastic pan containing aspen bedding (dig pan) were evaluated. Among the 5 enrichment items tested, tree shrews showed the strongest preference for the dig pans and least preference for the suet feeders with crinkle paper. The shortest time to first interaction was with the forage feeders at 2 minutes (m) 24 seconds (s) after initial introduction. However, tree shrews spent more time total interacting with dig pans (meaning 29 m 15 s). More importantly, 6 of 8 tree shrews displayed decreased stereotypic behaviors in cages enriched with dig pans. Overall, mirrors, dig pans, and artificial plant spheres decreased stereotypic behavior and increased natural behavior such as scent marking. To account for neophobia, subjects were evaluated for time to first interaction with novel items. Surprisingly, most subjects showed an immediate interest in novel items. In this study, we observed increased natural behaviors and decreased anxiety like behaviors highlighting the benefit of novel enrichment items for tree shrew. Items such as dig pans that elicit natural behaviors should be strongly considered as part of a behavioral management plan for tree shrews.
PS63 Marseille Declaration: Together We Prioritize Animal Welfare

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When animals are needed for research, high standards of animal welfare and quality of work are imperative for two reasons. First, because of the ethical imperative to minimize pain and distress. And second, because psychologically and physiologically healthy animals are necessary to provide meaningful and reliable data. To ensure these goals are met, husbandry conditions must meet species-specific needs for a complex environment that provides adequate space for movement, social contact, nutrition, stimulation, and freedom from stress and illness. Local legislation pertaining to animal care and use varies widely across the globe and cannot be solely relied upon to ensure consistent high standards are met. In addition, local regulations may or may not support a Culture of Care program, robust ethical review, post approval monitoring, and incident reporting for animal studies, continuous education and training for all staff working with animals, and a program of risk management. For this reason, and to enable coordinated action with our global partners in industry and academia, the signatories of the Marseille Declaration agreed to define their objectives and priorities for the welfare and husbandry conditions of laboratory animals. The declaration is named because this framework was initially drafted at the 2022 FELASA Conference in Marseille. The declaration does not claim to be a concrete guideline or audit standard, nor does it claim to be complete. Rather, it outlines the signatories’ shared expectations, that sometimes go beyond local legislation, for external partners working with animals on their behalf worldwide, including a commitment to applying and promoting the care and accommodation standards for dogs, pigs, and nonhuman primates consistent with those required by the European Union and United Kingdom. The signatories invite others to join our declaration and coalition. This presentation will share the principles of the Marseille Declaration, along with the goals for aligned and global support for high standards of animal welfare and quality of research results.

PS64 Pebble to the Metal: A Boulder Approach to Enrichment for Danio rerio

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Zebrafish are a widely used animal model, yet there is limited understanding of their welfare
needs. Despite an increasing number of studies on zebrafish enrichment, it remains unpopular among researchers to use in-tank environmental enrichment. Although supportive evidence is sparse, there is hesitancy to include in-tank enrichment due to perceived concerns of health/hygiene of the fish. To accommodate these concerns, we tested the potential benefits of enrichments presented outside the tank on 40 adult mixed-sex zebrafish. We hypothesized that zebrafish would show a preference for enriched environments and have lower cortisol levels than zebrafish housed in barren environments. We used two experiments to test our hypothesis. In our first experiment, we either group housed (8 tanks of 4 fish) or singly housed (8 tanks of 1 fish) the fish. Over 2 weeks, using a repeated-measures factorial crossover design, we quantified if their preference for a pebble picture located under half of their tank was as strong as zebrafish’s well-established preference for social contact. We used two positive controls: group housing, and visual access to conspecifics. Singly housed zebrafish displayed a significant preference for the enriched half of the tank (quantified as spending <50% of their time over the pebble picture); this preference was equivalent to the positive control of visual access to conspecifics. In our second experiment, using the same cohort of 40 zebrafish, alternating tanks received pebble enrichment or standard housing (barren) underneath the entirety of the tank floor, equally distributed between singly and group housed tanks. After one week, to quantify stress levels, we collected tank water to measure cortisol levels. Overall, being group housed decreased tank water cortisol levels by 25% and being enriched decreased tank water cortisol levels by 22%. These effects were independent and additive such that singly housed enriched fish did not differ in cortisol levels from group housed barren fish.

PS65 Skin Swabbing of Zebrafish (Danio rerio) as a Refinement for Genotyping: How to Make it Work

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Current practice when obtaining a tissue sample for genotyping zebrafish (Danio rerio) is to remove a small part of the caudal fin in a procedure known as fin clipping. During this procedure, zebrafish in the United Kingdom need to be anesthetized ahead of sample removal, and the use of analgesia is being phased in as a legal requirement. This procedure is invasive as tissue is removed and zebrafish have previously shown an aversion reaction to MS-222. In partnership with an outsourcing genotyping service, we trialed a refined DNA collection technique, skin swabbing. This project has been developed with the intention of validating this as a reliable method for collection of sufficient DNA to return accurate genotyping results via an external service provider. Over six separate skin swabbing sessions, we experienced a success rate of 84.6% while testing for 10 different genotyping assays. Common errors were failed internal control and signal between positive and negative results. The housekeeping Ct data indicated that the amount of DNA varies from a swabbed sample, with a lower Ct means there are higher amounts of DNA. There was no pattern of error detected when comparing the specific technicians who performed the sampling, the size of the animal, or the selected genotyping
We further refined our methodology to reduce error rates by letting the swabs dry on the plate for 60 minutes after the final sample was collected. From a 3Rs perspective, skin swabbing offers refinement benefits in improving welfare of the fish using a less invasive technique with a shorter procedure time when compared to fin clipping. Trial and error may be required depending on the genotyping assay used and external suppliers should be able to support this process.

**PS66 Incorporating Lidocaine as Analgesic During Fin Clipping using a Recirculating Housing System**

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Analgesia is one way to embrace the 3RS when working with laboratory animal models. While it is standard in mammalian care, only recently has it been put into practice with aquatics species in research, even though it is well established that fish can feel pain. Working with the veterinary team, scientists, equipment manufacturer, and the Aquatics team, the Francis Crick Institute has developed a way to provide analgesia pre- and post-procedure on zebrafish (*Danio rerio*) undergoing fin clipping for genotyping individuals. The developed methodology does not impact user workflow as there are no additional steps required to administer analgesia. The newly developed process maintains the ability for high-throughput sampling while maintaining excellent animal welfare. Analgesia is provided to zebrafish at the correct dosage and exposure time off the recirculating housing system, first in their home tank on the benchtop and then post-clip in a shallow tray where they will remain until the genotyping results are received. A wash step is performed before the tray is returned to the main system. This, in addition to a carbon filter, ensures that lidocaine is not detectable in the system water as verified by HPLC testing. The wash step and carbon filter prevent fish experiencing repeated exposure to lidocaine while housed on a recirculating system. This talk will outline the necessary steps to provide analgesia for fin clipping without impacting procedural session length, both with a recirculating system or in small static tanks, which can further be adapted to suit different facility set ups as required. Providing analgesia to zebrafish can now be standard practice by following these steps.

**PS67 Persistent Abdominal Distension in a Laboratory Guinea Pig**

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A 2-year-old, 3.5 kg, intact, singly housed female guinea pig was presented for growing abdominal distension. The animal was housed in the university’s training core and was utilized for purposes such as handling, restraint, and injections. No treatments or experimental manipulations were performed on this animal. On presentation, the guinea pig exhibited normal behavior and had an unremarkable examination other than a symmetrical, distended abdomen. On palpation, her abdomen was soft and nonpainful, with no evidence of organomegaly. Differentials included bloat, neoplasia, intestinal dysbiosis, and obstruction. Serial radiographs of
the abdomen and thorax revealed increased gas within the stomach and throughout the gastrointestinal tract. This clinical presentation waxed and waned over several months and was resistant to medical therapy, which included pro-kinetics, anti-gas medicine, and analgesics. Her mentation and food and water intake remained consistent and there were no abnormalities in her eliminations. After nearly 6 months from the initial presentation, a few days prior to euthanasia, the animal developed diffuse alopecia centered over the abdominal distension and displayed signs of pain including increased sensitivity to handling and picking at the abdomen. Necropsy findings included adrenal hypertrophy and hyperplasia, steroid hepatopathy, and sparse and inactive hair follicles. The clinical signs and histologic lesions were consistent with Cushing’s disease. Differential diagnoses should include Cushing’s disease in cases with nonspecific abdominal distension in the absence of other clinical signs. Furthermore, a full workup including blood profiles and hormonal assays should be considered in a guinea pig with a persistent distended abdomen that is otherwise asymptomatic.

**PS68 Unexpected Mortality in a Captive Wild Caught Crested Anole Colony**

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Crested anole (*Anolis cristatellus*) breeding colony was established with wild-caught individuals from Puerto Rico. Approximately 2.5 months after transport, investigators reported decreased reproductive performance, and two animals were found dead on same day. Two days after initial mortalities, two anoles were identified as sick, one with poor righting and the other moribund. A necropsy performed after euthanizing the moribund animal showed body condition 1.5/5 with no fat pads, and empty stomach/intestines. Differential diagnoses included metabolic bone disease, parasites, stress, unknown species-specific needs, or infection. Five days after initial mortalities, 5 anoles were found dead/euthanized, and multiple new sick cases identified. An animal with poor righting reflex was approved for diagnostics and histopathology. The blood glucose was 147 mg/dL, and a blood smear revealed normal red blood cells and platelets with severe leukopenia. This individual also had no fat stores, but feces was present in the distal intestine for fecal float, no parasites seen. With histopathology pending, housing conditions were optimized to decrease potential stressors by adding dividers between cages to decrease aggression, eliminating daily temperature fluctuations, increasing misting, and adding standing water for increased humidity and hydration, increasing UVB light, and changing enrichment to promote arboreal behavior. Feeding and weight logs were established to monitor response to treatments. Radiographs were performed to exclude metabolic bone disease but were inconclusive due to machine resolution. Histopathology revealed renal tubular mineralization and urate stasis, and metabolic derangements. Diet was modified to include calcium with D3, reptile vitamins, and mealworms for higher fat content. More severe cases were gavaged daily with critical care diet for several weeks and then gradually weaned. The number of insects fed gradually increased individually based on the feeding logs. Visible response to treatments was seen after 2 weeks with increased activity and more vibrant color patterns. Return to reproductive function visualized by eggs laid occurred seven weeks after changes initiated. Metabolic disease was the primary diagnosis. However, the cause was likely multifactorial.
PS69 Intermittent Lethargy and Epistaxis in a Rhesus Macaque

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A 14-year-old male rhesus macaque (Macaca mulatta) enrolled in a behavioral neuroscience study presented with a history of epistaxis and intermittent episodes of laying down in his cage. Physical examination revealed tachycardia and grade 3/6 left systolic gallop murmur, with normal respiratory auscultation and no abdominal distention. Survey radiographs revealed right sided cardiomegaly with a mild unstructured broncho-interstitial pulmonary pattern. Cardiac troponin was elevated (0.07 ng/ml). Differential diagnoses included valvular insufficiency, hypertrophic cardiomyopathy, septal defects, and dilated cardiomyopathy. At this time, the animal was removed from study due to concerns regarding fluid regulation in an animal with abnormal cardiac function. A follow-up echocardiogram and EKG one month later revealed dilated ventricles and decreased ejection fraction, consistent with a diagnosis of dilated cardiomyopathy (DCM). The EKG showed intermittent ventricular premature contractions (VPCs) and a gallop murmur that had worsened from grade 3/6 to 5/6. While off study, the animal started on Carvedilol (3.125 mg/day), which lowered the awake resting heart rate to within normal limits for one month without any dosage adjustments. Serial EKG and cardiac auscultation were performed in an awake chair-trained animal, a procedural refinement that enabled close monitoring of disease progression. Elective euthanasia was performed two months after treatment initiation when the Carvedilol therapy failed to control the tachycardia and VPCs. Histological findings were consistent with dilated cardiomyopathy, characterized by extensive cardiomyocyte hypertrophy with loss and replacement by mature fibrous connective tissue and fatty infiltration. This case presents a refinement to the management of cardiomyopathies in laboratory housed primates and highlights the importance of considering spontaneous DCM as a differential diagnosis for cardiac disease in adult rhesus macaques.

PS70 Suspiciously Swollen and Scaly Skin in a Recently Transported T-cell Receptor (TCR) Transgenic Mouse

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An experimentally naïve 2.5-month-old, male, homozygous C57BL/6-Tg(TcraTcrb)1100Mjb/J (OT-1) mouse presented with swollen digits on all paws and mild scaling along the pinnal margins. Seventeen days prior, this mouse along with four other male mice were transferred from another animal facility and housed in breeding trios with C57BL/6 female mice. On exam, all digits were swollen and mildly erythemic with mild scaling. Mild to moderate swelling was primarily over the metacarpals and metatarsals while swelling was not noted elsewhere. The pinnal margins were non-erythemic though mildly thickened bilaterally with scaling. The four
other male mice transported with this mouse were not similarly affected. Differential diagnoses included immune mediated disease, vascular disease, infection, or neoplasia. Despite a 7-day course of meloxicam in the water (1mg/kg/day PO), no improvement was noted, and euthanasia was elected. On necropsy, there was moderate diffuse erythema and dermatitis of the paws and multifocal missing nails on various digits. Internally, there was moderate enlargement of the mesenteric lymph nodes, mild splenomegaly, and moderate diffuse pallor of the lungs. Ectromelia virus serology and *Pneumocystis spp.* PCR evaluation of lung tissue was negative. Aerobic and anaerobic bacterial culture of the spleen and mesenteric lymph node were negative as well. A complete blood count showed lymphocytosis (8270/ul). On histology, diffuse enlargement with small to medium-sized round cells was noted in numerous lymphoid organs (thymus, mediastinal and mesenteric lymph nodes, and spleen) that were CD3+, CD4+ on IHC, and diagnosed as T cell lymphoma. Skin lesions of the paws consisted of mild to moderate multifocal epidermal hyperplasia with orthokeratotic and parakeratotic hyperkeratosis, interface dermatitis, and other histologic features typical of exfoliative dermatitis, and is presumably paraneoplastic in origin based on overall case findings and similarity to paraneoplastic exfoliative dermatitis described in cats and rabbits with thymomas and lymphomas. Spontaneous development of T cell lymphomas in TCR transgenic mice has been characterized previously, and cutaneous involvement should be noted as a possible overt clinical sign.

**PS71 Subcutaneous Swelling in a Laboratory Ferret**

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A 10 month old, 1.28kg, group-housed, experimentally naive, neutered, male ferret was examined for a non-painful right axillary subcutaneous mass approximately 2.0x1.2x1.0cm in diameter. As the ferret was afebrile and displayed no regional lymphadenomegaly, he was placed on monitoring for suspected local inflammation secondary to rough housing. On reassessment 7 days later, the mass had grown and become lobulated, now measuring 4.1x1.6x1.3cm in diameter. An FNA of the mass found mild to moderate mixed inflammation. The ferret’s clinical signs had also progressed; he was quiet, dehydrated, and febrile. Support in the form of fluids, critical care, meloxicam, and amoxi-clav was initiated. Bloodwork revealed mild neutrophilia (9.828-K/uL), monocytosis (0.546-K/uL), and basophilia (0.273-K/uL), and marked eosinophilia (13.65-K/uL). Differential diagnoses included an allergic reaction, parasitism, hypereosinophilic syndrome, eosinophilic gastroenteritis, autoimmune disease, or neoplasia. During this time, the right prescapular lymph node slightly enlarged, the right axillary mass decreased in size, additional masses were identified caudal to the left ear and at the right proximal caudal thigh, and abdominal palpation revealed splenomegaly and enlarged mesenteric lymph nodes. Despite aggressive supportive care, the ferret became progressively lethargic and dehydrated, although no additional clinical signs developed. A diagnostic necropsy was performed. Repeat blood work revealed marked leukocytosis (47.8-K/uL), with more extreme neutrophilia (17.208-K/uL), lymphocytosis (13.384-K/uL), monocytosis (1.434-K/uL), basophilia (0.956-K/uL), and eosinophilia (14.818-K/uL) than before, while gross examination showed severe splenomegaly (35.26g, 13x3.5x1.5cm), prominent lymph nodes, and multiple firm, subcutaneous and
intramuscular and subcutaneous masses. Histopathology revealed lymphoid hyperplasia and severe splenic extramedullary hematopoiesis, severe pyogranulomatous myofasciitis, and eosinophilia with severe eosinophilic gastroenteritis. This ferret’s presentation of severe idiopathic pyogranulomatous myofasciitis associated with the masses and other tissues and concurrent eosinophilic gastroenteritis – two rare conditions – represents an unusual clinical case scenario.

**PS72 High Mortality in Juvenile Rainbow Trout**

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500 juvenile rainbow trout fish were ordered from a reputable source slowly began to experience a mortality of ~3% within the first 2 hours after their arrival to our aquatics facility. This was presumed to be a typical occurrence due to shipping stress and acclimating to a new tank system. Modifications were made to the tanks to decrease the speed of the water flow rate to prevent the fry from fatiguing while swimming and could ultimately lead to their mortality. Routine water tests were performed to ensure that the water temperature, nitrate, and nitrite levels were appropriate. As the week progressed, the mortality increased over the next few days from 10% to 40% to eventually 80%. Fish were noted to have a corkscrew swim pattern, some of the fish had increased pigmentation, and fecal casts extending from their vent. Due to the likelihood that this was an infectious disease outbreak and some individuals remained asymptomatic, we elected to euthanize the remainder of the fry in the tank and the tank was disinfected using the lab's standard chlorination protocol. Our differential diagnoses for this rapid rate of mortality in fry included infectious pancreatic necrosis virus and infectious hematopoietic virus. Any potential survivors from this cohort could potentially serve as asymptomatic carriers if they were to reach adulthood posing a threat to any future fry brought into the colony that would be naive. Fry were submitted for viral culture, PCR, and histopathology. The results of our diagnostic testing were conclusive for an infectious pancreatic necrosis outbreak. We moved forward with contacting the vendor to warn them of the potentiality of disease in their colony.

**PS73 Facial Swelling in an Adult Sprague-Dawley Rat**

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A one-year-old intact male Sprague-Dawley rat was reported to have acute facial swelling on the right cheek and blood in the ear canal. Initial differentials included: trauma, neoplasia or abscessation of the Zymbal’s gland, parotid salivary gland, or extraorbital lacrimal gland, or a deep inner ear infection. The mass was 2.5x1.5x1.5cm (about 0.59 in), firm but slightly fluctuant, and warm to the touch. Under general anesthesia (2% isoflurane), the mass was lanced using a 20g needle. Approximately 1 ml (about 0.03 oz) of purulent exudate was drained from the mass. The abscess was not flushed with iodine for fear of spreading infectious material to deeper tissues or the ear canal. The rat was given a single dose of enrofloxacin and meloxicam.
and the husbandry staff was instructed to monitor daily. Six days later the abscess recurred, and
the rat was euthanized. Necropsy revealed a solid mass immediately caudoventral to the ventral
ear canal, with purulent material filling the interior of the mass. On histopathology, the mass was
identified as a Zymbal’s gland adenocarcinoma, likely leading to a secondary abscess formation.
Aerobic culture revealed a mixed population of bacteria, including *Proteus mirabilis*,
*Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus merionis*. Zymbal’s gland pathology
is common in rats used in research. Though abscessation was the most prominent feature in the
initial presentation, it is often secondary to another underlying pathology, in this case neoplasia.
The bacteria cultured in this case are common opportunistic infections. Interestingly,
*Streptococcus merionis* is most often associated with gerbils and other jirds; it has previously
been cultured from the oropharyngeal region of gerbils and other small burrowing rodents.
Though thought to be a commensal bacterium, not much is known about its pathogenic potential.
With respect to the present case, there have been no gerbils ever housed in this building.
Therefore, the origin of the *S. merionis* could not be determined.

**PS74 Mysterious Mass in a Mangabey**

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A 25-yr-old, 6.47 kg, intact, socially run-housed female sooty mangabey (*Cercocebus atys*) was
admitted to the hospital for oral trauma. Physical exam findings included: a missing incisor #101,
a complete crown fracture of incisor #102 with a retained root due to trauma, a necrotic incisor
#401, and a crown fracture of incisor #402. In addition to oral trauma, the uterus was moderately
enlarged and firm on abdominal palpation. Abdominal radiographs revealed a soft tissue mass in
the caudal abdomen. The geriatric mangabey also had a history of lumbar kyphosis and
decreased range of motion in both stifles due to chronic arthritis. Incisor #102 was addressed on
the exam table via manual extraction of the retained root, and a dental exam was scheduled for
further treatment of the remaining incisor traumas. The animal was started on antibiotics and
NSAIDs for trauma and arthritis. Complete blood count revealed neutropenia with monocytosis,
indicative of chronic inflammation. Chemistry did not reveal any significant findings. At this
point, a list of differential diagnoses included uterine leiomyoma, leiomyosarcoma, adenomyosis,
adenosarcoma, and endometriosis. Due to chronic arthritis and suspected endometriosis or
neoplasia associated with advanced age, the mangabey was euthanized. On gross exam, the
uterus was enlarged, and the uterine wall was uniformly thickened with tan mottling.
Histopathology showed that the myometrium was thickened by smooth muscle with multifocal
islands of endometrial glands and stroma, consistent with adenomyosis. The oviduct was
surrounded by endometrial glands and stroma with hemosiderin-laden macrophages, which is
pathognomonic for endometriosis. Immunohistochemistry (IHC) staining with CD10 stain was
performed to confirm presence of endometrial stroma in both tissues. While endometriosis is the
most commonly diagnosed nonneoplastic uterine lesion in the sooty mangabey, other conditions
such as adenomyosis can occur concurrently and must be considered.

**PS75 Subtle, Unilateral Hindlimb Lameness in a Lesser Egyptian Jerboa**
A 2-year-old female lesser Egyptian jerboa (*Jaculus jaculus*) presented with a discrete swelling on the right hindlimb. There was no known history of trauma and the animal had been singly housed since its arrival at our institution approximately 1 month earlier. The physical exam was normal except for a 2mm fluctuant swelling on the medial aspect of the right tarsus. When standing at rest, a subtle favoring of the right hindlimb was observed; however, lameness was difficult to appreciate during normal ambulation in the cage and behavior was otherwise normal. Fine needle aspiration of the tarsal mass produced a small amount of clear, viscous, acellular material consistent with synovial fluid. Differential diagnoses included acute soft tissue injury, a synovial cyst, and chronic degenerative joint disease. Conservative therapy was initiated with 0.5 mg/kg meloxicam administered subcutaneously once daily. Despite treatment, the lameness worsened, and the jerboa was sedated for imaging. Radiographs revealed soft tissue swelling around the right hock and a small, radio dense lesion associated with the tarsal joint space. Humane euthanasia was elected due to the poor prognosis for improvement. On necropsy, there were several gross changes of the right tarsal joint, including generalized soft tissue swelling and a small, spherical outpouching from the joint space. Histologic findings included a chronic, non-healing fracture of one of the tarsal bones with exuberant cartilage formation. While this case illustrates that fractures must be considered as a differential in instances of joint swelling and mild gait abnormality in this species, the fracture of single tarsal bones is relatively rare in humans and domestic animal species. It is unclear what the inciting cause was in this animal, though this species’ unique hindlimb biomechanics must be considered, as jerboas are desert-adapted rodents that demonstrate bipedal locomotion and explosive hopping as a mechanism for predator avoidance.

**PS76 High-mortality Epizootic Mycobacterium ulcerans ecovar Liflandii in a Colony of Zaire Dwarf Clawed Frogs (*Hymenochirus boettgeri*)**

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*Mycobacterium ulcerans* ecovar Liflandii (MuLiflandii) was identified as the causative agent of systemic mycobacteriosis in a research colony of approximately 300 Zaire dwarf clawed frogs (*Hymenochirus boettgeri*). Approximately four frogs per month over a five-month period presented with acute morbidity and mortality, including lethargy, coelomic effusion, multifocal cutaneous hemorrhages, and rarely cutaneous granulomas. Differential diagnosis included *Chytridium dendrobatidis*, Ranavirus infection, ovarian hyperstimulation syndrome, and bacterial septicemia. Coelomic samples tested negative for *C. dendrobatidis* and Ranavirus, documented water quality appeared sufficient, and there was no history of gonadotropin administration. Histologically, clinically affected animals showed multifocal necrotic and inflammatory lesions in multiple organs, which contained myriad acid-fast bacteria consistent with *Mycobacteria spp.* (n = 8). Identification and speciation of mycobacteria was performed using nucleic acid amplification and sequencing, as well as special mycobacterial culture...
techniques with mass spectrometry. These findings suggest that \textit{MuLiflandii} is a primary pathogen in \textit{H. boettgeri} and should be considered in the differential diagnosis of sepsis and coelomic effusion in amphibians. Mycobacterial speciation is important given the difficulty in diagnostic specificity, variability in pathogenesis within the family \textit{Mycobacteriaceae}, and the implications for both animal and human health as a potential zoonotic disease. \textit{H. boettgeri} is a species common in the pet trade and used increasingly in laboratory animal medicine, and these findings provide consideration for this pathogen as a potentially important public health concern. To the authors’ knowledge, this is the first report of \textit{MuLiflandii} infection in the genus \textit{Hymenochirus} and illustrates the diagnostic challenges of differentiating among mycolactone-producing mycobacteria, as well as between these species and \textit{Mycobacterium marinum}. Furthermore, we demonstrate the utility of environmental sampling for this pathogen reliably within the tank system, suggesting this mode of sampling could be used as a reliable method for direct frog surveillance.

**PS77 Armenian Hamsters (\textit{Cricetulus migratorius}): A New Host for \textit{Corynebacterium bovis} Infection**

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\textit{Corynebacterium bovis} (Cb), an opportunistic bacterial pathogen, causes skin disease in immunocompromised mice and possibly rats. Until now, Cb has not been reported to affect other rodents. In 2021, neonatal mortality, initially of unknown etiology, was observed in a small, newly established, Armenian hamster (\textit{Cricetulus migratorius/Cmig}) breeding colony. With successive litter losses in which pups presented with flaky skin, Cb was detected by PCR from caging as well as the dam of an affected litter. Affected 10d old neonates had mild to moderate hyperkeratosis with abundant Gram-positive coccobacilli within the excessively keratinized skin. These skin sections were PCR positive for Cb. Subsequently, a second colony of breeding hamsters was established at another institution as the sole vendor supplying \textit{Cmig} to the research community was disbanded. On arrival, 1 out of 4 nulliparous breeding pairs cultured positive for Cb. The first litter of this pair had 8/8 neonates with scaly skin/fur on day 7 that were otherwise bright, alert, responsive and nursing, but then found dead on days 10 and 11. Cb was isolated from acanthotic, orthokeratotic skin from these pups in which intra-corneal and -follicular bacterial colonies consisting of short rods compatible with Cb along with multifocal inflammatory infiltrates, dermal fibrosis, and serocellular crusts were observed. No further pups with clinical signs nor mortality were observed from subsequent litters generated by this or other breeding pairs, even though Cb was consistently cultured and microscopic lesions consistent with Cb were observed in pups of various ages as well as in post-weaning hamsters up to 2.5mos old, although the older hamsters had a lesser bacterial burden and lesion severity. Small numbers of \textit{Demodex} were concomitantly detected in some pups ≥8 days old. \textit{Demodex} causes scaly skin in heavy infestations of adults and is enzootic in all \textit{Cmig} derived from the single importation from Armenia in the 1960s. The role that \textit{Demodex}, the skin flora, and perhaps other unknown contributing factors have on the clinical presentation and mortality observed in these colonies
remains undefined, but evidence indicates that Cb can colonize and likely cause dermal pathology in Cmig.

**PS78 Novel Demonstration of Corynebacterium bovis-associated Lesions and Interface Dermatitis in NIH-Foxn1nu Rats (Rattus norvegicus)**

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*Corynebacterium bovis* (Cb), a short, pleomorphic, gram-positive rod, is well recognized to cause clinical disease and histopathologic lesions in immunocompromised mice and has been isolated from Sprague-Dawley and athymic nude rats. While rats can be colonized with Cb, there is no peer-reviewed literature describing clinical disease or histopathologic lesions resulting from Cb infection in this species. Clinically observable dermatitis developed in 2 naïve NIH-Foxn1nu (nude) rats from which Cb was isolated by aerobic bacterial culture and PCR. Shared use of behavioral equipment by Cb-infected immunocompromised mice is the suspected origin of the bacterium in the colony. Affected rats presented with a pruritic dermatitis characterized by urticaria, macules, and pinpoint abrasions on the dorsal thoracocapular and ventral abdominal regions, as well as bilaterally thickened and flaky pinnae. Rats failed to respond to a series of treatments, including the provision of amoxicillin-impregnated feed (0.12%), application of topical silver sulfadiazine ointment, or gentle cleansing of the affected areas with chlorhexidine (4%) solution along with weekly nail trims. Histopathology showed typical Cb-associated lesions, such as acanthosis and orthokeratotic hyperkeratosis with intracorneal and intrafollicular colonies of short rods compatible with Cb. Additionally, a distinct pattern of interface dermatitis was observed with and without an association with bacteria, characterized by a lymphocytic mural folliculitis and epidermitis, with lymphoplasmacytic and histiocytic peri-adnexal to interstitial dermatitis, and occasional neutrophilic luminal folliculitis, superficial neutrophilic pustules, and dermal fibrosis. Based on the isolation of Cb and select histologic features, which resembles Cb infection of nude and highly immunocompromised NSG mice, Cb likely contributed to the clinical disease observed in these cases. While it is possible that Cb and/or other components of the skin microbiome may have contributed to the development of the interface dermatitis, it is not possible to determine which, if any, of the clinical features are solely attributable to Cb, as interface dermatitis has also been observed in both nude mice and rats devoid of typical Cb lesions.

**PS79 Incidence of Dystrophin Mutations in Swine (Sus scrofa domestica): Novel Porcine Stress Syndrome Implications for Physiology during Anesthesia**

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Swine are increasingly being used for biomedical research as appropriate animal models given similarities to humans including size, arterial capacity, and cutaneous structure. From November 17, 2021, through February 15, 2022, 11 out of 11 swine (Sus scrofa domestica) exposed to isoflurane inhalant anesthesia over two different research protocols were euthanized after exhibiting symptoms like malignant hyperthermia including hyperthermia, hypercapnia, skeletal muscle rigidity, dyspnea, tachycardia, and hypotension. This group of 2 males and 9 females was composed of intact Yorkshire/Landrace crosses females with weights between 68 to 91 kilograms and ages 3 to 5 months purchased from a research breeder. While malignant hyperthermia is caused by mutations in ryanodine receptor 1, another novel stress syndrome in pigs involves a mutation in the dystrophin gene. We analyzed the incidence of ryanodine receptor 1 and dystrophin mutations in 7 of the original 11 clinically effected pigs and in 56 subsequent non-clinical research swine using a combination of blood and muscle samples. All animals tested negative for the ryanodine receptor 1 mutation, while the dystrophin variant was found in 2 out of 7 clinical (28.6%) and 22 out of 46 (47.8%) subsequently tested female pigs. During procedures in female swine, creatinine kinase, an indicator of muscle damage, was measured while under isoflurane anesthesia. Creatinine kinase was slightly elevated in dystrophin mutation positive carriers (625.0 ± 81.8U/L) compared to those negative (543.8 ± 109.5U/L), but this did not reach statistical significance (P=0.088). Body temperature was slightly decreased in dystrophin mutation positive carriers (37.7 ± 0.1°C) compared to those negative (37.8 ± 0.4°C), but this, also, did not reach statistical significance (P=0.368). After unsuccessfully trying to prevent the clinical signs utilizing intravenous administration of dantrolene, one research group switched anesthesia protocols from primarily inhalant anesthesia to primarily total intravenous anesthesia, and the clinical signs did not return. Taken together, choice of anesthesia should be carefully considered when performing longitudinal porcine studies.

PS80 Efficacy and Effects of High-dose Carprofen after Plantar Incision in B6 Mice

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Carprofen is a nonsteroidal anti-inflammatory drug commonly used to provide analgesia to mice. The recommended carprofen dose for rodents is 5-10 mg/kg once or twice daily. However, recent research indicates a need for higher doses. In this study, we first compared the efficacy of carprofen at 5, 25, and 50 mg/kg for attenuating postoperative mechanical and thermal hypersensitivity in B6 mice. Then we evaluated clinical safety through serum chemistry, CBC, fecal occult blood, and histopathological evaluation. Additionally, we measured the pharmacokinetics of these 3 doses over a 72-hour period. We hypothesized that both 25 mg/kg and 50 mg/kg would effectively attenuate postoperative mechanical and thermal hypersensitivity resulting from a plantar incision in B6 mice. Male and female C57/BL6 mice (n=38) were assigned to 1 of 4 treatment groups: saline (0.9% NaCl, 10 mL/kg SC SID for 3 d); C5 (carprofen 5 mg/kg SC SID for 3 d); C25 (carprofen 25 mg/kg SC SID for 3 d); and C50 (carprofen 50 mg/kg SC SID for 3 d). Mechanical and thermal hypersensitivity assessments were
performed 24 h before surgery and at 2, 6, 24, 48, and 72 hours afterward. All groups showed mechanical and thermal hypersensitivity postoperatively. Compared to the saline group, only carprofen at 50 mg/kg attenuated mechanical hypersensitivity and thermal hypersensitivity at all study timepoints. No clinically significant dose-related toxicity was found on serum chemistry, CBC or histopathological analysis; however, from 6 hours until 72 hours, fecal occult blood was present in all experimental groups. These findings suggest that 50 mg/kg provides effective attenuation of mechanical and thermal hypersensitivity compared to saline and there are minimal toxic effects when given once daily for 3 days.

**PS81 Don't Stand So Close to Me: Do Aging Adult Male Rats Prefer to be Alone or in Pairs?**

GLAS: Yes
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This study was designed to look at the space requirements for aging male Sprague Dawley rats. These rats quickly outgrow Guide floor space requirements, leading institutions to have to decide if it is better to house animals in pairs, but crowded, or separated, but alone. This study therefore seeks to assess the behavioral and physiologic responses of rats in varying conditions of caging floor space and group vs. individual housing. We obtained 276 male Sprague Dawley rats which were each assigned to one of three types of caging: standard sized Lab Products rat IVC (Alt Design), Allentown NexGen 1800 rat IVC (Allentown), or Tecniplast GR1800 rat IVC (Double Decker) and initially pair housed. Once both rats in a cage reached 600g, they were assigned to either the paired housing condition, and remained in their initial caging, or assigned to the single housed condition and separated. Three days after this assignment, rats were assessed for acute effects of their experimental condition with one individual undergoing behavioral testing such as the Elevated Plus Maze (EPM) or Forced Swim (FS). One month later, rats were assessed for chronic effects of their experimental conditions with the opposite assay from their acute assessment (i.e., if a rat completed EPM for acute assessment, they would undergo FS for their chronic assessment). After the chronic assessment, blood samples were taken for CBC and Corticosterone analysis, and rats were euthanized. We found that rats made more open-arm entries on the EPM if they were from a Double Decker cage compared to either the Allentown or Alt Design and that caging type did not affect blood markers of stress. We also found that paired vs single housing made no difference to behavioral or blood results.

**PS82 Effect of In-house Permethrin-soaked Enrichment Bedding on Mouse Nesting Scores, Body Weights, and Body Condition Scores**

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Fur mites are an excluded agent in most contemporary laboratory mouse colonies but remain a persistent issue with infestations complicating animal health and research outcomes. Wild
rodents are a likely source, and infestations may be clinically silent or result in pruritis, dermatitis, weight loss, and decreased fertility. To address potential mite contamination, our institutional rodent quarantine practice was to provide animals with permethrin-treated nesting material manufactured by an outside vendor. However, this product is no longer commercially available. Methods to create in-house permethrin-soaked enrichment bedding have been published. Before implementing this technique, we wished to assess for impacts on mouse health and nest building. Female C57BL/6 mice (n=10/group, 3-7 mo, pair-housed) were provided permethrin-soaked or untreated enrichment bedding and body weights, body condition scores, and nest scores were measured over 5 weeks. Treated enrichment consisted of a 4 g bedding “puck” saturated with 5 mL of 0.5% permethrin and left to air dry for 24 hours before use. Control cages received one untreated bedding “puck”. Both treated and control cages were also provided with an untreated cotton nesting square as additional enrichment. Initial body weights and body condition scores were collected before the experiment began and then twice weekly for the duration of the experiment. Nest scoring was performed twice weekly. There were no significant differences in body weight at any time point between the mice receiving permethrin-soaked or untreated enrichment bedding. At one time point, mice receiving treated bedding had significantly higher body condition scores than control mice (p=0.03). At three of ten nest score assessments, cages with treated bedding had significantly lower scores than control cages (p=0.031, p=0.02, p=0.03. Despite this, all cages demonstrated interaction with the enrichment material and built nests consistently throughout the study. Permethrin-soaked enrichment bedding did not negatively impact the health or well-being of mice as measured by body condition, body weight, or nesting scores. Our institution plans to implement permethrin-soaked enrichment bedding made in-house for fur mite treatment of rodents in quarantine.

PS83 Mice Make Moisture: Comparing Relative Humidity in the Housing Room and Home Cage

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Relative humidity (RH) is commonly measured in mouse housing rooms. It is typically assumed that measurements in the room reflect what the mice experience in the cage. However, there is limited data comparing RH measurements at the room level and inside of ventilated cages. The purpose of this study was to compare how RH levels taken at the room level compare to those inside of ventilated cages containing breeding trios and same sex groups of laboratory mice. We also assessed how RH is impacted by the age of a single mouse litter, the time since cage change, and the group size of same sex cages. RH was measured using a temperature/humidity sensor attached to a solid top caging lid. The lid was rotated across N=24 breeding trio cages, equally divided across litter ages (no litter, PD1, PD10, PD18; n=6). The lid was also rotated across N=15 same sex holding cages, containing either 1, 3, or 5 mice. Measurements in holding cages were repeated 3x per cage in relation to time since cage change (1 day, 1 week, 2 weeks). All measurements lasted for five minutes and were done following a two minute acclimation period in each location. Data were analyzed with general linear models and blocked by cageID where appropriate. In breeder cages, RH was impacted by litter presence and age (F4,20= 29.74; P<0.001): it was higher in all mouse cages than the room and it was higher in cages with
weanlings than cages with no pups. In holding cages, RH was higher in mouse cages than the room ($F_{2,32} = 10.36; P<0.001$). It was also impacted by time since cage change ($F_{2,28} = 6.14; P=0.006$) and an interaction between sex and group size ($F_{2,28} = 5.29; P=0.011$). RH was highest in cages holding five males and lowest in cages holding a single mouse of either sex. This data shows that RH is not constant between mouse cages in a single room, nor within a single cage over time. Further, measuring RH at the room level may not be a valid assessment of what the mice experience in ventilated cages.

**PS84 Efficiencies of Different Genetic Modification Techniques in Rat Embryos**

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CRISPR-Cas9 has revolutionized the creation of genetically modified animals. Preconstructed DNA templates along with CRISPR-Cas9 reagents can be inserted into embryos by pronuclear injection (PNI), electroporation (EP), and delivery via adeno-associated virus with electroporation (AAV+EP). Currently, no published literature compares the efficiency of these techniques as it relates to embryo development and knock-in (KI) rate in rats. We used a 401 base pair (bp) short artificial intron targeting exon 2 of the *Crh* gene as the DNA construct. Superovulated Sprague Dawley (SD) female rats mated to SD stud males were used to generate zygotes. Ten SD females were superovulated per collection, five collections were performed for a total of 50 female rats. Ten SD stud males were used for mating. Zygotes were randomly assigned into four groups: culture only control, PNI, EP, and AAV+EP. Manipulated embryos were cultured to blastocysts in 500 µL of KSOM-R media. Embryos were collected individually and submitted for genomic sequencing to detect evidence of genome editing. Embryo survival after one day in culture was 98% (109/111) for culture only control, 58% (101/175) for PNI, 100% (106/106) for EP, and 95% (124/130) for AAV+EP. Cleavage rate after one day in culture of surviving embryos was 99% (108/109) for culture only control, 88% (89/101) for PNI, 99% (105/106) for EP, and 94% (116/124) for AAV+EP. Development of embryos to 4-cell stage after three days of culture were 90% (98/109) for culture only control, 62% (63/101) for PNI, 77% (78/101) for EP, and 52% (65/124) for AAV+EP. Knock-in rates for manipulated embryos were 67% (12/18) for PNI, 3% (1/35) for EP, and 63% (22/35) for AAV+EP. From our results, we conclude that PNI decreases embryo survivability. All three gene editing techniques have similar embryonic development to a 2-cell and 4-cell stage for rat embryos that survive 24 hours in culture. With a 401 bp DNA template, we found PNI and AAV have similar KI rates while EP had a much lower KI rate. We speculate that this lower KI rate is related to the size of the DNA repair template. Our work is important since optimizing gene editing techniques greatly reduces total animal numbers and saves both the time and money associated with rat model generation.

**PS85 Facilitating Mouse Studies of Post-Acute Sequelae of COVID-19**

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The chronic form of COVID-19, Post-Acute Sequelae of COVID-19 (PASC), remains a significant public health concern. The B6.Cg-Tg(K18-ACE2)2Prlmn/J (hACE2) mouse has been widely used to study acute COVID-19 studies, but its suitability as a model for PASC remains uncertain. The long-term goal of this research is to establish whether the hACE2 mouse is an effective model for PASC research. Previous work by our lab has shown experimental inoculation of the hACE2 mouse with SARS-CoV-2 results in persistent infection up to 16 weeks, but their infective potential was unclear. The primary objective of this study was to assess viral infectivity in persistently infected mice with the aim of transitioning them from animal biosafety level 3 (ABSL-3) containment to ABSL-2. To this end, a sub-genomic E RNA (sgE-RNA) RT-PCR assay, an assay that detects replicating virus, and sentinel mice were employed to determine SARS-CoV-2 viral infectivity. It was hypothesized that infected mice would be free of replicating virus by 16 weeks post-infection (WPI) and that sentinel mice would only become infected when exposed to recently inoculated mice. Six to 18-week-old, hACE2 mice (N= 78 females + 75 males) were intranasally inoculated with the USA-WA1/2020 strain of SARS-CoV-2 in ABSL-3 containment. Acutely ill mice were euthanized, and their tissues collected, while cohorts of surviving mice were necropsied at weekly intervals up to 16 WPI. Naïve, female, homozygous hACE2 sentinel mice (N =5) were exposed to previously inoculated mice at 0 DPI (N=3) and 8 WPI (N=2), and euthanized 2 weeks post-exposure, when lungs were harvested. Viral RNA and sgE-RNA were present in lungs up to 16 WPI and viral RNA was also present in the lungs of all sentinel mice. Together, these results suggest that intranasal inoculation of hACE2 mice results in persistent infection with replicating virus present up to 16 WPI, hindering removal from ABSL-3. However, the presence of viral RNA throughout the study suggests that this experimental model may be valuable for assessment of PASC pathology associated with viral persistence.

**PS86 Chlamydia muridarum: Insights into the Effectiveness of Automated Cage Washing and Infectiousness as Assessed by Intercage Transmission During Cage Change**

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*Chlamydia muridarum* (Cm) has reemerged as a prevalent contaminant in academic laboratory mouse colonies causing persistent infection and immune activation, potentially confounding research. The susceptibility of Cm to inactivation and/or removal (IAR) from cages via heated, pressurized water and its’ transmissibility as it relates to modern husbandry practices are unknown. To evaluate IAR, we assessed the ability of Cm to survive autoclaving and/or cage wash and subsequently infect naïve animals. Cages which had housed Cm-shedding mice were
assigned to 1 of 3 groups: sanitization in a tunnel washer (82.2°C [180°F] final rinse for an average of 17 seconds per run, n=10), sanitization in a tunnel washer followed by autoclaving (121°C for 20 minutes; n=10), or control (bedding change only; n=10). The interior of each empty soiled cage was swabbed pre- and post-treatment and assayed for Cm by PCR. All pre-treatment swabs were PCR positive, while post-treatment swabs in all cages (excluding controls) tested negative. To determine if any residual elementary bodies in cages were infectious, a Swiss outbred (SW) and an NSG mouse was co-housed for 7 days in each cage type (n=10 pairs of mice/group). This process was repeated weekly for 4 weeks after which the mice were housed in sterile cage units for 4 weeks. At the end of the 4-week observation period, feces was tested and determined to be negative by PCR. To assess transmissibility, 6 IVC cages (1 cage per rack side) each housing a SW and an NSG mouse were randomly placed amidst cages housing Cm shedding mice (as confirmed by PCR from pooled soiled bedding from each cage per rack) for 35 days. These cages were only manipulated by animal care staff during weekly cage change in an animal transfer station using microisolator cage technique. All mice remained fecal PCR negative when tested for Cm 14- and 35-days post placement. Collectively, these results indicate that Cm does not remain in cages after mechanical washing and even when present (empty soiled cages), cages do not transmit Cm. Further, modern husbandry practices appear to be sufficient to prevent cage to cage transmission.

**PS87 Group 2 Innate Lymphoid Cells Are Required for Protective Immunity in Helminth Infected Mice**

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Experimental studies employing the murine hookworm *Nippostrongylus brasiliensis* are widely used to elucidate the pathogenesis and immunology of helminth infection, which is a major cause of morbidity in human and animal health. Hookworms parasitize the host through skin penetration by third stage infectious larvae (iL3), migration through lung tissue and entry into the gastrointestinal (GI) tract for egg production by adult stage worms. Type 2 immune responses, characterized by Interleukins (IL’s) 4, 5, 9, 13, 25, and 33, drive host protection through worm clearance and tissue repair, while Interferon gamma (INF-g) and IL-17A responses can drive susceptibility and disease exacerbation. The relative contributions of CD4+ T helper (Th2) cells versus group 2 Innate lymphoid cells (ILC2s) to host immunity and tissue repair remain unclear, largely due to the lack of genetic systems that selectively eliminate only one of these populations. The recent generation of mice deficient in Locus Control Region 1 (LCR1 –/-), which allows a selective loss of ILC2 with an intact Th2 compartment, provides a critical tool for addressing this long-standing controversy. In this study, LCR1 –/- mice or wild-type controls (C57BL/6, n = 15/group) were subcutaneously infected with *N. brasiliensis* iL3 and evaluated for parasitological impact and extent of lung injury. Data show 144- and 49-fold higher fecal egg loads and intestinal worm numbers and significantly more red blood cells in the lungs of LCR1 –/- mice vs. controls, indicating greater host susceptibility and lung damage in mice lacking ILC2s. LCR1 –/- mice produced higher IL-17A levels than WT controls. As expected, LCR1 –/- mice had significantly fewer GATA3+ST2+ILC2s, as well as fewer eosinophils, but Th2 cells were...
equivalent between groups. This study sheds new light on the mechanisms of resistance against hookworms and supports the idea that ILC2s are essential for both host protection and tissue repair independently of Th2 cells.

**PS88 Linoleic Acid: An Omega-6 Fatty Acid Essential for Liver Regeneration in Rats.**

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The laboratory rat is currently the most used experimental model in hepatic surgical resection studies to investigate liver regeneration, chronic liver disease, and hepatic cancer. Our previous studies showed that the dietary consumption of linoleic acid (LA), an omega-6 fatty acid, stimulates the growth of rodent and human liver tumors in vivo. We tested the hypothesis that increased LA intake in animals fed a 5% corn oil semipurified diet (control Diet I) or an essential fatty acid deficient (EFAD; deplete in LA) diet but supplemented with an equal amount of LA as in Diet I (experimental Diet II), compared to EFAD diet alone (Diet III), would elevate plasma levels of LA and stimulate regeneration of 70% partial hepatectomized (HPX) rat livers that show metabolic similarities to hepatomas. Three groups of randomized male (N=60/group) and female (N=60/group) Buffalo rats (BUFF/CrCrl) were fed either diet I, II, or III and water ad libitum under an IACUC-approved protocol. After 8 weeks on the respective diets, rats were subjected to HPX. At Day 4 post-HPX (maximum regenerative period) arteriovenous (A[carotid]-V[inferior vena cava]) samples (0.2ml) were collected across regenerated livers (Diet III HPX livers did not regenerate) and measured for LA-, glucose-, O2-uptake, and lactate- and CO2-output; harvested remnant livers were measured for liver LA, total protein, cAMP, DNA content, and [3H]thymidine incorporation into liver DNA (TI-DNA). Regenerated liver A-V consumption of LA, glucose and O2, and lactate and CO2 output, were significantly elevated by over 200% in dietary groups I and II, compared to III; and, LA-, protein-, cAMP-, DNA- and TI-DNA-content were significantly elevated (P<0.0001) in I and II (male>female) by over 6000%, 400%, 250%, 800%, and 60% (I = II). HPX livers regenerated to 60% original size in groups I and II, but not in III. Understanding the mechanism of LA-dependent liver regeneration in the laboratory rat will strengthen our current efforts to enhance successful surgical resection therapies in humans.

**PS89 Dietary Approaches to Combatting Obesity: An Investigation of High Fiber and High Protein Diets in Spiny Mice (Acomys cahirinus)**

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Spiny mice (Acomys cahirinus) are a unique species with a diverse research portfolio. Although they have been a laboratory model for over 20-years, little is known regarding nutritional requirements. Studies of dietary salinity, fats, and sucrose have been performed with the aim of inducing pathologies. This original research expands on the knowledge of dietary effects in this
species with a focus on fiber and protein. We hypothesized that increased dietary protein or fiber will regulate weight gain and improve glycemic control, while a combination diet of increased protein and fiber will achieve an ideal body weight with appropriate glycemic control. Spiny mice were fed either a commercially available rodent diet, a high fiber diet, a high protein diet, or a combination of high fiber and high protein diet for 8 weeks. Physiologic data including body weight, body condition scores, and peripheral blood glucose were collected throughout. A complete blood count, select chemistry panel, organ weights, and histopathologic data were obtained at endpoint. There was no significant difference in the consumption rate between diets. Data shows weight management was obtainable with added fiber. Weight gain was similar between animals on the high protein and the control diet. No diet modification proved best in controlling blood glucose, including during stress-induced hyperglycemic episodes. Ultimately, no diet proved best in managing both weight and blood glucose. While the high fiber diet was effective in controlling weight, it often resulted in a decrease in weight among a growing population. No diet was able to significantly impact blood glucose within the 8 weeks. Surprisingly though, the combination diet, while able to maintain weight with a slow increase in weight gain, showed a trend in elevated blood glucose, warranting a longer diet trial prior to recommending this specific combination.

PS90 Welfare Wednesday: A Weekly Installment for Continuous Animal Welfare Training

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Employee time is a highly valued resource, but direct training of staff either individually or in small groups exhausts the availability of both the trainers and trainees, who could otherwise be dedicated to animal care. When the veterinary group realized there was a gap due to the insufficient cadence of annual training, we developed a new method to disseminate information efficiently and rapidly in a way that was easy for staff to grasp the presented material quickly and asynchronously across multiple production sites of a large animal vendor. Welfare Wednesday was created to review the top clinical observations reported to the veterinary team through a visual aid, using one slide to outline the critical topic points. This slide is displayed on monitors throughout the vivariums and employee break rooms in conjunction with an email that includes a copy of that slide along with more detailed information. A new topic is covered weekly. The learning platform utilized to communicate with animal caretakers about these common clinical observations quickly evolved into a way to educate the entire company on issues noted with our models and created discussion between different teams at all levels. A virtual assessment performed companywide determined that Welfare Wednesday was a successful training mechanism, with many positive responses received. This tool might be useful at institutions with large staffs and/or multiple geographically separated vivariums.

PS91 Reducing Errors and Increasing Operational Efficiency Using Modern Cloud Study Management Solutions

A Lanham*
As pharmaceutical research becomes infinitely more advanced, pre-clinical data capture must catch up. Many researchers still rely on outdated paper and spreadsheet systems that slow down the entire research process, increasing the risk of data error and wasting already constrained time and resources—impacting both animal welfare and scientific integrity. A digital study and colony management system centralizes data and massively reduces the use of paper systems and the risk of mistakes. In order to improve study efficiency and reduce error, we introduced a study and colony management solution to a group that previously used spreadsheets and paper almost exclusively. Over time we tracked metrics around the reduction of errors made in data collection and the overall reduction in time and paper spent in study management. Users were trained to use the new system and then asked to compare how long similar tasks took using the new software solution compared to their previous workflow methods. Overall we saw a drastic reduction in errors made and almost eliminated the use of paper daily. Modernizing a vivarium's workflow by switching to a digital study and colony manager increases efficiency and reduces error within research leading to both an improvement in animal welfare and a reduction in overall cost.

**PS92 The Clinical Evaluation and Management of a Nonhuman Primate Model of Multiple Sclerosis**

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The Common marmoset (*Callithrix jacchus*) is an increasingly popular translational model of neurologic disease. We utilize the marmoset model of experimental autoimmune encephalomyelitis (EAE) to further understand the pathogenesis of human multiple sclerosis (MS), a chronic inflammatory disease of the central nervous system. The marmoset EAE model presents unique husbandry and management challenges, including the support of animals with neurologic deficits ranging from blindness to paresis or paralysis. To refine experimental endpoints and clinical management, and to allow for consistency in collection of clinical data, we adopted a standardized neurologic examination based on a previously published EAE score system and the expanded disability scoring scale (EDSS), the primary scale used in MS in humans. The scale includes muscle tone of each limb and tail, grip strength, sensitivity to touch, eye movement, and pupil dilation. These examinations are performed in parallel with regularly scheduled MRI procedures, allowing for both clinical and imaging-based measurements of disease progression. In addition to monitoring disease progression, these scores also help inform and dictate clinical support needed for each individual animal, such as removal of hanging enrichment once vision is impaired and/or addition of food enrichment as mobility decreases. The creation and implementation of a standardized neurological scoring system in marmoset EAE has allowed us to successfully maintain animals throughout their disease progression and complete numerous studies, providing valuable knowledge about human MS pathology.
**PS93 The “Aunting” System: Improving Survival in Immunocompromised Mouse Strains Post-Weaning**

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Immunocompromised mice can be challenging to breed even when housed using specific pathogen free (SPF) conditions. Breeders cannibalize pups, pups fail to thrive including post-weaning, and litter sizes are generally small for NSG and NOD.SCID strains. Important factors such as increased enrichment, temperature, and socialization can be utilized to increase production and optimize colony health. The “Aunting” system in post-weaning period provides warmth and socialization to runted pups that are often cannibalized by parents or fail to thrive when utilizing traditional delayed weaning. In addition, post-weaning aunting improves weanling health while allowing breeders to continue production without having to nurse multiple litters, increasing breeding production overall. Mice are housed on ventilated racks with room temperature of 74 degrees and provided acidified water and breeder chow. At seven weeks of age, one male and two female mice are housed in breeding cages which includes bedding, nesting material and igloo enrichment items. If the first litter is unsuccessful, animals receive additional enrichment rotated to maintain novelty. On 10-14 days, pups are provided with chow on floor and a dietary gel supplement. Pups are weaned at ~20 days depending on health and appearance followed by housing with designated aunts. Enrichment items and dietary gel supplementation are maintained. Aunts, CB17SCID females, 8 weeks of age and older, are housed four mice per cage. Depending on how many mice are being weaned, anywhere from one to four aunts are used for the aunting period in single or multiple cages, without exceeding five mice per cage total. After pups are healthy enough to be removed from their aunts, the aunts are recombined into their original cage. The “Aunting” system is also effective when weaning singly housed mice and helping them adjust. After three to five days post-weaning aunting, pups have gained weight with improvements in health, potentially demonstrating the importance of additional warmth and socialization versus primary nursing in a delayed wean scenario. In conclusion, weanling survival improves when post-weaning care is provided by an “Aunt” between 18-21 days of age.

**PS94 Thinking out of the Box to Get into the Dirt: Constructing A Visible Burrow System Habitat for Rats**

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Researchers studying the sociality of rodents have long been confronted with the challenges of translating a rich array of natural behaviors into an ethologically relevant context that can be manipulated in a laboratory setting. Confining animals to standard cages and conducting artificial social experiments has often been the approach. To provide animals with a semi-naturalistic environment and researchers with an alternative model for sociality studies, a visible
burrow system was constructed, featuring interconnected tunnels, home cages, and an open arena for rats. However, many challenges and creative solutions were needed for husbandry and health monitoring. For example, pathogen testing was conducted on two dirt-like substrates to confirm their safety for use. Extensive environmental parameters measuring temperature, humidity, ammonia, and adenosine triphosphate levels were recorded at regular intervals to gauge the effectiveness of cleaning methods, as well as to determine a threshold for cleaning that would minimally disturb the animals' environment without posing health concerns. An iterative process was utilized to upgrade the system from a hand-wash-only design to a rack-washable and autoclavable system. Researchers, animal care staff, and the attending veterinarian collaborated to produce new guidelines for the acclimation of animals to the system, criteria for removal, and standards for health checks. By analyzing environmental parameter data and maintaining open lines of communication among all stakeholders, the system has housed different cohorts of animals with varying experimental needs. Cages and tunnels were changed on a tri-weekly interval, and valuable behavioral and husbandry knowledge was gained from the arena and its burrows. By providing the animals a naturalistic environment that promotes social interaction, researchers can study behaviors and the neural mechanisms underlying them from a new perspective, while the vivarium continues to gain knowledge that can be applied to the construction of other novel housing paradigms in the future.

PS95 Dealing with A Flood in an Aquatic Facility: How to Keep Your Head above the Water

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When a vivarium is hit by a natural disaster, animal research becomes vulnerable to loss. The disruption that follows may halt or disrupt research projects over months or even years, since unique, irreplaceable, long-term animal models could be at risk. Therefore, catastrophic events require a coordinated and fast response to face the destruction and minimize the negative consequences, both to animals and to research projects. In December 2022, because of heavy rain fall, part of the IGC vivarium was flooded and three satellite aquatic animal rooms were affected. Most of the facility equipment was destroyed and zebrafish life support systems failed. To save the experimental animals, a prompt coordinated response was required, where the researchers and the animal care staff had pivotal roles as first responders. The animal rescue strategy included animal evacuation and shelter in place, following a priority list elaborated in the first hours of the incident. Coordination, communication, teamwork, and solidarity were essential to deal with disaster, from which we are still recovering 6 months later. Nevertheless, we were able to draw important lessons to incorporate in emergency planning and training.

PS96 Musings on the Microbiome: Does Water Delivery Source Make a Difference?

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Nuances of the gut microbiome have led to investigations of environmental parameters and their influences. To date, the water delivery method largely is underreported in animal research and is hypothesized to have an impact on microbiome. Water is typically provided to laboratory mice by reusable autoclaved bottle (RAB), by autowater mechanism (AW) from the housing rack, or by use of a single-use disposable plastic pouch (DPP). This study hypothesized that controlling water delivery source would positively stabilize gut microbiomes of mice following arrival from approved vendor to our facilities, within either immunocompetent (n=36 B6; 18M:18F) or immunocompromised (n=36 NOG; 18M:18F) genetic backgrounds over an 8-week study period. Mice were housed on a single IVC rack in sex-specific groups and provided with autoclaved caging/bedding and irradiated feed, while receiving one of three routes of reverse-osmosis, chlorinated water (8 cages per water source). Fecal pellets (n=2) were collected from each animal biweekly and water samples were collected from each cage weekly or from the AW rack for analysis of potential bacterial load. Animal care was provided by dedicated staff (n=3) that performed daily checks and changed cages biweekly. The results indicated that over the course of the study, water from ~11% RAB cages (7 of 63 samples) had bacterial detection and ~4% of water samples from AW cages (1 of 25 samples) had bacterial detection. No DPP water samples had detectable bacteria during the study. Shotgun metagenomics highlighted obvious shifts in gut microbiome in all groups over the course of the study, regardless of water delivery source, except for NOGs on RAB. No clinical concerns were reported, except one cage of fighting male mice; histologic examinations of gastrointestinal tracts and organs from representative mice (n=12) were unremarkable. While microbiome shifts ultimately stabilized within cage cohorts by 8 weeks, the changes in gut composition over time, despite strict control of husbandry elements, indicated that nuances in water delivery methods still result in microbial variability and should be documented in animal studies involving microbiome assessments.

**PS97 Neuroblastoma Cell Line Engraftment in 48 Hours Post-FertilizationPF Zebrafish Larvae**

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Neuroblastoma is the most common solid extracranial tumor in children, accounting for ~8-10% of all childhood tumors and ~15% of childhood cancer-related deaths. Zebrafish are commonly used for xenotransplantation tumor studies to evaluate therapeutic interventions, but little information is available regarding which neuroblastoma cell lines can be successfully engrafted in zebrafish larvae and if injection location plays a role. To evaluate the ability of BE(2)-C neuroblastoma cells to engraft in zebrafish larvae, two groups of five 48 hours post-fertilization (HPF) zebrafish larvae were microinjected with approximately 35 red fluorescent protein-labeled BE(2)-C cells into either the yolk sac or hindbrain. Larvae were imaged at Days 1 and 3 post-
injection via fluorescent microscopy to evaluate the presence of cells at the site of injection, size of tumor, and spread from the initial injection site. Tumor cells were present at the site of injection on Days 1 and 3 in both groups, indicating the ability of BE(2)-C cells to engraft at either site. BE(2)-C cells injected into the yolk sac were static in number without evidence of metastasis at Day 3, while cells injected into the hindbrain were static to increase in number with evidence of metastatic spread in one fish. These preliminary results indicate that BE(2)-C cells can successfully engraft in 48 HPF larval zebrafish when injected into the yolk sac or hindbrain, with a potential advantage to tumor growth and metastatic potential when injected into the hindbrain.

PS98 Age at Intravenous Administration of AAV9 and an Engineered Variant, AAV.CAP-Mac, Influences Transduction Efficiency in the CNS of C57BL/6J Mice

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Adeno-associated virus (AAV) capsids are used for central nervous system (CNS) gene therapy. Direct administration of AAV into the brain is invasive and often associated with local transduction. However, some conditions require widespread diffusion throughout the CNS; intravenous (IV) administration of AAV represents an attractive alternative. Newborn and juvenile animals are believed to have more permeable blood-brain barriers (BBBs), and age-dependent variation in neuronal transduction has been described with AAV9, the neurotropic gold standard, and an engineered variant AAV.CAP-Mac. We aimed to better characterize this phenomenon following systemic dosing in rodents. We dosed C57BL/6J mice (n=106) with AAV9 or AAV.CAP-Mac (5E13 genome copies/kg) IV. Animals were dosed once at P1, P9, P16, or P25 and sacrificed after 14 days. We hypothesized that the amount of vector and efficiency of transduction in the CNS would be highest in animals dosed at P1 and would decrease as age-at-dosing increased. Immunohistochemistry showed the greatest transduction of neurons and astrocytes in the brain of mice dosed at P9, supported by vector genome quantification, with both capsids. Following this peak, transduction of the brain decreased with increasing age of administration. Conversely, transduction of the liver (a potential site of AAV toxicity) by both capsids was lowest when dosed at P9. Superior BBB-crossing properties for AAV.CAP-Mac compared to AAV9 were not observed at any age. AAV.CAP-Mac demonstrated less neuronal and more endothelial transduction compared to AAV9, particularly at P16 and P25 dosing. Transduction of thoracic dorsal root ganglia was also lower by AAV.CAP-Mac compared to AAV9. Overall, our results demonstrate that CNS transduction via IV dosing of AAV9 and AAV.CAP-Mac is highest in young B6 mice when performed at P9. Because P9 in mice better correlates to the brain development of a newborn human than does P1, groups studying neonatal gene therapy with AAV9 via the IV route in mice to target the CNS should
consider dosing at P9 rather than P0-1. This would also allow for baseline assays and genotyping to be performed prior to dosing, more efficient injection technique and anesthesia, and less disruption of neonatal mice immediately following birth.

**PS99 Histocompatibility as A Function of Inbreeding in Miniature Swine**

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Pigs are potential organ donors for humans. Our laboratory is developing highly inbred lines of miniature swine to obtain animals that can provide donor organs to recipients of preparative regimens in which cells or thymi from other, histocompatible animals with different genetic modifications may be used to induce transplantation tolerance. The aim of the present study was to test the survival of split thickness skin grafts (STSG), exchanged without immunosuppression, between pigs from a subline bred to a coefficient of inbreeding (COI) of 92%, acceptance of which would represent a very stringent test for histocompatibility. Two 5-month-old pigs (one male 28 kg, one female 27 kg), each received one STSG from self and one from the other animal. Grafts were inspected daily for evidence of rejection from day 3 until day 28. The day of rejection was defined as the post-operative day on which less than 10% of the skin appeared viable, as judged by color, texture, and warmth to touch. For both pigs, the self-graft showed a normal appearance (pink, warm and soft) throughout the study. For the male pig, the allograft demonstrated hyperemia on day 9 but remained warm and soft to touch. The hyperemia resolved spontaneously by day 15, and the graft showed normal appearance for the remainder of the study. For the female pig, the allograft demonstrated more severe hyperemia starting on day 9 and continuing until day 21, at which point it darkened to purple, progressing to full rejection by day 25. Swine leukocyte antigen-matched swine from our herds that are not further inbred (COI \(\leq 60\%\)) reject skin grafts in less than 10 days, indicating that the survivals of both allografts were markedly prolonged. Since skin grafts are among the most difficult tissues for which to prolong survival, it is likely that organ transplants between these animals would be accepted indefinitely, as we have previously demonstrated for another highly inbred subline of our swine. In addition, this study revealed a sex-dependent difference in the histocompatibility of skin grafts, likely due to male-specific, Y-chromosome encoded antigens, analogous to the H-Y antigens, defined previously in mice and humans. This finding demonstrates the importance of including both sexes in animal research.

**PS100 Comparing Different Strategies to Reduce Hepatocellular Damage in Obese Common Marmosets**
Obesity is a common problem in captive common marmoset colonies (*Callithrix jacchus*), with institutions reporting up to 40% prevalence. Obesity can lead to hepatocellular damage secondary to hepatic steatosis and hepatitis. Reducing caloric intake is a common treatment strategy; however, it is unknown whether weight loss also corrects hepatocellular damage. In other species, hepatic damage is often treated with a combination of S-Adenosylmethionine (SAMe) and milk thistle extract, which supports liver function and repair. No published studies investigate using SAMe and milk thistle extract in marmosets. We hypothesized that pharmaceutical therapy (SAMe + milk thistle extract, or SMT) and caloric restriction, both alone and combined, would reverse hepatocellular damage in obese marmosets, with combination therapy reducing enzyme levels more quickly. Fifteen animals (nine males, six females) were randomized into three groups: 1) 12.5% daily caloric restriction (reduction from 45g to 40g feed daily), 2) 10mg/kg SMT PO SID, or 3) 12.5% daily caloric restriction and SMT. Subjects were adults with body conditions greater than 3 out of 5 and with elevated serum levels of the hepatocellular leakage enzyme alanine transaminase (ALT). Monthly bloodwork and weights were collected for 3 months of treatment and for 3 months after removal of pharmaceutical treatment. Across all groups, there was a significant effect of treatment over time on ALT ($p=0.036$). Evaluating each treatment alone, ALT was significantly decreased at 6 months compared to baseline in the SMT group ($p=0.043$). Combination therapy did not result in a faster reduction in liver enzymes. Liver biopsies were also collected from one animal in each group at baseline, 3-, and 6-months. All liver biopsies revealed glycogen hepatopathy, which remained consistent throughout the study except for the combination treatment animal who showed a marked reduction in glycogen deposition after 3 months of treatment. The results of this study support the use of both caloric restriction and SMT therapy in obese marmosets for reducing hepatocellular damage. Though all treatments are effective at reducing enzyme levels, the combination of caloric restriction and SMT may be necessary to reverse glycogen deposition in the liver.

**PS101 Effects of LED Lighting on Fecundity in C57BL/6 Mice**

GLAS: Yes
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Light emitting diode (LED) lighting is a new energy-efficient technology that is quickly replacing standard fluorescent lighting in animal research facilities as vivaria upgrade or are newly constructed. The impact of LED lighting on the health and welfare of research animals has not been thoroughly investigated. The goal of this study was to compare the effects of LED and
fluorescent lighting on fecundity of C57BL/6 mice, paired at 6-8 weeks old, over a six-month period under four conditions: standard fluorescent (10 pairs), low intensity (~half normal intensity) LED with instantaneous light-dark phase transitions (8 pairs), normal intensity LED with instantaneous light-dark phase transitions (9 pairs), and LED with gradual light-dark phase transitions (9 pairs). We hypothesized that there would be no difference in fecundity between these lighting conditions. All breeding pairs under all lighting conditions successfully mated and became pregnant during the study period. The production of the breeding pairs was not significantly affected by type of lighting or intensity as there were no significant differences between treatments in litter numbers (average 4.6±1.4 litters per dam), or litter size at birth or weaning (average 7.3±1.7 pups per litter). While pup weights at parturition were nearly identical across treatments averaging 1.4±0.04 g/pup, there was a significant effect seen with lighting treatment on weanling weight with the fluorescent control group having the smallest weanlings averaging 9.3±0.6 g. A higher than anticipated frequency of dystocia occurred during the study with significantly greater frequency in the normal intensity instantaneous and gradual phase transition LED treatments. The increased frequency of dystocia correlated with an overall significantly decreased survival of pups to weaning in the gradual phase transition LED treatment. For all treatments, only 6% of dystocias occurred in primiparous females, while 85% of dystocias occurred in multiparous females where the interval between litters was less than or equal to 25 days. The significant differences in weanling weight, dystocias and pup survivability between lighting conditions warrant further investigation as vivaria change to almost exclusive LED lighting.

**PS102 A Comparison of Fluorescent Versus LED Lighting on Reproductive Success in Laboratory Zebra Finches (Taeniopygia guttata)**

GLAS: Yes
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The zebra finch (Taeniopygia guttata) is an important animal model for biomedical research, especially in the field of neurobiology (e.g., auditory learning). However, limited evidence-based husbandry recommendations exist for laboratory zebra finches, including appropriate light sources. While fluorescent lighting is commonly used for captive avian species, it may negatively affect aspects of physiology and behavior. Light-emitting diode (LED) technology has been shown to be superior to fluorescent lighting for some laboratory animal species, such as mice and rats, but a study assessing the impact of LED lighting on zebra finches has not been published. We compared the effects of “daylight” spectrum fluorescent and LED lighting on the reproductive success of indoor-housed research zebra finches. We hypothesized that use of LED lighting would maintain or improve zebra finch fecundity compared to fluorescent lighting, demonstrated by improved hatching rates and hatchling survival. Over 26 weeks, 54 male-female
pairs housed in breeding cages under either fluorescent or LED lighting were monitored twice weekly for an average of 37 consecutive days. The number of days to produce the first egg of the clutch, maximum clutch size, percent hatching rate (eggs hatched per maximum clutch size), and percent hatchling survival to 11-days-post-hatch (11-dph nestlings per maximum clutch size) were recorded. Five pairs were excluded due to aggression or infertility. Results (n = 23–26 pairs per light source) showed no statistically significant difference in the timing of the first egg produced, clutch size, or percent hatching rate, but percent hatchling survival was higher in the LED group (mean 97.8% vs 88.5%; Mann-Whitney U test, p = 0.049). These results support our hypothesis, and additional studies are being performed with more breeding pairs to confirm our findings. Based on our results, we will provide evidence-based lighting recommendations, which will include specific photometric values, for zebra finches used in biomedical research.

**PS103 Effect of Tunnel Handling to Reduce Fighting in Aggressive Male Mice**

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Fighting in laboratory mice has long been a discouraging issue for animal research personnel. Separation of animals is often employed to resolve fighting, but increased cages and per diem rates can be frustrating for researchers. Tunnel handling has been shown to reduce anxiety when compared to tail handling in mice. Mice of a particular strain housed at our institution display aggressive behavior; we explored whether tunnel handling could reduce fighting incidence. Male APP+PS1 mice (n=81) were weaned at 4 weeks of age into 27 cages with 2 to 5 mice per cage. 14 cages contained a PVC tunnel, and 13 cages did not have a tunnel. All cages contained standard nesting enrichment including one shredded paper “puck” and one cotton square. Mice were monitored for 14 weeks and active fighting or fighting wounds observed by husbandry staff were reported to veterinary staff. Mice in cages containing a tunnel were strictly handled and moved during cage changes using gentle guidance into the tunnel. Mice in cages without tunnels were handled by the tail base. Five of thirteen cages without tunnels were reported for fighting; two cages had one instance of fighting and three had multiple reports despite separation of aggressor animals. Seven of fourteen cages with tunnels were reported for fighting, three with one report and four with multiple reports. The proportion of cages in the tunnel and no tunnel groups with no fighting reports at days 30, 60, 90, and 95 (final study day) were compared with no significant differences between the groups (all p>0.3). Similarly, the proportions of cages with multiple reports of fighting at the same time points were compared between the groups with no significant differences found (all p>0.3). Tunnel handling did not have an apparent benefit for reducing aggression in APP+PS1 male mice.

**PS104 Treatment of Ulcerative Dermatitis Restores Immune Cells to Homeostatic Levels in Mice**

GLAS: Yes
Ulcerative dermatitis (UD) is characterized by epidermal pruritic lesions of the upper body, enlarged spleens, and an inflammatory immune response affecting the widely used C57BL/6 (B6) mouse strain and mice on a B6 background. The underlying cause of UD is still not fully understood, although it has been shown that a high fat western diet potentiates the disease. Several studies have investigated palliative, but not curative, treatment for UD, but it is not known if mice treated for UD are immunologically like mice without UD. To determine the immunological profiles of UD mice treated versus untreated, we fed 120 B6 males and 120 B6 females high fat western diet (HFWD) to induce UD. Symptoms of UD started developing around 16-20 weeks after the start of HFWD. None of the 120 males developed UD, 16 of the females did develop UD. Once diagnosed with UD they were assigned into 3 groups: no treatment (4 females), toenail trimming (4 females), or topical application of triple antibiotic ointment containing bacitracin zinc, neomycin sulfate, and polymyxin B sulfate (4 females). Toenail trimming was done once a week and mice completely healed between 8-12 weeks. Topical triple antibiotic ointment was applied to lesions once daily for 8-12 weeks, but they never fully healed. Once fully healed (toenail trimmed) they were euthanized along with a control and a mouse with UD, and blood, lymph nodes, and spleen were harvested for multicolor flow cytometry analysis of immune cell composition and their activation status. After analysis we found that B6 mice with UD have altered systemic frequencies of neutrophils, monocytes, B cells, CD4+ T and CD8 T cells in Blood, lymph nodes, and spleen compared to unaffected controls. For the treated mice, toenail trimmed mice fully recovered from UD and their immune cell profiles were like mice with no UD. Topical triple antibiotic ointment mice never fully recovered, and their immune cell profiles had altered systemic frequencies. In summary, we found that mice treated with toenail trimming once weekly is a method to control UD, whereas topical antibiotic ointment was not. Once mice are completely healed, they have no significant difference in immune cell composition and activation status to that of a control mouse.