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Abstracts of Scientific Presentations

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Animal Welfare, Training, and 3Rs Posters

P1 Use of Doppler Ultrasound to Confirm Euthanasia of Nonhuman Primates in Maximum Containment

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When euthanizing an animal, it is important to confirm death before proceeding to necropsy or carcass storage. In maximum biocontainment labs (ABSL-4), common methods of confirmation, such as auscultation with a stethoscope or palpation of pulse, are not possible. Our previous approach was to use a pulse oximeter to monitor for loss of signal and observe cessation of respiration. In many cases, we would lose signal from the pulse oximeter prior to administering euthanasia solution, so this reading could not be used to confirm death. As an improvement to this approach, we decided to try confirming euthanasia using Doppler flow detector since absence of pulsatile arterial flow should more accurately represent death than loss of a peripheral pulse oximetry signal. To start, we used a portable Doppler device on sedated, noninfected nonhuman primates to verify accurate placement of the probe to hear a heartbeat. After practice outside of maximum containment, we used the Doppler device on moribund animals and also during end-of-study procedures on infected animals. While using the Doppler device simultaneously with pulse oximetry on all animals, we observed a longer duration of signal using the Doppler device. We were also able to determine that a heartbeat was present after cessation of respiration. While the majority of these comparisons have been in non-moribund animals, we plan to gather more data on moribund animals as they present to quantify further this improvement. In conclusion, we were able to confirm euthanasia more confidently with the Doppler device under maximum biocontainment conditions compared with our previous method of confirmation.

P2 Effectiveness of Self-assessments for Semiannual Inspections during COVID-19

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As a mechanism to maintain compliance with federal regulations and guidance while also considering personnel health and safety during the COVID-19 pandemic, the IACUC authorized the Office of Animal Research Support (OARS) to develop a "self-assessment" program for semiannual inspections and postapproval monitoring (PAM) activities. During most of 2020 and portions of 2021 and 2022, the Animal Resources Center self-assessed central rodent and aquatic vivaria and laboratory personnel self-assessed surgery areas and satellite housing in their laboratories, in lieu of the IACUC or OARS PAM staff. The self-assessment program resulted in a 74% increase in total findings per inspection in central vivaria and a 112% increase in findings per inspection in satellite housing facilities. Self-assessments of surgical laboratories resulted only 26% findings per inspection compared to the IACUC or OARS. Categories of findings were similar, regardless of the mechanism. We believe that a combination of targeted self-assessments and focused monitoring by the IACUC or OARS may improve investigator engagement in the inspection process and could lead to improved compliance in the long term.

P3 Method for Addressing Rodent Tail Entrapment in Cage Lids in a Multiuser Vivarium

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Rodent tail entrapment is an unfortunate byproduct of the frequent opening, closing, and docking of disposable cages in a busy research vivarium. It poses a direct health and welfare concern, and in severe cases can result in animal loss and the need for replacement study animals. After noting an increase in reported cases in our vivarium, we sought to identify possible causes and look for new methods of mitigation. Cage docking technique was found to be unsuited to less rigid disposable caging and proved to be a common cause of entrapment, paired with 'sign blindness' to previously posted visual aids. We were able to substantially reduce incidents by implementing the following: making improvements to our onboarding training in cage handling and docking, reducing sign fatigue by rotating visual aids, and by addressing this issue during regular retraining of staff and research personnel. In implementing the additional steps of new and updated training and the rotation of visual aids, we were able to successfully improve animal welfare by mitigating this issue.

P4 Rat Refinements: Housing and Handling Practices to Improve Welfare

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Standard housing may hinder species-typical behaviors and postures which could impact the health, welfare, and study outcomes of research rats. The objective of this study was to compare the behavioral and physical measures of rats in standard cages (C: 24.1 cm L x 45.7 cm W x 20.3 cm H; 733.9 cm^2; 3 rats per cage) and in modified primate cages (T: 81 cm L x 83 cm W x 93 cm H; 10,416cm^2; 5-6 rats per cage) while incorporating gentle handling to habituate rats to restraint. Seventy Crl:CD(SD) Sprague Dawley rats (34 males, 36 females; 5 wk of age) were randomly assigned to housing treatment. Rats were video recorded for 18 d. To habituate rats to restraint, all rats received 15 s of gentle handling 3 days/wk. At the end of the study period, rats were assessed for levels of anxiety (elevated plus maze (EPM) for 5 min) and response to humans (novel human approach test for 1 min) before and after restraint for blood collection. Blood glucose levels (mg/dL) were measured to assess response to restraint. Body weight (g) was also monitored throughout the study period. Duration (s) of general behavior and posture were scored daily during the morning active period for 10 min. Data were analyzed using linear mixed models. Treatment, sex, and time period were included as fixed effects and cage was the random effect. There were no weight differences between rats C and T rats ($P > 0.05$). There were no differences in blood glucose in response to restraint ($P > 0.05$) and no difference in latency to touch

novel human before or after blood collection ($P > 0.05$) between C and T rats, suggesting regular gentle handling was effective habituation for restraint regardless of housing type. T rats visited the open arms of the EPM more frequently than C rats ($P = 0.039$), suggesting less anxiety-like behavior. For general behavior, T rats were less inactive ($P < 0.0001$), spending more time on locomotion ($P < 0.0001$) and resource exploration ($P = 0.0003$) than C rats. T rats also spent less time in lying ($P < 0.0001$) and sitting ($P = 0.0006$) postures than C rats. The results of the study suggest that more complex housing is beneficial to rats, allowing more active behaviors and postural changes than standard housing. Future studies will explore the long-term effects of housing on rat welfare.

P5 Mice Just Want to Have Fun: Playpens Facilitate Faster Training in Noninvasive Techniques for Improved Refinement and Wellbeing

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There is a growing impetus to refine rodent handling and husbandry, including the use of environmental enrichment and noninvasive scientific techniques, both to improve upon animal welfare and to ensure the reproducibility and translatability of preclinical results. Mounting evidence in multiple laboratory animal species indicates that providing intermittent access to playpens enhances generalized wellbeing. Moreover, studies in rodents have illustrated playpens are rewarding as they allow for the expression of natural behaviors, and induce a positive affective bias, which enables lower emotional responses to aversive stimuli; however, if playpens themselves can serve as a training tool has yet been investigated. We examined whether mice given access to playpens could be trained more easily than those kept in standard rodent housing, specifically on the task of oral vehicle administration (0.1 mL of commercially available chocolate drink mix), which serves as a noninvasive alternative to gavage. C57BL/6 (n=3) and Swiss-Webster (n=3) male mice were provided twice weekly access to a playpen containing deep cellulose bedding, chocolate chips, plastic hut and tubes, nesting materials, tissues, and a toy. All mice exhibited a wide array of natural behaviors in the playpen (e.g. burrowing, foraging, nesting, climbing), thus confirming via proxy indication its positive benefit. Following acclimation to the handler, vehicle, and syringe, we noted the frequency to consume the vehicle within a maximum of 10 session whilst cupping the mouse in one's hand versus administrating directly in the playpen. Only 1 mouse learned to consume the vehicle by hand, requiring 3 sessions; however, all 6 mice readily consumed on the first attempt in the playpen. Ultimately, our preliminary results demonstrate that using playpens for training substantially expedites the overall time required for mice to learn a novel task, which may incentivize a greater willingness for rodent users to employ alternative techniques, and simultaneously promote increased animal welfare.

P6 Using Microphysiological Systems to Improve Translational Neuroscience Research and Support the Replacement of Animal Testing

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Currently, translational research of neurodevelopmental diseases (NDDs) remains challenged by a dearth of valid preclinical animal models and poor reproducibility of studies. Indeed, the NIH estimates that between 80-90% of research fails to enter clinical trials, with attrition rates even higher for neuroscience. Given that NDDs comprise complex etiologies and uniquely human symptoms, establishing animal models that recapitulate the essential components relevant to the disease often undermines validity and success. Thus, human relevant models for NDDs are greatly needed, both to better understand the underlying molecular mechanisms, and more effectively guide drug discovery for efficacious treatments. Currently, microphysiological systems (MPS), including organ-on-chip and 3D organoid technologies, hold the potential to overcome the barriers of animal models and improve translational research while also serving as a replacement model. Such systems have already been successfully implemented to expedite drug discovery and clinical trials. Our lab was one of the pioneers in developing a human induced pluripotent stem cell (hiPSC) derived cerebral organoid model specifically for use with NDDs, which is marked by high uniformity, standardization, and reproducibility so to enable high throughput assessment of neurotoxicant characterization. Additionally, we have successfully enhanced our model through the co-culture of hiPSC-derived microglia, thus encapsulating all major cell types within the brain and further increasing its clinical relevance for NDDs especially with an aberrant neuroimmune link. The development of an immune competent system allows investigation into microglia-dependent processes such as synaptic pruning, apoptotic cell debris clearance, and the production of cytokines. As these processes are crucial to normal neurodevelopment, creating an MPS in which to interrogate their role in the context of disease emerges as an invaluable tool. In summation, the incorporation of MPS, whether as a complete replacement method or as a complement to animal studies, can significantly improve the translational value of NDD research.

P7 Experiences with Employing Positive Reinforcement Training for Vaginal Swab Collection in Rhesus Macaques (Macaca mulatta)

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Positive reinforcement training (PRT) was used in rhesus macaques to facilitate collection of voluntary daily vaginal swabs to reduce required sedation events, as such frequent events have been shown in the literature to have a negative impact on welfare and exacerbate stereotypical behaviors. Here, daily voluntary vaginal swabs were desired as a noninvasive technique to track menstrual cycles of females in a study of HIV sexual transmission that included investigational oral drug administration. Few resources were found in the literature documenting shaping plans for macaques for vaginal swabbing, therefore a shaping plan was subsequently developed in the facility. Twelve animals were identified by the investigator for possible use on this project. Duration for training for the desired behavior was limited, and formal temperament testing was not performed. Consequently, 4 animals were deemed as poor candidates due to highly inhibited temperaments incompatible with end goals of the study (daily menstrual cycle tracking and/or drug administration in a treat taken from hand). Of the remaining 8 animals, 4 were successfully trained for voluntary daily swab collection, 1 did not complete training but successfully underwent drug treatment, and 3 were used as controls because they would have required extensive desensitization to hand treating before beginning PRT, which was impossible within investigator-driven time constraints. Duration to successful training completion (defined as voluntary rump presentation and cooperation with daily swabbing) ranged from 4 wk to more than 4 mo. This was consistent with the natural discrepancy in animal temperament and fitness for training as previously documented in the literature. Going forward, a proposed pool of animals should be evaluated for temperament prior to enrollment on such studies, and highly inhibited animals should not be considered for this procedure. These results highlight the importance of communication between investigators and animal facility staff prior to study initiation.

P8 Using Cage Ammonia Levels to Validate the Change Interval in Individually Ventilated Caging System

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We conducted a study to validate a 14-d cage change interval, with the goal of reducing disruption to the animals while still maintaining the highest-quality husbandry. A GM460 gas monitor probe was inserted through the water bottle port on the cage lid to measure cage ammonia levels. The animals were housed in individually ventilated cages, on corn cob bedding, on a 180-cage double-sided rack filtered at the rack level set to 75 air changes per h. Cages were left on the rack during testing to ensure uninterrupted air flow. To prevent cross contamination from the probe, individually wrapped disposable straws were slipped over the gas monitor probe before measuring ammonia levels for each cage. The cages were measured on day 0 and Day 5-14. Five cages of males and 5 cages of females consisting of 3-5 C57BL/6J mice varying from 4-9 mo in age were tested. It was found that cages containing 3 mice stayed under 50ppm, and cages containing 4 or more mice resulted in ammonia levels exceeding 50 ppm by day 10. As a result of the studies, the cage changing frequency for cages with 4 or more mice was set to 10 d. With this cage-changing schedule, we are confident that our mice are in a safe living environment.

P9 Canine Training: It's Not Just for Kicks and Tricks Anymore!

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Having a dog struggle during a dosing event, bleeding event, or even just while trying to capture a body weight or observations can not only have a negative impact on the technical staff, but can also result in poor data congruent to the stress incurred. After taking a look at the reasons for why a dog might struggle, it was determined that a habituation program could greatly reduce the number of dogs that struggle during the aforementioned events and could also help to provide better data as a result of the reduced stress. We developed a program that would not only train the dogs to the various tasks that we require of them for day-to-day events, but also would allow us to evaluate them and their eligibility for study work. Over a 4-wk period, we trained them to separate and sit for initial restraint, sit and stand on the table for various events, and to allow us to manipulate them for various other techniques and restraint methods. Our training methods included both verbal and visual cues for the dog with the sessions building up on each other (i.e., learning to separate prior to sitting, learning to sit immediately following separating, etc.). During training, the dogs were evaluated for social compatibility, human/dog interactions, cooperation, and compliance. The evaluation process allowed for determination of study fit dogs that would be willing coworkers in the laboratory setting. Throughout the program it was observed that a trained, or habituated dog, was far more likely to comply and allow for various restraints and techniques than a naïve, nonacclimated dog.

P10 Nonhuman Primate Enrichment Evaluation Project (NHPeep): An Exploration into How NHP Enrichment Encourages Natural Behavior and Reduces Stereotypy

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Stereotypy is something that every institution deals with when it comes to nonhuman primates. Industry wide there is a struggle to find the right treatment, the right environmental enrichment, and the right cage mate to alleviate some of the issues presented. When looking at the current procedures and treatments for stereotypy, a new approach seemed to be needed to tackle the problem at the source. At our facility we use a committee to look at suggested enrichment items, where item cost, animal welfare concerns, and feasibility are carefully considered. We wanted to take it a step further and get the animals input on the devices they would use. We developed a scoring system with ratings from 1 to 5 that looked at 5 different areas to help to determine if a device was worthy of consideration for use in our facility. We looked at percentage of time used, wear and tear, mastery, behavioral changes, and impact on social dynamics. Using animals with good, established social pairs or trios, including individuals with stereotypy and without, both facility-accepted toys, as well as new enrichment devices were scored to determine their viability at the facility. In a short amount of time, it became evident that the new enrichment devices were received with greater appreciation by the animals than the older, established toys. It was also noted that the older devices were viewed as a part of the cage, rather than enrichment, whereas the new devices offered opportunities for foraging, game play, brain exercises, and naturally allotted behaviors. While some of the new devices failed to live up to our expectations, others far exceeded them. This program shined light on new ideas that would direct us to change the types of devices used, as well as how often they were given and changed for the nonhuman primates' wellbeing.

P11 Snuggle Your Bunnies! An Alternative to Manual Restraint for Rabbit Gavage, Intravenous Injection, and Ear Vein Blood Collection

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Restraining a rabbit for various dosing routes and blood collection can be difficult with a myriad of items needing consideration. These considerations include the animal's fragility, the animal's ability to kick and injure technicians, technician safety, and animal welfare. All of these items need to be carefully calculated when looking at a manual restraint method for rabbit dosing and blood collection methods. For years we have used a manual restraint method that was effective in restraining the animals for gavage dosing, but in recent years it has been correlated to ergonomic injuries to technicians, which has caused strains on resources due to time off, restricted numbers of technicians to restrain, and the financial cost due to workman's compensation and associated healthcare fees. We began to search for a new way to effectively restrain the animals safely, while also helping to reduce the injuries and stress added to the technicians due to such a physical restraint. The method that we settled on uses a mechanical restraint that will wrap the animals in a way that they cannot easily react in a physical manner, protects technicians from kicking, and allows for the technicians to have a more relaxed grip on the animal's head for the various tasks directed by protocol. While initially looking to reduce injury and stress to technicians, we also found that this mechanical restraint device also served to replace other restraint devices used for intravenous injection and ear vein blood collection. This not only helped to reduce the strains incurred from the manual restraint, but also to help to keep the animals calm by making them feel secure and restrained further reducing their stress while being dosed or having blood collected.

P12 Refinement of Weight Algorithm Method for Pregnancy Detection in Mice

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Research involving mouse embryonic or perinatal development typically requires mice at defined developmental stages. The use of weights for pregnancy detection is an effective method to reduce false positives from being used in studies. Previous published data was based on inbred strains. We implemented a weight-based algorithm for transgenic colonies and observed false negative rates as high as 26% for pregnancy detection on gestational days 7.5 and 8.5. The purpose of this study was to focus on the 3Rs by refining our current algorithm to improve pregnancy detection and lower the false negative rate. We examined weight gain in a population of 394 transgenic mice from 21 different colonies on various backgrounds, between the time of plug detection and gestational ages of 7.5 and 8.5 d. The colony animals used in our study were divided into 5 groups from initial weight at the time of plug detection: less than 17g, 17-19.75g, 19.76-21.5g, 21.6-25.5g, and more than 25.5g. Using refined weight gain scale thresholds, we were able to track trends within groups, as well as overall pregnancy determination for the population. Out of 394 females weighed after a copulation plug, the false negative rate lowered to 16% while the false positive rate only increased 0.5% using the updated methods. This refinement process offers overall improved pregnancy detection rates (9.4% of increased correct calls to 77.4%) at 7.5 and 8.5 d gestation. This helps with an efficient transfer of pregnant animals to investigators for research, as well as retaining nonpregnant animals to use for future timed matings improving animal welfare.

P13 Biomedical Research and the Roadmap to Resiliency: Can You Become Resilient?

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Laboratory animal professionals (LAPs) include all persons involved with animal research at any level. Such professionals experience many positive and rewarding interactions when caring for and working with research animals while contributing to scientific and medical advancement. However, these professionals may also experience conflicting feelings and exhaustion when the work is stressful due to limited resources, making end-of-life decisions, dealing with conflicting priorities, and their own capacity for emotional investment in the human-animal bond which is characterized as compassion fatigue. The first step to becoming resilient against compassion fatigue is learning how to take care of oneself. The Roadmap to Resiliency with its signs, detours, and destinations, is presented as a guide to lead LAPs on a path towards resiliency. The authors share successful strategies to evoke or bolster resilience in the following six dimensions: physical, interpersonal, emotional, cognitive, behavioral, and spiritual. The Roadmap to Resiliency will map routes to physical health through multiple self-care strategies, emotional well-being through interpersonal connections, mental satisfaction through the discussion of ethics, as well as suggest specific action plans on how to safeguard mental health in this line of work. By providing a multimodal approach, LAPs can benefit from these step-by-step resiliency strategies to maintain personal health. The objective being that they may be able to function more effectively and gain satisfaction in their essential work of caring for animals, humans, and for science. Can one become resilient? The Roadmap to Resiliency will serve as a training tool/resource to enhance the LAPs level of resilience by helping them to cope with the past to better their futures.

P14 A Tale of 2 Tails: Comparing Partial versus Full Tail Amputations in Spiny Mice (*Acomys sp.*)

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Spiny Mice (*Acomys sp.*) are a relatively new model used in biomedical research, and are of particular interest due to their unique ability to regenerate skin and other tissues without the formation of scar tissue. Impressive still is their ability to regenerate all parts of their skin, down to hair follicles and glands. As a social species, the default standard is pair or group housing. Studies have shown that chasing, not fighting, is the primary form of aggression, however fighting still occurs. A common site of injury is the tail. Injury may be mild or severe to the point of vertebra exposure; which necessitates amputation. Animals observed in the wild frequently lacked tails, and this loss did not appear to affect fecundity. When amputations are required, all attempts are made to conserve tissue, where possible. Early experiences taught us that partial tail amputations often resulted in repeat injury to the tail from either self-mutilation or conspecific traumatization, warranting further amputations. Moving forward, to avoid multiple amputations in the same animal, full tail amputations have become the recommended approach at our institution. Two years' worth of surgical retrospective data was analyzed to determine the average time to case resolution in partial versus full tail amputation. Partial tail amputations typically required additional amputations as tails became further traumatized. On average, it took 30.6 d from the first amputation to case resolution. By comparison, a full amputation approach had a mean case resolution time of 15 d. Full amputation resulted in quicker operation times, less surgical site complications and faster healing times. In the interest of animal welfare, a full tail amputation should be considered over a partial tail amputation, and is here recommended as a standard approach.

P15 Characterization of Food Grinding in a Population of Sentinel Mice

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Food grinding is an abnormal behavior seen in laboratory rodents characterized by the chewing of food pellets into very small pieces or powder with little or no ingestion. This behavior creates excessive food waste known as orts that can have multiple effects on the cage environment impacting both the animals and husbandry staff. The increased amount of material in cages negatively impacts the microenvironment and can lead to clogged lixits, cage flooding, and more frequent cage changing. The exact etiology for increased food grinding behavior in mice remain unclear and has been postulated to be stereotypic or compulsive. We aimed to better characterize the factors that contribute to food grinding behavior in our mouse colony. We sampled 6,898 cages including 102 sentinel mice cages from multiple vivaria across the campus. Results of our study showed that 7% of investigator cages had evidence of food grinding, while 79% of sentinels demonstrated food grinding ($P < 0.0001$). At the cage level, we saw no apparent difference in sentinel mice food grinding behavior with different bedding or food type. A majority sentinel cages (62%) had 2 or more forms of enrichment (only 25% of colony cages had 2 forms), suggesting that number of items alone is not sufficient to mitigate this behavior. We found that sentinel mice in ventilated caging had higher rates (87%) of food griding compared with those in static caging (23%, $P < 0.0001$). Furthermore, the density of cages on which the rack which the sentinel was housed did not appear to have an impact on food grinding. Understanding the factors that gives rise to this behavior has potential to improve the husbandry and welfare of sentinel mice.

P16 Improving Animal Welfare Assessment by Means of Electronic Score Sheets

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Reliable animal welfare assessment is of paramount importance to enable experimenters to evaluate the health status of each experimental animal ensuring that potential harm or suffering is minimized. Commonly this goal is achieved by thorough clinical observation and documentation on classical score sheets on paper or data file. For longitudinal studies, this may result in a large amount of data difficult to evaluate clearly. Based on classical score sheets a powerful, flexible electronic tool for scoring mice in surgical cardiologic intervention was developed. This module consists of a data sheet template and a report tool integrated within a commercially available animal management software. Scoring schemes can be entered in advance during experimental design. The template allows online mapping of individual score sheets in the animal room. During clinical examination, the experimenter is guided through the scoring process by clicking several selection fields before confirming sum score value, from which severity grade and treatment measures for animals are derived. This easy-to-use IT tool allows fast and reliable data collection with automatic reminder function via email or personal task list. The electronic score sheet improves documentation and offers multiple report functions for single animals, experimental groups at one time or over the course of the study. This enables principal investigators, animal welfare officers, clinical veterinarians, and IACUC members as well as the veterinary authority to gain a fast overview. Apart from being convenient it offers an effective control capability. Handwriting mistakes are avoided, and information can be exported to different file formats on demand making them easy to read, archive or send to other stakeholders. Overall, this electronic score sheet greatly improves animal welfare evaluation of experimental animals and the transparency of scoring processes.

P17 A Program to Address Compassion Fatigue in a Large Contract Research Organization

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Compassion fatigue, sometimes called empathy burnout, is a common occurrence in veterinary medicine and laboratory animal science. Few, if any, workers in this industry have not been impacted by this condition either directly or indirectly through coworkers and colleagues. Here, a two-pronged approach was taken in attempt to address compassion fatigue among the technical staff. First, specific focus was placed on addressing how animal euthanasia contributes to compassion fatigue in the lab environment. New hires were acclimated through implementation of new training materials and exposure to the pathology department prior to participating in euthanasia in the lab. Second, a companywide program, termed the "culture of care," was introduced to staff to highlight enrichment and research improvements throughout the company as well as to celebrate the successes of in-house staff in pursuit of enhancing animal welfare. Feedback was collected from technicians participating in these training activities and used to assess the effectiveness of the trainings. The site is using this feedback to tailor more effective trainings designed to combat compassion fatigue as well as implement cultural changes throughout the organization that can contribute to the reduction and management of compassion fatigue in staff throughout animal operations.

P18 Handle with Care: Effect of Acclimation of Rats to Frequent Handling on Stress Behaviors and Task Efficiency

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In the animal research field, we are constantly looking for improvements to animal models by exploring new methods and practices that enhance comfort and decrease stress on our animals. One of the ways to achieve this goal may be through more frequent, noninvasive human contact and handling. Our research aimed to collect data supporting the hypotheses that more regular handling of rats, prior to the commencement of a study, would result in less stress behaviors displayed by the animals and more efficient technical execution for our scientists. To test these ideas, we performed a 3-wk study in which 26 Sprague Dawley rats were separated into 2 groups; 1 group that was handled daily by a technician, and the other not handled at all (save for cage changes). Before randomization, each rat was tested for baseline stress behaviors observed during subcutaneous (SC) injection and tail bleeding (TB) procedures, and each task was timed. The stress behavior variables measured were audible vocalizations, escapes during the procedure, and tail spinning. After the 3-wk handling period, each rat was tested again to examine any differences in the stress behaviors between the handled and control groups, as well as the efficiency of the task. The handled group presented significantly less escapes during TB, as well as significantly less vocalizations during SC dosing than the nonhandled group on day 21. Additionally, the nonhandled group showed significantly more escapes during TB on the day 21 test than the day 0 test. This effect was not seen in the handled group. There was no significant effect on the time taken to complete each task. In summary, the handled group demonstrated a decrease in stress behavior after more frequent handling and these data signify a promising step toward improving the welfare of our rats.

P19 Comparison Study Employing Conventional Blood Collection Methods and Microsampling Techniques in Cynomolgus Macaque: A Progressive Approach to Sample Collection

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The purpose of this study was to evaluate the suitability of whole blood microsampling procedures in NHPs to support toxicokinetic assessments of biotherapeutics in NHPs. Application of microsampling procedures reduces total blood volumes required for analysis and improves animal welfare during the collection process in support of the 3Rs. A 1-mo single dose intravenous pharmacokinetic (PK) study was performed in male cynomolgus monkeys with a human IgG1 control monoclonal antibody (mAb) as a surrogate biotherapeutic. Whole blood for serum sampling was collected via femoral vein using the vacutainer method. Whole blood for plasma microsamples were collected via saphenous vein, lateral heel or tail vein using a commercially avaiable collection device and a positive displacement pipette for comparison. The drug concentrations from all sample types were determined using a quantitative ligand binding assay (LBA). The PK parameters obtained from all samples were examined using a standard PK analysis method. PK parameters from both sample types were analyzed statistically and compared. Similar profiles of drug concentrations versus timepoints from all sampling procedures were observed. The correlations of PK concentration data obtained from serum and plasma microsamples were ≥ 0.97 using Brand Alman Plot analysis. Key PK parameters obtained from plasma microsamples were similar to those obtained from conventionally collected serum samples (the % differences of mean PK parameters obtained from both sample types were within ±25%). This study confirmed that PK parameters obtained from samples using microsampling were similar to that of conventionally collected serum samples in cynomolgus

monkeys. Therefore, the microsampling procedures described can be used as a substitute for conventional sampling procedures to support PK/TK studies of biotherapeutics in nonclinical product developments.

P20 Institutional Officials Consortium: An Industry Consortium for Collaboration in Animal Welfare, the 3Rs, and Openness about Biomedical Research

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Animal care and use programs in industry (pharmaceutical and biotechnology companies, contract research organizations, breeders of animals needed for research) face many of the same challenges and opportunities. While industry organizations could be reticent to share best practices, optimizing animal welfare, advancing alternatives and the 3Rs, and communicating to the public why and when animals are still needed for the discovery and development of new therapies is pre-competitive. In this spirit, the Institutional Officials Consortium (IOC) was created as a formal organization where industry peers in animal welfare can work together to support and synchronize programs ensuring ethical engagement with animals in pharmaceutical and biomedical research, highlight alternatives to animal research and support implementation of the 3Rs principles, be public and factual around research within our organizations and communities, broaden public understanding about the benefits and necessity of animal-based research, and to align support for pro-research advocacy organizations. Each strategic priority is associated with a working group. IOC holds biannual meetings to assess progress on these goals by each working group, to provide strategic input to research advocacy organizations, and to assess deliverables. Among many deliverables from the working groups in 2016–2021 are strategy tools for animal rights challenges, guidelines for implementing an Animal Welfare Office, benchmarking and communication platforms, recommendations for internal communication on animal-based research, an annual summit for communications, public relations, and government affairs professionals at member organizations on best practices, and 2 new public websites on the direct benefits of research to animal and human health. IOC member companies benefit from a network to benchmark challenges from activists to 3Rs activities, access to exclusive materials on encouraging a supportive research environment, and participation in setting recommendations and building resources for organizations and communities on positive, thoughtful, and open sharing within this topic. The IOC welcomes the opportunity to engage in open dialog through benchmarking and best practice sharing for all research settings.

P21 Surveillance of Plasma Fentanyl Levels Achieved with Transdermal Patches in Surgical Sheep (Ovis aries) Models

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Appropriate analgesia is an essential component of invasive, surgical models in sheep and often requires continuous administration of opioids. Transdermal fentanyl patches (TFP) are a user-friendly and low-stress method of continuous opioid administration when applied at least 12 h prior to a painful procedure. However, their efficacy in research settings may be dependent upon several factors including breed, placement technique, procedure, environment, and individual variability. Therefore, we conducted surveillance for the ability of fentanyl patches used as part of multimodal analgesia to achieve generally accepted, therapeutic plasma concentrations (0.6 ng/ml) in sheep. A total of 18 male and female sheep used by 2 experienced research groups were studied. Seven miniature sheep (13-18.5 kg) undergoing artificial lung implantation had 50 µg/hr TFPs applied to the antebrachium or dorsal scapula for an average dose of 3.47 µg/kg/hr. Additional analgesia included local lidocaine blocks, 0.01 mg/kg intramuscular (IM) buprenorphine, and 1-2 mg/kg intravenous (IV) flunixin meglumine at the time of surgery with redosing as needed postoperatively. Eleven Polypay-cross sheep (46.3-53.2 kg) in an orthopedic implant study had 75 µg/hr TFPs applied to the dorsal neck for an average dose of 1.54 µg/kg/hr. In addition, flunixin meglumine (0.5-1.0 mg/kg, IM) was administered every 24 h for 48 h following surgery. Blood was collected from all sheep prior to TFP application and 24 h following application but prior to surgery. Plasma fentanyl levels were analyzed using a validated ELISA kit. There were no statistically significant differences in the mean 24-h postpatch plasma fentanyl levels based on research group, fentanyl dose, or sex. However, 3 out of 7 miniature sheep and 2 out of 11 Polypay-cross sheep failed to reach ≥ 0.6 ng/ml plasma fentanyl by the time of surgery. In addition, 1 miniature and 1 Polypay-cross sheep had levels that exceeded the upper therapeutic range (≥1.5 ng/mL). Further investigation revealed some differences in application technique between the research groups, but this did not fully explain the low fentanyl levels found in individual sheep within each group. These results suggest that TFP in sheep may not always achieve therapeutic levels within 24 h and should be used in multimodal regimens to ensure optimal analgesia. Overall, the efficacy of TFP may be easily assessed with commercial ELISA kits and it is worthwhile to perform periodic surveillance of fentanyl levels to optimize analgesia in sheep.

P22 Rearticulation of Skeletal Remains as a Tactile Training Instrument

Withdrawn

P23 Gnotobiotic Mice Welfare at a European Facility

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European and national legal requirements consider that animal welfare should be given the highest priority in the context of animal keeping, breeding, and use. All establishments should assure animal welfare by performing improvements in housing, husbandry, breeding, and care. Our mouse facility aims to establish and maintain an appropriate culture of care among the animal users community, ensuring the best practices and following all the available recommendations on mice welfare. A germ-free/axenic service is available since 2005 for production and maintenance of germ-free mice, which expanded in 2013 into a gnotobiology facility for gnotobiotic related research. No specific or detailed recommendations for gnotobiotic mice welfare are available, apart from the general ones for rodent facilities, which lead us to develop our own response for situations inherent to gnotobiotic housing or husbandry conditions. Since 2009 we have changed to yellow-colored bottoms (opaque to mice) and cage lids, changed the bedding from paper to corn cob, have been using different nesting and environmental enrichment materials according to cage type (breeding, stock, experimental), diminished the exposure to light and noise inside isolators, and refined our protocol of fostering fetus inside an isolator for germ-free derivation, avoiding foster mother stress and nest disturbance. An acclimatization period is also in place when transferring mice from our production isolators to the experimental positive pressured individual ventilated cages. In these cages, mice are more exposed to external noise than in isolators, besides the normal stress related to mouse transfer. By changing simple elements, we have achieved better breeding performances, higher adoption rates on germ-free rederivations, and have greatly decreased the stress of experimental animals. Better welfare for sure means high gnotobiotic standards and a general good research outcome.

P24 Harmonizing Animal Care and Compliance in a Large Decentralized Federal Research Program

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The National Institutes of Health's (NIH's) Intramural Research Program (IRP) portfolio includes more than 2,100 active protocols that involve animals. This work is performed by scientists at 24 institutes and centers (ICs) who have access to more than 1,000,000 ft^2 of animal housing and procedure space in over 75 facilities in 3 states. Program management and oversight is the responsibility of 20 Animal Care and Use Committees (ACUCs), 18 animal program directors (APDs), and an animal care support staff of over 1,000 skilled individuals. Our office contributes to the success of this decentralized organizational structure by advocating global strategies to ensure compliance with regulations and accreditation standards and enhance the humane treatment of research animals across NIH while maintaining an appreciation and respect for the unique differences between institutes. Core functions are primarily performed through advisory committees and specialized services focused on (1) maintaining effective communication networks within and outside NIH; (2) coordinating the development of cross-cutting institutional policies and performance standards; (3) providing quality training and educational opportunities; (4) negotiating inter-agency collaborations and agreements; (5) administering quality improvement programs; (6) promoting regulatory compliance; (7) navigating worker safety and facility maintenance challenges; (8) managing accreditation and external oversight assessments; and (9) cultivating a robust culture of care by raising awareness for the pivotal impact that animal research has on scientific discovery. We summarize current strategies in maintaining autonomy of the 20 IC animal programs within the framework of the overarching NIH IRP animal program.

P25 Design and Evaluation of an Artificial Intelligence Model for Automated Tail Vein Administration in Rodent Models

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Drug injection via the tail vein is an essential biological tool for animal experiments modeled on rodents. However, since tail vein administration is done by humans, it takes a lot of time and cost for an unskilled person to obtain accurate experimental results. In response to these problems, we propose a Vision machine learning technology of a Vessel-constraint network (U-Net) that uses tail vein information with infrared (730nm, 840nm) light emitting diodes. The artificial intelligence (AI) model was designed by training a venous vascular imaging library of rodent tails using a U-Net model. U-net is a powerful tool for biomedical image segmentation, such as recognizing blood vessels in tissue via Convolutional Neural Networks (CNNs). It is a high-precision V/T (vessel/tissue) classification model based on data fusion. Based on the learned U-Net and AI model, we introduced an automatic administrator that uses a camera to determine the ideal position of the needle based on recognition of the venous blood in the rat tail. It is composed of an attached anesthetic delivery device and collaborative robot that moves the needle to the correct insertion position. A terminal study was performed using randomized Sprague-Dawley (SD) rats at 5 wk of age to confirm accuracy of the robot. The control group (n=10) used standard injection techniques and the experimental group (n=30) used the robot for automated injection. Each group was anesthetized with isoflurane (3cc/min) prior to injection and 2% Evans blue dye was used to confirm injection accuracy. The tail vein was correctly recognized by the device in all rats in the robot injection group and the positional accuracy between animals was improved dramatically with an injection error range within 0.01mm. The overall rate of successful intravenous injections rate was 90±5%. These results indicate that U-Net-based AI technology is a cost-effective and convenient method for researchers to easily administer tail vein injection to rodents and can reduce the stress and sacrifice of experimental animals.

P26 Refinement of Handling and Dosing Methods for Rats and Mice

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Many scientific procedures involving animals require the administration of test substances. This almost always requires the animal to be restrained which is known to cause stress. Our research group has developed and implemented several techniques to reduce the stress caused during substance administration. Oral dosing of rats and mice is commonly carried out using an esophageal cannula. This procedure is distressing for the animals requiring physical restraint. There are also risks of incorrect placement, tracheal dosing, and esophageal trauma. An alternative approach is to use voluntary ingestion of test substances in palatable solutions. This approach has previously been reported by several research groups, but is not widely used, and it is not clear if this is due to a lack of awareness or whether researchers have encountered problems using this approach. Our experience has been that rats and mice readily take palatable solutions from a syringe but can quickly form negative associations with ingestion of some drug solutions which arises from conditioned aversion, where any aversive effects of the drug become associated with the palatable solution. To reduce the potential for this to develop, we have developed a modified protocol. Using this approach, we have administered a wide range of psychiatric drugs without issue. Although the approach will not be compatible with all test substances, this method offers both welfare and scientific benefits and could reduce variability between subjects and improve the quality and reproducibility of scientific studies. For the purposes of injections, we have modified our handling techniques for rats and mice to eliminate the need for "scruffing" in rats or tail handling in mice prior to injecting the animal. We have been able to use objective measures of affective state and the stress response to show that these methods offer significant improvements in welfare. Importantly, these techniques have been taught to and used by researchers with a range of prior experience levels and all have demonstrated competency within a short period of time. These methods offer both welfare and scientific benefits and could reduce variability between subjects and improve the quality and reproducibility of scientific studies.

P27 Precision Cut Lung Slices: A Versatile 3R Method for Pulmonary Research

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In recent years, the paradigm that in vivo experiments are the gold standard for translatable basic and clinical research has changed. Validation of alternative experimental models that regard concepts like the 3Rs is increasingly being pursued, and methods like organ-on-a-chip, organoids, and complex in vitro cell models are being developed and refined constantly. For pulmonary disease modeling and pharmacology/toxicology studies however, many of the alternative models still lack the ability to mimic the complex

structural make-up and functional mechanics of lung tissue. A more suitable alternative model are precision cut lung slices (PCLS). This ex vivo method features viable slices of lung tissue which retain the 3-dimensional structure of the lung, comprising all resident cell types and maintaining parenchymal functionality. Animal involvement is reduced to harvesting of lung tissue, as the slices serve as the actual experimental sample. Total animal numbers are decreased due to a high yield of technical replicates that are generated. Unique endpoints can be examined in PCLS, that are challenging to analyze in vivo or in vitro. Insights on immediate functional (airway responsiveness and ciliary beating), cellular (viability, metabolism, immune responses) and structural (histology, immunohistochemistry) changes connected to pulmonary disease or responses to pharmakons/toxicants can be provided. Slices can be prepared from animal or human lung tissue, which contributes to the refinement and even replacement of animal experimentation, but also enhances the translatability of results. Current literature demonstrates the use of PCLS for investigation of immune responses of resident lung cells after challenge with immunoactive compounds, assessment of genotoxicity and evaluation of toxicity of a broad range of chemicals. PCLS were also used to investigate the onset of fibrosis, pathogenesis of asthma/COPD and other pulmonary diseases. Here, the PCLS model will be showcased, and examples for endpoints and its use in pulmonary research will be presented.

P28 Location Matters: Data Logger Implantation in Nonhuman Primates

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Implantable devices capable of measuring and recording physiologic parameters such as temperature, heart rate, electrocardiogram (ECG), and activity can be useful tools in laboratory animal research in support of the 3Rs through reduction of animal stress for regular collection of physiologic measurements without human interaction. Data loggers were explored as alternatives to our traditionally used telemetry systems as a further refinement, decreasing surgery duration and invasiveness thus shortening recovery times, extending battery capabilities to preclude the need for multiple implantations with longer studies, and minimizing associated equipment and personnel effort. HRT and HRT-ACT data loggers were surgically implanted subcutaneously on the dorsum and intramuscularly between the external and internal abdominal oblique muscles in macaques to determine the optimal location for use in infectious disease studies. Subcutaneous implants were found to record higher quality ECGs and heart rate measurements but recorded lower overall temperatures with greater diurnal temperature variation compared to intramuscular devices. The intramuscular approach required greater surgical expertise, was more invasive with a longer postoperative recovery, and had higher costs associated with the procedure compared to subcutaneous implantation. On radiographs, dorsal subcutaneous implants may obscure portions of the right or left lung field on ventral-dorsal views, and intramuscular implants may obscure abdominal organs on lateral views. Pros and cons were identified for each potential implantation site, so study-specific needs must be considered prior to selecting the optimum data logger location. Both subcutaneous and intramuscular data logger implantation sites have now been used with success in different nonhuman primate infectious disease studies.

P29 Relocation-associated Diarrhea, Microbiome Composition, and Probiotic Intervention in Cynomolgus Macaques (*Macaca fascicularis*)

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Social housing changes can be associated with diarrhea in macaques. We characterized changes in microbiome composition of juvenile primates (*Macaca fascicularis*) that exhibited diarrhea after a change in social housing by comparing matched-case samples (n=30 per group, 2 time periods) collected when diarrhea outbreaks began to baseline samples via 16S rRNA next-generation sequencing microbiome analysis. Significant changes in alpha and beta diversity and in abundance levels of several taxa were seen across time points. Diarrhea was associated with differences in beta diversity and abundance levels of several taxa. We then evaluated the effectiveness of a species-specific probiotic on prevention of relocation-associated diarrhea (n=120). We compared microbiome composition before and after administration to characterize effect of the probiotic on gut flora. Significant changes in abundance at the phylum, genus, and species level were noted in animals that received the probiotic compared to those who did not. No significant differences were noted in changes in alpha diversity. A significant effect of group ($P = .05$) was noted in changes in beta diversity between baseline and relocation samples. There was no significant effect of probiotic on diarrhea incidence following relocation. Characterization of the changes in abundance levels will direct future interventions aimed at reducing the incidence of diarrhea that occurs after shifts in social housing.

P30 3rs Resources for Animal Technicians

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Implementing the 3Rs is essential for conducting humane animal research and achieving high-quality scientific outputs. Everyone involved in animal research is responsible for putting the 3Rs into practice, but animal technicians hold an important front-line position for advancement of the 3Rs. Their day-to-day involvement in animal breeding, housing, husbandry, and scientific procedures means they can have a significant, beneficial impact on improving laboratory animal welfare and optimizing animal care and use. Therefore it is of great importance that animal technicians are supported in staying informed of, and implementing, 3Rs practices. Their 3Rs achievements should also be celebrated and disseminated via peer-to-peer channels. We present an overview of practical resources from the NC3Rs created to support, train, and champion animal technicians within the international research community. Web-based content launched in the past 24 mo includes advice on managing aggression in laboratory animals, evaluating environmental enrichment, best practice in colony management, malocclusion in mice, playpens for rats, refining zebrafish genotyping, and e-learning modules on anesthesia and pain assessment. Our free online resources, events and webinars offer accessible and effective learning opportunities and complement those in the AALAS Learning Library. Tech3Rs, our dedicated newsletter for animal technicians, is a crucial resource for staying up to date with the latest 3Rs opportunities and learning from other technical staff who are championing the 3Rs at their institutions. Subscribers can have Tech3Rs delivered straight to their inbox and all issues can be viewed online. Join us to learn more about these globally accessible resources and the 3Rs opportunities they offer.

P31 Practicing the 3Rs: A Novel Approach to Euthanasia Training Using Nonanimal Models

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Our organization is committed to the 3Rs, and the dedicated lab animal scientists here are always on the lookout for new and innovative ways to reduce the number of animals we use in our research. The Animal Welfare and Compliance Training Team has developed a novel approach to practicing pup decapitation as part of its euthanasia training: using 3D printing to create a model that simulates the process. Euthanasia training can be a difficult subject for laboratory personnel, even for those who have been working in the industry for years. It is imperative that euthanasia be performed appropriately for all animals in our care and using a nonanimal model to expose personnel to the sensitive topic of euthanasia has helped to reduce potential trauma to trainees while also reducing the number of live animals needed to meet competency and proficiency standards. The use of nonanimal models to supplement training is heavily supported by our organization. The pup decapitation models are already in use and have received high praise from internal personnel and external visitors who have had the opportunity to work with them. The 3D-printed pup model is an excellent example of how technology can be used to reduce the number of animals used in research while still providing a high-quality training experience. We hope that other laboratories will adopt this innovative approach to animal welfare.

P32 Does Acclimation to Dosing Apparatus Using Syringe-fed Treats in the Pre-dose Phase Reduce the Struggle Response of Canines when Preforming PO Dosing?

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Oral gavage dosing in canines can be a source of undue stress to both technicians and animals, especially when a struggling canine is involved. Typically, animals are only exposed to the dosing apparatus (i.e., syringe) at the initiation of the dosing phase. Acclimating canines by exposing them to the dosing apparatus using syringe-feeding techniques during the pre-dose phase may reduce the struggle response during the dosing period. Over the course of 5 d, 12 animals were introduced to a syringe filled with a high-value treat to familiarize them with items used during oral gavage dosing. Following the acclimation period, animals received water via oral gavage daily for 5 d. They were evaluated for struggling during dosing by the restrainer, the technician dosing the animal, as well as an observer. Animals were evaluated on a scale of 1 to 5 following a scorecard. Animals were assessed based on whether or not they vocalized, if they moved while being dosed, how much they moved, during dosing, and if alternate restraint methods or a secondary restrainer had to used. With the aid of positive reinforcement training, acclimation of canines to the dosing apparatus by use of syringe-feeding techniques during the pre-dose phase of a study can help to reduce the struggle response during the dosing phase of a study. The animals that were acclimated to the dosing apparatus via syringe-fed treats showed a significant reduction in struggling during dosing and overall struggled less than the animals that did not receive syringe-fed treats.

P33 Evaluating Nest Quality of 2 Nesting Materials in Various Bedding Substrates

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Regulatory guidelines for laboratory animal facilities state that animal housing should provide sufficient space and supplementary structures to allow for animals' physical, physiologic, and behavioral needs. Environmental enrichment allows animals to express species-typical behaviors, which enhances their psychological well-being. Mice naturally build nests to aid in reproduction, heat conservation, and protection. In laboratory animal facilities, nesting material in mouse cages provides environmental enrichment for the animals and allows them to express naturalistic behaviors, which can enable them to better cope with stress. Additionally, the quality of nests that mice build can indicate pain or distress and thus aid in welfare assessment. In the wild, mice create multilayered, heterogeneous, domed nests. In contrast, laboratory mice are typically given 1 type of material to create nests. Here, we assessed the quality of nests our colony mice build with our current nesting material, cotton squares, and investigated whether welfare could be improved with a different nesting material, crinkled paper. We conducted a crossover study comparing the 2 materials in cages with corncob or virgin wood pulp bedding. Using previously published nest scoring systems, we scored the robustness of nests created by 10 pairs of male C57BL/6NCrl mice daily for 2 wk in each bedding type. Each pair of mice received 1 type of nesting material for a week, after which the bedding remained the same, but the nesting material was switched for the second week. For both bedding types, nest quality was highly variable throughout the week with no nesting material having significantly higher scores. Additionally, the final scores for each nesting material were similar by the end of the week, indicating that mice build similar quality nests over time when given either 2 cotton squares or a bag of crinkled paper. In conclusion, we can provide our mouse colonies either nesting material for environmental enrichment. While it is crucial that animal care and use programs provide environmental enrichment, it is also important that the program ensures the enrichment serves its purpose of improving animal welfare.

P34 Competence Assessment Methodology for Laboratory Mice Handling

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Compliance with European and national legal requirements and ethics is essential to promote high standards of good research and animal welfare. People are required to be properly educated, trained, and supervised, and the acquired competence for specific techniques must be assessed. Thus, the methodology for training and competence assessment must be well-defined, reliable, and consistent among different assessors, facilities, and institutions to ensure an appropriate application of the learned techniques, minimizing animal suffering and promoting a good research outcome. Based on the EU Common Education and Training Framework document, a thorough methodology of competence assessment was developed within the CONGENTO, a Portuguese research infrastructure composed of 4 research institutions in the Lisbon area that synergize technology development across different animal models. Assessment tables were developed with scored items for the most common laboratory mice techniques, organized into 5 major categories: handling and restraint, injections, blood collection methods, Identification methods, and euthanasia. Each table covers different learning outcomes related to professional attitude, skills, knowledge, and application of the 3Rs. Clear guidelines and assessment criteria were defined contributing to standardizing this process. The implementation of the competence

assessment will start in July 2022, first with tutors (users with the most experience in each research group) proceeding then with senior and junior researchers. Assessment criteria will be provided to the users for all the techniques to be assessed. Until now, we have had meetings with the users of the Mouse Facility, explaining the whole process, which has been very well accepted and has helped to develop a general culture of care, self-confidence, and mutual help among users. We believe that the competence assessment will facilitate the free movement of people among institutions, with equivalent standards of competence.

P35 Positive Reinforcement Training Facilitates Veterinary Care of 2 Adult Male Pigtailed Macaques (Macaca nemestrina)

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Positive reinforcement training can be a valuable tool for refining nonhuman primate veterinary care and improving welfare during care. We present 2 case studies in which adult male pigtailed macaques (*Macaca nemestrina*) were trained to reliably shift between enclosure areas, allowing for effective clinical treatment and preventative health care. Case 1 was a singly housed 11-y-old male that required regular weight checks but had a history of recovering poorly from ketamine sedation. With the goal of weighing this male while awake, we first desensitized him to the wand target and weighing scale. Using positive reinforcement training techniques, we successively trained him to touch and follow a target, which facilitated shifting him between cage areas, and finally to sit and station on a scale placed in the bottom of his cage. Once trained, we could weigh him weekly without sedation or restraint. Obtaining weekly weights revealed a 3 kg weight loss in the 5 wk after a vasectomy procedure, prompting a sedated veterinary examination. On examination, a cystic epididymis and an inguinal hernia were diagnosed and surgically repaired by hemicastration and closure of the inguinal ring. This animal showed no behavioral changes; therefore, the weekly weights were an integral part of disease detection and prompt treatment in this case. In case 2 a group-housed 12-y-old male that was unable to be reliably examined during facility-wide health exams due to his aversion to shifting into transfer cages. Using positive reinforcement training techniques, we first trained him to touch, follow, and station to a target, and then desensitized him to the presence of a transfer cage. Ultimately, we trained him to follow the target into a transfer cage connected to his home cage. As a result, he voluntarily entered the transfer cage for sedation and exam. Here, the training ensured his capture, reduced capture stress, and reduced the number of personnel required to capture him. Postcapture, he continues to participate in target training with a transfer cage in view. We recommend positive reinforcement training for the refinement of veterinary care, as these case studies demonstrate the investment can have substantial benefits for primate health and wellbeing.

P36 Adopting an Extended-release Buprenorphine Postoperative Analgesic Regimen in Transgenic Mice

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Postoperative pain management for mice receiving headpost and craniotomy surgery includes the administration of NSAID analgesics. Carprofen or ketoprofen are administered during surgery, then BID for 2 days following surgery. To refine our postoperative process, we evaluated a single administration of 72 h extended-release buprenorphine in place of currently used NSAIDs. This modification would refine our process by reducing the number of postsurgical injections. A previous pilot study of buprenorphine SR found that some mice developed irritation and subdermal nodules around the injection site in the days following injection. These findings led us to maintain our current use of carprofen and ketoprofen. The newly released pharmaceutical grade extended-release buprenorphine reportedly provides 72 h of postoperative pain relief after a single administration. Replacing current analgesics with this would yield the same benefits as buprenorphine SR by reducing the number of postoperative injections and animal handling time, thereby reducing stress for the animals during recovery. To further evaluate a switch to extended-release buprenorphine, we conducted a follow-up pilot study to test an extended-release regimen against our current analgesic regimen for headpost and craniotomy procedures in transgenic mouse lines. We first tested the tolerance of extended-release buprenorphine on transgenic animals that had not undergone surgery. Next, we administered extended-release buprenorphine or carprofen regimens to surgical animals, comparing their weights and recovery times over a 14-d postoperative period. Our findings did not show significant differences in the tolerance of extended-release as compared with Carprofen. The negative effects previously observed with buprenorphine SR were not present in mice that received the extended-release treatment. Comparing the extended-release buprenorphine and carprofen groups, we found no difference in either the amount of weight lost by day 14 or the average number of postoperative days to reach bright, alert, and responsive status. Given these findings, we have incorporated extended-release buprenorphine into our postsurgical care protocol and have reduced the surgical impact on transgenic mice.

P37 Perceptions of Laboratory Animal Veterinarians Regarding Institutional Transparency

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Institutions typically have a veterinarian who is responsible for the veterinary care program and compliance with regulatory obligations. These veterinarians operate at the interface between the institution's animal care and research program and senior management. Veterinarians have strong public trust and are well positioned to share information about animals used for scientific purposes, but their perspectives on sharing information with the broader public are not well documented and their perceptions of transparency may influence how institutional policies are developed and applied. We analyzed the perceptions of institutional transparency among laboratory animal veterinarians working at different universities. Semi-structured, open-ended interviews were used to describe perceptions of 16 attending veterinarians relating to animal research transparency. Three themes were drawn from the interviews: 1) reflections on transparency described processes to either selectively limit information flow and guide the narrative towards a preferred conclusion, or processes that foster engagement with diverse perspectives to inform institutional decisions, 2) reflections on culture described the context to interpret actions (or lack thereof) of individuals or groups within that university relating to the sharing of information about animals used for scientific purposes; participants did not identify institutional transparency surrounding animal research as a priority for their university but believed it became so if influenced by internal and external pressures, and 3) reflections on self where attending veterinarians used the interview to engage in self-reflection about their own actions and how these actions have influenced their university's culture of transparency. The personal priorities of the veterinarians regarding transparency, combined with barriers to change within the institutions, were perceived to result in inaction. For example, sometimes veterinarians chose not to pursue available opportunities for change at seemingly willing universities, while others had their initiatives for change blocked by more senior administrators. The sharing of information regarding the animals

used for scientific purposes varied in how it was conceptualized by attending veterinarians. Transparency was not perceived as an institutional priority by many of the veterinarians and a cohesive action plan to increase transparency that involves multiple universities was identified as a promising avenue to overcome existing barriers.

P38 Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy: A Sustainable and Rapid Monitoring Tool for Monitoring Welfare and Stress in Breeding

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In laboratory animals, providing welfare is crucial for more reliable experimental results and greater reproducibility. Welfare problems in laboratory mice can be observed by stereotypic behaviors, barbering of whiskers or fur, and aggression between group-housed males. The measurement of corticosterone, a main stress-related hormone in rodents, is critical to detect in laboratory animals. There are a lot of poor welfare indicators, but our biggest challenge is quantifying the absence or reduction of welfare without causing more stress. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy has been used to detect changes in physiological conditions. In this study, we tested the hypothesis that ATR-FTIR is suitable to measure catecholamines and other stress markers in male C57BL/6J mice (n=30) related to mouse-rat cohousing (male Sprague Dawley rat). The diagnostic tool we are developing is based on the characterization of spectral signatures by ATR-FTIR in low volume of blood samples (1 uL) collected from the tail by a single puncture. All sample collections, both from breeding and experimental animals, were approved by our IACUC. Our results showed that the infrared spectral signatures are different in mouse–rat cohousing than in the control condition. In this sense, we are searching to determine, by increasing the number of samples evaluated, the spectral signature that determines different degrees of stress. Additionally, our goal is to develop learning machine algorithms for the determination of stress. ATR-FTIR can be a sustainable, reagent-free, rapid, low-cost analysis platform using ultra-low blood volume without euthanasia and minimal sample preparation for stress detection. In this context, we believe that the ATR-FTIR platform has innovative potential and can greatly contribute to monitoring animal welfare.

P39 Refinement and Direct Training Strategies of Intragastric Catheter Placement in the Mouse to Improve Animal Welfare and Surgical Outcomes

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Microsurgeries performed in rodents are common for generating research models of human disease. These surgeries in mice possess risk factors for animal welfare if not performed appropriately due to the small size of the animals. Microsurgeries require a specific technical skill set. Equipment and training opportunities are often costly and may not be available for less common procedures. Thus, having the access and resources for such surgical training in an academic setting would be beneficial for animal welfare and advancing the availability and survival of research models in biomedical research. We used the intragastric catheter (IG) infusion model to assess the functional physiology of feeding circuits. This surgically complex procedure was used to compare whether direct training, that included new refined approaches, would improve the surgical outcome. Intragastric catheter placement was performed in 165 C57Bl6 mice of 4-8 wk of age; 88 were performed by surgeon 1 and 77 by surgeon 2. Both surgeons had basic to advanced understanding of aseptic techniques, suture methodology, and IG microsurgery procedure technique. Surgeon 2 received direct training by a university trainer, who is also a veterinarian with focus on refined surgical approaches on the IG, while surgeon 1 was trained by a former lab manager. The new changes included the sequence of procedure, choice of materials, animal monitoring, and postoperative maintenance. After assessing the training approaches on the surgical outcome, surgeon 1 was retrained by surgeon 2. The mice were followed for 2 wk and the outcome was assessed as follows: no complications, minor complications with recovery, and mortality. The outcome for successful recovery was 73% for surgeon 1 and 85% for surgeon 2, while mortality was higher with 20% compared to 6%, respectively. The success rate improved for surgeon 1 from 67% to 80% after applying refined methodology. These results confirmed that the inclusion of refined approaches in the IG model under direct training is beneficial to the surgical outcome, animal welfare, and research outcome.

P40 Effect of Genetic Background on Foster Survival Outcomes in Inbred Laboratory Mouse Strains

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One of the major concerns in transgenic mouse breeding colonies is pup survival. Several transgenic strains are fertile and able to give birth but do not care well for their pups or lack maternal instincts completely. Consequently, researchers are having to keep more breeders, thereby, increasing the number of mice needed for studies, or use outbred mice as foster mothers. Since mutant mice are most often created using inbred strains, differences in maternal behavior can lead to changes in offspring behavior and physiology, thus, introducing potential study confounds. The aim of this study was to use closely related background strains (BALB/c, C57BL/6N, C57BL/6J, 129S1, FVB/N) as fosters and compare pup survival to that of an outbred strain (CD-1). We used a cross-fostering within strain study paradigm. Four pairs of experienced dams from each strain were time mated with experienced males over 3 consecutive d. Following litter births, 60% of the pups, with dates of birth within 3 d of each other, were cross fostered between the dams of the same strain. Foster pups had a toe cut on the right foot and natural litter on the left foot to distinguish between the two. Preliminary data (n=4 dams per strain) demonstrated no differences in pup survival rate between the inbred and CD-1 dams (99.4% vs 100% survival rate). Therefore, at this time, we found that under ideal housing conditions (isolated room with minimal amount of traffic and noise) inbred mice are as successful at rearing foster pups as CD-1 dams. We are currently extending the study to include conventional housing conditions in a large bay containing multiple mouse colonies and a higher amount of traffic.

P41 Evaluation of Postsurgical Trauma in Mice under Different Housing Conditions

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Though mice are social animals, investigators sometimes request that they be housed individually following surgery. We tested the hypothesis that pair housing of mice following a skin incision and closure with wound clips will result in greater occurrence of trauma to the surgical site compared to single housing. We further evaluated the effect on animal wellbeing of individual housing of formerly paired mice following surgery. Female mice (C57Bl/6),

approximately 6-8 wk old were housed as follows: Group A-single housed before surgery/single housed after surgery (n=10 all surgery); Group B-pair housed before surgery/single housed after surgery (n=10 all surgery); Group C-pair housed before surgery/pair housed after surgery (n=20, 10 surgery/10 no surgery); and Group D-pair housed before surgery/pair housed after surgery (n=10 all surgery). Parameters measured included body weight, body condition, grimace (live scoring approach), nest building, time to incorporate material into the nest (TINT), wound trauma scores, and occurrence of missing staples. No difference in mean body weight was noted, except for increased weight of Group A versus Group C before surgery and for days 5 through 14 after surgery. Group B demonstrated increased mean grimace scores versus Group C. Nest building and TINT scores were lower before and following surgery for single-housed mice (Groups A and B) versus pair-housed cohorts (Groups C and D). Mice housed singly before and after surgery (Group A) had greater wound trauma scores compared to pair-housed cohorts Groups C and D. The greatest incidence of missing staples occurred in mice housed singly before and after surgery (Group A) with 2 mice, while all other groups included a single mouse each with at least 1 missing staple. There were no statistically significant differences mean body condition scores between groups. Taken together, these results suggest that single housing of mice following surgical incision of the skin and closure with surgical staples resulted in increased trauma to the surgical incision site and reduced overall wellbeing compared to pair-housed cohorts. In summary, pair housing of mice following surgery resulted in increased wellbeing and reduced trauma to the surgical site compared to individually housed cohorts.

P42 Evaluation of Surplus Animal Requirements for Preclinical Rat Studies

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In preclinical research, surplus (spare) animals are obtained to ensure adequate numbers of healthy, eligible subjects for study. This may be at odds with efforts to reduce animal use. We hypothesized that those invested in study success generally overestimate the number of spare animals needed, and that there is opportunity for reduction. Using rats as a model species in preclinical research, we retrospectively assessed use of spares in toxicology, pharmacokinetic, and efficacy studies conducted at one institute according to Good Laboratory Practice (GLP) standards (19 studies; 5691 rats) or non-GLP standards (84 studies; 6476 rats) in 2018-2021; rats were 7-12 wk old, and 90.4% were Sprague-Dawley. Across studies, 6.9% spares (StDev 3.9%) were acquired. Spares were used when study-assigned animals were excluded due to pre-study health concerns or due to abnormal findings during baseline measurements (e.g., ophthalmic exams). 14.6% of studies required at least 1 spare, and 1.9% of studies required over 4% spares. GLP studies more frequently required at least 1 spare (47.4% GLP, 7.1% non-GLP; Fisher's Exact $P < 0.0001$) and required more replacement rats than did non-GLP studies (0.77% of rats on GLP programs required replacement; 0.14% non-GLP; Mann-Whitney $P < 0.0001$). Greater frequency of spare use may have occurred in the GLP studies due to increased pre-study screening. Although 2/103 studies in our retrospective analysis used >4% spares (1 each of GLP and non-GLP), both of these studies were relatively small (≤60 animals). Of studies with over 100 animals, none required replacement of over 3.6% of animals. Though researchers and IACUCs must critically evaluate details of each research program (e.g. risk tolerance for having insufficient study subjects, subject characteristics, pre-screening procedures, study size), we recommend 4% as a benchmark for large preclinical studies in rats (>100 animal), with larger studies likely requiring less. 92.9% of non-GLP studies reviewed required no spares; a risk assessment may find that acquisition of no or very few spares is warranted for some programs. Future work will evaluate the relevance of other subject and study characteristics in determining best practices for spare allocation.

P43 Implementation of an Alternative Model in Mouse Cardiac Blood Collection Training

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The training of terminal procedures with laboratory animals presents a variety of challenges, both logistically and with regards to animal welfare. Especially for facilities without designated training stock colonies, technicians are typically limited in the amount of practice animals available to use without compromising animal welfare. This makes procedures such as these ideal for evaluating the application of the 3Rs. After assessing various training procedures at our facility, it was decided that the mouse cardiac blood collection route would be most appropriate for the implementation of an alternative training model. The model would aim to reduce the number of animals used during training, improve a culture of care, as well as potentially help to refine the sample collection technique. We developed our model to closely mimic the procedure using commonly available components at a cost of less than $15 per reusable model. To test the efficacy of our model, 2 training curriculums were conducted concurrently for 5 mo with consistent trainers; one used our model to introduce the technique, and one was conducted with our traditional live animal curriculum. Our aim was to compare the number of animals used by each style of class, as well as the rate of proficiency earned to evaluate reduction. A necropsy evaluation of proficiency animals would also be conducted to evaluate refinement. We found that the training group that used the model for initial introduction of the skill had a proficiency rate of 91%, as opposed to 67% where no model was used as the introduction training. Using the model also allowed for a minimum of 10 less animals used per technician, which was compounded by model trained technicians reaching proficiency with fewer attempts than non-model technicians. Necropsy evaluation also determined that technicians had a higher understanding of the skill, as the model group's animals showed significantly fewer lesions noted. Due to these results, training using the alternative model has become standard at our facility. We hope that the success of this study demonstrates that alternative models can serve as a valid option in training curriculums to supplement learning and replace some live animal training.

P44 Short-term Socialization and Habituation Program Reduces Fear and Improves Experimental Handling in Laboratory Beagles

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A good welfare and behavioral management program habituates animals to routine husbandry and experimental procedures, which minimizes fear and anxiety, promotes cooperative behavior, and ensures consistent, reliable study data. We developed a daily 2-tiered socialization and habituation program (SHP) consisting of 1) out-of-cage play in a novel environment, stimuli, and individuals; and 2) counter-conditioning training focused on desensitization and habituation to handling. Our program maximized the7- to 10-d stabilization period after arrival, but prior to study procedures, with a goal of eliciting increased presentation of relaxed and compliant behaviors and decreased presentation of fear and stress behaviors. An ordinal scoring scale of 1-5 was developed whereby dogs were scored with baseline and end of program intruder test and handling, along with a daily score assigned for 1-on-1 handling. A total of 88 male and female laboratory beagles approximately 5-6 mo of age were used over 2 trials. Results indicated a statistically significant reduction in fear during the intruder test in both trials and sexes. For trial 1 males/females and trial 2 males/females the corresponding P values were 0.0042/0.0001, and 0.0079/<0.0001, respectively. The

changes in invasive procedure handling scores at initial and final scoring were not statistically significant; the average score changes were -0.238/0.810, and 0.435/-0.167. The rates of daily progression or regression, as compared to scores on the first day of socialization and handling, varied by animal in both trials; when aligned with behavioral observations and summaries of specific days' sessions, this subset of data indicates that introductions to certain new/increased restraint led to regression, and highlighted the necessity of individualized approaches. A significant behavioral change was observed in habituation to the presentation of treats at the conclusion of all sessions; by the third day of the program, all animals had become accustomed to approaching their bowl immediately upon return to their home cage. This unexpected benefit decreased the risk of dogs attempting to come back out of their cage and improved safety and welfare of both the animals and staff. Overall, our SHP demonstrated that, even with limited sessions, dogs can become less fearful and more cooperative. Our program helped to identify dogs that required extended pre-study training, which in turn increased their overall wellbeing and welfare.

P45 Rabbit Replacement: A Novel Rabbit Training Simulator

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While working in ABSL-3, ABSL-3AG, ABSL-4, and ABSL-4AG, it is extremely important that technicians are well trained, confident, and safe during bio-sampling procedures. Our training plan embodies the replacement of animals through simulators to decrease risk of exposure and injury while allowing initial hands-on experience for learners. Our unit facilitated a clinical skills training laboratory for animal care employees to train and fine tune their technical abilities using a variety of models and simulators. To our knowledge, no full-size simulators are available for exercises on handling and routine procedures on rabbits. A novel rabbit simulator was created by animal health technicians to practice safe sharps handling, animal restraint, and blood collection from peripheral ear vessels. This identified specific areas for trainees to gain proficiency prior to contact with live animals. After working with live animals, trainees were surveyed informally and the responses were positive, especially from inexperienced employees. Trainees felt the simulator improved their overall confidence when approaching new technical abilities while in a low-risk environment without live animals or pathogens present. The model has been successful in refining technical skills for experienced trainees as well. Animal care employees will use the simulator, "Reggie," for continued training of new and established personnel. While simulators such as "Reggie," allow for improvement of sharps safety, blood collection, and restraint, we are not yet able to completely replace live animal training experiences.

P46 Use of Low-level Laser Therapy in Rhesus Macaques (Macaca mulatta) with Chronic Cranial Implants

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Rhesus Macaques (*Macaca mulatta*), are frequently used in neurobiological studies. Multiple survival surgeries are often indicated to stage placement of cranial devices needed to record data. Implanted hardware may include headposts, chambers, and screws. Dental acrylic is typically used around the chambers to create a water tight seal. Despite use of best surgical and implant practices, bacterial contamination, chronic inflammation, bone and soft tissue loss, and acrylic-associated complications may still occur. When persistent, these conditions can result in severe dehiscence at skin-implant interfaces, compromised tissue perfusion and early termination of studies. Highly effective, minimally invasive approaches for the chronic management of cranial implant-associated complications are currently limited in this species. To slow the process of these skin-related issues, and improve animal welfare, we incorporated low-level laser therapy (LLLT) to our cranial care routine. LLLT has been used to restore cellular function, promote healing, and diminish pain in a variety of disorders. It is known to increase microcirculation and enhance tissue oxygenation and tissue nutrition. In accordance with the fundamental concepts and applications of laser therapy, we performed LLLT once per day, three times weekly for 17 mo. Following a standard margin cleaning regimen, LLLT was systematically applied at 1-250hz for 5 min around the chambers, headpost and along the midline to 2 chair-trained male rhesus macaques. LLLT has been well tolerated in both animals treated. We have noticed a decrease in inflammation, a reduction in pain, and no major skin infections or tissue necrosis. Overall, we have found the use of LLLT in macaques with chronic cranial implants to clearly improve the overall quality of implant-tissue healing as well as the general clinical health and wellbeing of these animals.

P47 Reporting Critical Events at Online as Part of the Culture of Care in Laboratory Animal Science

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Transparency and an open error culture with regard to critical events that can happen during an experiment in breeding or in animal husbandry should be a matter of course and must be supported from the employee up to the management level. Open communication about mistakes and incidents is still associated with the fear of consequences and shame. The web-based database CIRS-LAS provides a platform to collect and analyze critical events that occur in every context of laboratory animal science and to make them available to others working with lab animals. On CIRS-LAS.de, anyone can enter a case report on a critical event. For this purpose, keywords for a later search in the database are requested, as well as information on animal species, background of the experiment or general information, a short description of the incident, and a classification in a subject area. If improvement or refinement measures have already been taken, these can also be indicated. Registered users can search the database in advance of a planned experiment, preventing the repetition of failed experiments and thus reducing the number of lab animals. At the same time, the quality of one's own work increases, since the findings from the reported critical events and named refinement strategies can be incorporated into one's own work. Open communication should therefore be a main part of an institutional culture of care. Fear and inhibition to talk about one's own mistakes are still great, especially in the field of LAS. However, through project presentations, explanations, and constructive discussions, many people can be persuaded worldwide to actively participate in CIRS-LAS. The number of registered members is steadily increasing and currently stands at 240, with 50 cases entered in the database. The optional anonymous case reporting on CIRS-LAS.de is an important tool to enable all people working with lab animals to deal transparently with errors and thus contribute to improved animal welfare and a reduction in the number of used lab animals. Mutual open and constructive communication about critical events on the CIRS-LAS platform helps to increase confidence and supports the culture of care.

P48 Cross-pollination across Animal-care Related Fields: Learning Together to Develop a Culture of Care for Human and Animal Wellbeing

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A culture of care involves creating healthy workspaces, attention to psychological wellbeing, benefits of gratitude and mindfulness, addressing bullying and toxicity, and systemic causes of stress and burnout. It is equally important to hold space for people to grieve,

have rituals, and feel connected. Animal care professionals across different disciplines, such as laboratories, shelters, zoos, aquariums, sanctuaries, and farms, can learn from one another and provide support when dealing with the satisfactions (joys) and occupational stressors (sorrows) of their work. There are commonalities amongst the joys which may be supporting good animal wellbeing, the connection with the animals, species conservation, connecting to the public, animal and environmental protection, and contributing to other goals such as research and education and the sorrows which may include extended workloads, dysfunctional teams, limited time and budgets, grief, loss, and euthanasia. The authors will expand on previously presented frameworks that emphasize the interconnectedness and demonstrate how they can be used across the disciplines to identify personnel and the important role that each of us play in our animal care programs. While differences between the professions exist, there are also large overlaps grounded in common humanity. The knowledge that we are not alone, that we have a larger support network, that life is all about relationships and connections, and that we can find support and strength in each other unites us. The cross-pollination of stories across these disciplines and fields, as well as learning and contributing different proven strategies and methods will benefit all of us, both the animals and the people in these professions. What all people need most is to feel a sense of belonging and support and a deep and true culture of care and understanding in the workplace.

P49 Increasing the Reuse of Protein Non-naïve Nonhuman Primates in Pharmaceutical Drug Discovery and Development: An Overview and Industry Position on the Challenges and Benefits

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The IQ Consortium NHP Reuse Working Group (WG) comprises members from 15 pharmaceutical and biotechnology companies. In 2020, the WG developed and distributed a detailed questionnaire on protein non-naïve NHP reuse to the WG member companies. The WG received responses from key stakeholders including principal investigators, facility managers, animal welfare officers, and research scientists. This paper's content reflects the consolidated opinion of the WG members and the questionnaire responses about NHP reuse within nonclinical programs at all stages of research and development. Many of the pharmaceutical companies represented in the working group or participating in the questionnaire have already achieved some level of NHP reuse in their nonclinical programs, but the survey results suggested that there is significant potential to increase NHP reuse further and a need to understand the considerations involved in reuse more clearly. The WG has also focused carefully on the inherent concerns and risks of implementing protein non-naive NHP reuse and has evaluated the best methods of risk assessment and decision-making. This paper presents a discussion on the challenges and opportunities surrounding protein non-naïve NHP reuse and aims to stimulate further industry dialogue on the subject and provide guidance for pharmaceutical companies to establish roadmaps and decision trees enabling increased protein non-naïve NHP reuse. In addition, this represents a solid basis for collaborative engagement between pharmaceutical and biotechnology companies with CROs to discuss how the availability of protein non-naïve NHP within CROs can be better leveraged for their use within nonclinical studies.

P50 Placement of Human Hand in Cage Induces 22-khz Ultrasonic Vocalization In Male Albino Rats

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Rodents emit ultrasonic vocalizations (USVs) to communicate emotional states and coordinate their social interactive behavior. Twenty-two kHz USVs emitted by adult rats have been reported in a variety of aversive social and behavioral situations. They occur not only under painful or restraining conditions but can also be evoked by cutaneous touch or airflow. This study aimed to test if the introduction of a human hand in a cage can evoke 22-kHz USVs. The emission of USVs was captured, processed, and analyzed with a professional bioacoustics sound recording and analysis system. The study was performed on 2 consecutive days and each session took 1.5 mi per animal (baseline for 1 min, hand in cage for 15 s, and post hand introduction for 15 s). It was found that 36% of the adult male Sprague-Dawley (SD, n=14) and 13% of the adult male Wistar Han (WH, n=48) rats emitted 22-kHz USVs when a gloved hand was introduced into the cages. Average vocalization onset latencies were 5.0 ± 4.4 s (SD) and 7.4 ± 4.0 s (WH) and the USVs had a stable frequency (22 kHz) across the calls, ranging from 0.1 to 2.3 s in duration. Surprisingly, no 22-kHz USVs were found in any female WH rats (n=51) tested. To further explore the mechanisms underlying this observation, we compared retinal function, basal serum corticosterone and testosterone levels between the responders and non-responders. None of these parameters or endpoints showed any significant differences between the 2 cohorts (all $P > 0.05$). The results suggest that the introduction of a gloved-hand inside the cage can trigger adult male albino rats to emit 22-kHz ultrasonic vocalizations. This response should be considered in USV studies and animal welfare.

P51 Evaluating Serotonin Levels in Rhesus Macaques *(Macaca mulatta)* and Its Effects on Pairing

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The behavioral and psychological benefits of pair-housing Rhesus macaques (*Macaca mulatta*) are abundant and essential for lab animal welfare and wellbeing. The pairing process is continuously studied and improved on, such as determining what factors play a significant role in the compatibility of potential partners. Understanding the animals' characteristics and having the ability to predetermine good compatibility allows for a pairing process that limits the stress, anxiety, or injuries experienced by the animals. The role of serotonin and its influence on Rhesus macaque behaviors has been previously studied, however, its uses as a predicting factor in the pairing process have not been. Low serotonin levels negatively correlate with aggressive behaviors and social hierarchy. Individuals with higher levels of serotonin are more likely to maintain a high social dominance ranking, whereas those with low levels showcase spontaneous aggression more often. In this study, serotonin levels of 58 female Rhesus macaques were analyzed in order to determine if there was any correlation between high/low levels and the compatibility of the pairs. Serotonin concentration levels were determined via blood draw prior to pairing, and another blood draw was done once all pairs were deemed successful. Pairs were chosen by the behavioral technician, and the pairing process was done in the normal 3-step process: visual contact, protected contact, and full contact. The results showed a significant positive shift in serotonin levels (93%); however, it did not yield the data needed in order to determine whether or not preselecting pair mates based on their serotonin levels would be beneficial to the process. Therefore, the study was unsuccessful in its attempt in determining a new method for predicted compatibility of pair mates. Future studies may benefit from using subjects that are not familiar with one another,

collecting specific behavioral data during the process, and pairing subjects based on serotonin in place of other know predictors (i.e., temperament, weight, and age).

P52 How the IACUC Worked with Business Partners to Develop a Safety Review Process to Reduce Regulatory Burden

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Our organization performs contract research for external clients who wish to investigate the efficacy of their test articles. Principal Investigators conduct these studies under comprehensive animal use protocols and needed a review process that allowed them to add new test articles to meet study initiation timelines and reduce regulatory burden. The Animal Welfare and Compliance Office, principal investigators, and attending veterinarian formed a subcommittee to evaluate ways of adhering to customer study initiation timelines and reducing regulatory burden. The team worked together to identify pain points in our existing processes, evaluated safety concerns, enforcement of compliance, and the regulatory burden impact on all parties (customers, principal investigator, and IACUC members). The results of this collaboration led to the development of the Safety Review process which permits the broad use of monoclonal antibodies (including bispecifics), small molecules, CART cells, and recombinant proteins on established comprehensive animal use protocols. The proposal was presented to the IACUC for discussion, a vote, and was ultimately adopted. Previously, the principal investigators were required to submit an amendment, safety plans to Environmental Health and Safety, and receive approval from the IACUC and EHSS before they could initiate the customer study. The Safety Review process allows the principal investigator to initiate the study after the EHSS review is completed and submit one IACUC amendment at the end of the month with all new test articles added to the animal use protocol. We provide additional detail on the collaboration process, concerns evaluated, and implementation of the process. The Safety Review process may not be used for any test article or cell therapy that has a known potential for an adverse effect; vehicle that is not currently approved test article that is not a monoclonal antibody (including bispecifics), small molecules, CART cells, and recombinant; that will be administered via a route, dose, frequency or duration not already described in the animal use protocol; or the addition of new procedures/changes to the animal use summary.

Clinical Posters

P100 Wing Paresis Associated with a Unilateral Mass over the Keel in 2 Zebra Finches (*Taeniopygia guttata*)

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Over 20 mo, two experimentally-naïve adult (6 and 12 mo old) female zebra finches (*Taeniopygia guttata*) from a ~300-bird breeding colony (adults up to 3 y old) presented with acute onset of unilateral wing drooping and paresis. Examination identified a lateralized firm swelling under the pectoral muscle and an absence of fracture or trauma. Each bird was euthanized and submitted for necropsy 3 d after initially presenting due to progressive weakness and lethargy. Differential diagnoses included neoplasia, xanthoma, pectoral abscess, mycobacteriosis, peripheral neuritis, myopathy, or a viral etiology. Case 1 postmortem examination revealed a large (2.4 x 2.5 x 1 cm), pale pink-to-white, semi-firm, multinodular mass diffusely expanding the right pectoral musculature and extending into the left pectoral muscle and the coelomic wall. Histopathology demonstrated a poorly demarcated and invasive neoplasm composed of pleomorphic spindle-to-spindeloid cells arranged in streams and interlacing bundles completely effacing pectoral musculature. Metastatic foci in the lung were composed of atypical spindeloid-to-polygonal neoplastic cells. The neoplasm was diagnosed as a poorly differentiated sarcoma. For case 2, there was a large (1.5 x 1.2 x 1 cm), white, firm, smooth mass effacing and distorting the left keel bone. The mass invaded and expanded the left pectoral muscle and protruded into the coelomic cavity, displacing the heart caudally. Histopathology demonstrated an unencapsulated, densely cellular, infiltrative mass comprising streams of spindle cells multifocally surrounding small aggregates of osteoid material, and the center had a woven bone composed of pale eosinophilic trabeculae lined by osteoblasts and osteoclasts interlaced with neoplastic spindle cells, consistent with an osteosarcoma. A separate neoplastic mass within the dorsal intracoelomic cavity, unattached to adjacent visceral or musculoskeletal tissue, was interpreted to represent tumor seeding or metastasis. The neoplasms in both cases were determined to be the cause of wing dropping and paresis. Spontaneous sarcomas are rarely reported in passerine and other avian species, and prior reports of malignant tumors of any kind in passerine birds have generally featured aged animals.

P101 A Mixed Germ Cell-Sex Cord-Stromal Tumor in a Galago

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A 7-y-old female galago (*Otolemur garnetti*) presented for annual physical exam. On abdominal palpation a firm, round, approximately golf ball-sized mass was palpated in the cranial abdomen. The animal appeared to be in good health, and the presumptive diagnosis of a trichobezoar was made, which is a relatively common incidental finding seen in our galago colony. The animal was monitored closely for any sequelae but exhibited no signs of illness. Three weeks later, the animal underwent general anesthesia for a craniotomy and intracerebral tracer injections. The procedure was uneventful; however, recovery was prolonged, and the animal's breathing and heart rate rapidly declined. Due to lack of response to intervention and poor prognosis animal was euthanized. On necropsy, a 4 x 6 x 5cm pedunculated mass was located centrally within the abdomen. It had a mottled appearance, and the stalk was thick, firm, and white. The mass contained ~15-20ml of red viscous fluid which encapsulated a solid tissue structure. On histology, the solid tissue structure inside the mass was diffusely necrotic. The stalk portion of the mass was composed of a mixed cell population, including very large cells resembling oocytes, and what appeared to be ovarian stromal tissue intermixed with duct-like structures. The germ cell portion of the tumor was characterized by aggregates of large uniform cells surrounded by large amounts of connective tissue stroma containing a few lymphocytes. The sex cord portion consisted of irregular and branching tubules surrounded by large amounts of cellular ovarian stroma. Using immunohistochemistry, a mixed germ cell-sex cord-stromal tumor, which was immunopositive for inhibin, SALL4, Oct3/4 and calreticulin was diagnosed. When this tumor occurs, it is typically seen in infants and young children, causing abdominal pain, ascites, and uterine bleeding. This is a rare tumor in humans that has not been previously reported in any nonhuman primate species.

P102 A Comparison of 2 Direct Blood Pressure Measurements in Rabbits Anesthetized with Sevoflurane

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Direct blood pressure measurement is the gold standard for obtaining accurate blood pressure in rabbits under anesthesia. This study investigated the agreement between 2 direct blood pressure measurements obtained from the femoral artery (Fem, a major vessel) and the auricular artery (Aur, a commonly used peripheral vessel) in rabbits under sevoflurane anesthesia. We hypothesized that Aur would accurately estimate Fem in both hypotension and normotension. Four intact female New Zealand White rabbits were induced with ketamine (35 mg/kg)/xylazine (5 mg/kg) via intramuscular injection and maintained with 1.5-2x minimal alveolar concentration of sevoflurane in 100% O2. The femoral artery was surgically exposed and directly catheterized, and the auricular artery was percutaneously catheterized. Both catheters were connected to calibrated pressure transducers zeroed at the level of the heart. Blood pressure (BP) measurements (systolic, diastolic, and mean BP) were obtained using each method every 1 min for up to 120 min. Agreement between Aur and Fem was assessed using the Bland-Altman method of analysis. During both hypotension and normotension, Aur systolic, diastolic and mean BP underestimated Fem systolic, diastolic and mean BP, respectively. These results indicate that while direct blood pressure monitoring via auricular artery catheterization may be useful for detecting general trends in blood pressure in sevoflurane anesthetized rabbits, Aur BP consistently underestimates Fem BP in both hypotensive and normotensive states.

P103 Can Toxic Bone Marrow Initiate Tumorigenesis?

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Multistage chemical carcinogenesis, a 2-step process requiring an initiator and promoter, provides an effective way to study the stages of tumor development. In skin, tumors are induced using both the initiator, dimethylbenz[a]anthracene (DMBA), and the promoter, 12-O-tetradecanolyphorbol-13-acetate (TPA). This 2-step model was used to study the recruitment of bone marrow-derived epithelial cells into the skin during tumorigenesis using allogeneic bone marrow transplantation. DMBA exposed "toxic" bone marrow from male donor mice was transplanted into naïve X-ray irradiated female recipient mice. Naïve female mice that received bone marrow transplants from DMBA-treated male donors exhibited benign squamous papillomas and malignant squamous cell carcinomas after TPA promotion. Immunofluorescence staining of the tumor epithelium showed small and large clusters of 5 or more adjacent cells that were double positive for both GFP and pan-cytokeratin, indicating that the cells were of donor bone marrow origin and expressed keratin. Flow cytometry analysis of the tumors confirmed their GFP donor-derived and epithelial origin. Tumor cells double positive for pan-cytokeratin and the Y chromosome were confirmed using XY FISH, again indicating they were of male bone marrow donor origin. Results were validated in 45 tumors from 21 mice. Recipient mice that received a bone marrow transplant from corn oil-treated donor mice followed by TPA promotion did not develop lesions or tumors. Based on these results, we conclude that bone marrow derived epithelial cells contribute to the development of papillomas and chronic cutaneous ulcers.

P104 Short-term Management of Streptococcus equi Subspecies zooepidemicus in a Research Chinchilla (*Chinchilla lanigera*)

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Streptococcus equi subspecies zooepidemicus is a potentially zoonotic infection that has been identified in many animal species, including chinchillas (Chinchilla lanigera). Unfortunately, there are not many documented reports of treating and managing these infections in this species, particularly in research animals that are on study. Recently, one of the research chinchillas at our institution was found to have submandibular lymphadenomegaly suggestive of S. equi zooepidemicus infection during experimental implantation of novel intratympanic membrane devices. This was later confirmed via oral swab PCR testing. In order to get the animal to experimental endpoint, which was 4 wk post device implantation, we surgically removed the enlarged lymph node 4 d after it was first noted and started the animal empirically on oral enrofloxacin 10 mg/kg twice daily for 14 d and meloxicam 0.3 mg/kg SC for 10 d for analgesia and to reduce inflammation The surgery site was visually monitored daily and more closely examined during the animal's weekly research related anesthetic events. While there was evidence of other regional lymph nodes becoming enlarged and abscessing on necropsy, the surgical site itself had healed well. Additionally, the animal did not demonstrate discomfort related to the treatments utilized and was able to successfully make it to their experimental endpoint. At this time, it does not appear that this disease or its treatments affected the expected research outcomes assessing tympanic membrane healing post novel intra-tympanic membrane device implantation. In conclusion, it appears that surgical removal of the affected lymph node in addition to systemic antibiotics can be used to manage chinchillas with S. equi zooepidemicus infection until they can reach short-term experimental endpoints.

P105 Erythema and Swelling of the External Urethral Orifice in a Rat Sciatic Nerve Injury Model

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Eleven of 14 pair-housed female adult Lewis rats on a sciatic nerve injury study presented with mild to moderate erythema and swelling of the skin involving the external urethral orifice at approximately 1 to 2 mo after receiving unilateral right-sided nerve injury. All affected rats had mild stiffness of or involving the right hind limb and walked with a sunken hind end, occasionally dragging their ventral surface of the rear end in the cage. The clinical signs were unexpected and developed at various time points without distinct pattern. The differential diagnoses include irritation from physical contact, allergy, or infection. Swabs of the urethral orifices and free-catch urine samples from representative animals were sent out for bacterial culture and were positive for Escherichia coli. All affected animals in 7 cages were treated with enrofloxacin at 0.17 mg/ml in autoclaved drinking water for 1 wk. There was no consistent improvement of the observed erythema/swelling after enrofloxacin treatment. Then we evaluated alternative enrichment material to see if there was a physical contact component to the occurrence of this lesion. The nesting material was switched from a crinkled natural paper to a softer cellulose nesting material alternative and found that 4 out of 11 rats showed improvement from the provided treatment, including 2 rats that fully resolved. Histological analysis of the terminal urethra from a representative animal revealed mild focally extensive mural and serosal hemorrhage with no evidence of urinary bladder infection. Given the partial response to the change in nesting material, the tentative diagnosis is chronic physical contact with

cage substrate material secondary to postural and gait abnormalities associated with this model. We plan to partner with the investigator on the next cohort of rats on this study to further characterize this condition and pilot the response to various treatment and prophylactic measures. This study highlights the importance of understanding the variety of husbandry factors that may influence research models.

P106 Identification and Successful Eradication of *Ophionyssus* sp. Mites in Wild-caught Laboratory-housed Italian Wall Lizards (*Podarcis siculus*)

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Italian wall lizards (*Podarcis siculus*) were caught in the wild for a study on the ecology and evolution of nonnative species. The colony consisted of equal numbers of male and female lizards from 3 different cities on the east coast of the United States. Animals were immediately pair-housed in large rat cages with timed heat lamps and maintained on a 12:12 light cycle. Cages contained artificial grass, cardboard huts, petri dishes of water, and mealworms. Approximately 5 wk after arrival, 1 lizard was found dead with no prior clinical signs. The cage-mate was found dead the following morning with mites crawling along the dorsum; this animal was sent out for necropsy. When a third lizard was found lethargic with visible mites, a tape preparation of the skin was sent out for diagnostic identification. The decision to begin treatment for ectoparasites was made prior to receiving test results. Both the lethargic lizard and 1 healthy lizard were treated with 1 dose of topical selamectin (~36 mg/kg). No adverse effects were observed in either lizard and the affected lizard appeared more active 24 h after treatment. Although all affected lizards were from one city, the entire colony was treated with topical selamectin twice, 2 wk apart, to prevent spread. Cages and their components were sanitized after each treatment. Histopathology identified ectoparasitosis and multifocal hyperkeratosis. Additional nematode and larval parasites were found in the colon, which was suggestive of pinworms. The mites on the tape preparation were identified as *Ophionyssus* sp, which is a blood-sucking mite of reptiles. Following the second round of treatment, tape preparations and fecal samples were analyzed from previously positive animals. No mites, ova, or parasites were observed. In conclusion, wild-caught Italian wall lizards can be infested with ectoparasites, which may thrive in a controlled laboratory environment. Selamectin at ~36 mg/kg is a safe and effective treatment for *Ophionyssus* ectoparasitosis in this species.

P107 Palmoplantar Nonpustular Psoriasiform Dermatitis in a Rhesus Macaque (*Macaca mulatta*)

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A 6-y-old, male experimentally naïve rhesus macaque (*Macaca mulatta*) presented with a thickened dermal lesion surrounding one of its nail beds (onychosis) on the foot. On physical examination, the skin was raised and erythematous with scaly plaques and marginal fissures. Over the course of the next 4 mo, the lesions spread to additional sites including another toe, the plantar aspects of both feet, and 1 palm. Differential diagnoses included fungal, allergic, or atopic dermatitis. Punch biopsies and fungal cultures were taken of the affected sites under sedation. No growth was noted on fungal cultures. Histopathology revealed diffuse hyperkeratosis with prominent rete pegs that extended into the dermis. In addition, immune cell infiltration and coccoid bacteria were noted. The clinical presentation and histopathology findings were consistent with a diagnosis of palmoplantar nonpustular psoriasiform dermatitis with secondary bacterial infection. Psoriasis is a commonly diagnosed chronic immune-mediated skin condition in humans but reports in nonhuman primates are scant. The nonpustular plaque form of this disease is characterized by well-demarcated, thickened, erythematous skin lesions with associated scaling. Psoriasis can be further classified based on location of the lesions. In most cases, psoriasis is a life-long disease with periods of relapses and flares. Various treatment modalities have been tried in macaques, including topicals and systemic immunosuppression or modulation. In this case, only transient remission could be achieved with oral corticosteroids. Treatment with vitamin B12 supplementation and a topical plant-based treatment have resulted in stable, mild disease in this macaque. Despite the lack of published reports of psoriasis in nonhuman primates, it should be considered as a differential diagnosis for primates presenting with hyperkeratotic dermatitis.

P108 Atypical Dermatitis in a Laboratory-housed Wild Turkey (*Meleagris gallopavo*)

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A 1.5-y-old male wild turkey (*Meleagris gallopavo*) presented with severe dermatitis and scabbing of the neck several months after arrival. Ten wild turkeys (7 toms, 3 hens) were acquired from a breeder and group housed. Upon arrival, animals were treated with selamectin and tested negative for *Salmonella pullorum* and avian influenza through the state veterinarian. Physical examination revealed multifocal blackened, scab-like cutaneous lesions and proliferation affecting the caruncles and wattles. None of the other turkeys appeared to be affected. The turkey was anesthetized with isoflurane and punch biopsies of the affected skin were taken for histopathology. In addition, a dose of ceftiofur crystalline free acid and meloxicam were given to control secondary infections and pain. Differential diagnoses for skin lesions in adult turkeys include viral dermatitis (fowl pox), bacterial or fungal dermatitis, neoplasia, and trauma. Fowl pox was considered the most likely diagnosis due to the appearance and distribution of the lesions as well as the unknown health status of the wild turkeys. The veterinary staff prepared to vaccinate the remaining animals, pending results. Histopathology demonstrated that the dermal lesions were composed of increased amounts of organized collagen and fibroblasts in the dermis and epidermal ulceration with overlying hemorrhage. No evidence of a neoplastic or infectious agent, including eosinophilic intracytoplasmic inclusions or Bollinger bodies, which would be consistent with avian pox, were detected. Dermal fibrosis due to trauma or unknown origin was the most likely diagnosis. The lesions resolved within a month of initial presentation. This case highlights the importance of early diagnostics and caution when faced with unusual clinical presentations in laboratory-housed wild populations of animals.

P109 Continuous Dosing of Buprenorphine Hydrochloride with A Subcutaneously Implanted Osmotic Minipump for Post Laparotomy Analgesia In Ferrets

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To enable long-term in vivo physiologic monitoring of freely moving animals, four 3- to 4-mo-old ferrets were implanted with telemetry devices. Standard postoperative analgesia consisted of buprenorphine (0.03mg/kg) every 8 h, and 0.2mg/kg of meloxicam once daily. Sustained-release buprenorphine (SR) was not approved for use due to prevalence of injection site reactions. However, within the first 24 h, combined observations of orbital tightening, hunched posture, whisker retraction, and ear changes were compared to the Ferret Grimace Scale, and it was determined that the analgesia was

not sufficient. Buprenorphine dose was increased to 0.05mg/kg every 6 h, and meloxicam frequency was increased to twice daily. This change in dose and/or frequency of analgesics appeared to provide appropriate pain management, yet it did not eliminate the "peak and valley" effect, with technicians observing minor grimaces between doses that resolved within 30 min. When the telemetry study was repeated with eight 3- to 4-mo-old ferrets, buprenorphine injections were replaced with a subcutaneously implanted osmotic pump. This pump delivered at a continuous rate of 10 microliters/hour and delivered buprenorphine hydrochloride at 0.002 to 0.00215 mg/hr for 7 d. We found that this alternative method provided superior analgesia based on the Grimace Scale scoring system. Comparison of preoperative and 24-h postoperative photos of each ferret show that according to the Grimace Scale, all animals experienced what appeared to be a pain free postoperative period, a refinement of previous studies at our institution using intermittent dosing of buprenorphine. These results suggest using subcutaneously implanted osmotic pumps for continuous dosing of buprenorphine in ferrets, post laparotomy, provided improved pain relief when compared to a repeat dosing protocol.

P110 Morbidity and Mortality in Zebra Finches (*Taeniopygia guttata*) Associated with Circovirus and Avian Gastric Yeast Infection

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Zebra finch (*Taeniopygia guttata*) hatchlings and fledglings exhibited an acute increase in morbidity and mortality in a well-established breeding colony used in neuroscience research. Affected birds presented with diarrhea, lethargy, and sudden death. Differential diagnoses for these signs included infectious (bacterial, viral, protozoal, fungal), toxicity, and husbandry-related causes. Histopathology showed proventriculitis and ventriculitis with mucosal erosion, koilin disruption, and abundant intralesional, rod-shaped yeast also seen on fecal wet mounts. This led to a diagnosis of *Macrorhabdus ornithogaster* or avian gastric yeast (AGY) overgrowth. The efficacy of amphotericin B (AmB) in drinking water to treat for AGY was tested in a subset of birds compared to untreated controls. Four-week treatment of the subset of animals, followed by the entire colony, was well-tolerated with no major changes in body condition, water consumption, or number of offspring per breeding pair. Fecal AGY counts did not differ between treated and control animals, but significantly increased after one week of treatment, suggesting killing of organisms with passage into feces. Morbidity and mortality fluctuated during the course of treatment but decreased at 4 wk of treatment. Differential diagnoses for persistent clinical signs included adverse reaction to AmB, continued morbidity due to AGY, or other underlying infectious disease, including viral causes. Feces, liver, small intestine, lung, and spleen were PCR-negative for *Atoxoplasma* spp. Serum was negative for avian reovirus, rotavirus, adenovirus I and II, avian encephalomyelitis virus, and Newcastle disease virus. Feces were PCR-positive for circovirus. Zebra finch circovirus replicates in dividing cells, including immune cells, and is associated with immunosuppression and secondary infections, most commonly AGY. Circoviral treatment focuses on managing these infections, and resistance to environmental degradation makes eradication difficult. In the months following AmB treatment, offspring survival remained improved. This report highlights the need to consider circovirus infection in zebra finches exhibiting signs of opportunistic infections, and supports consideration of developing specific-pathogen free colonies.

P111 Treatment of Self-injurious Behavior in a Rhesus Macaque (*Macaca mulatta*) with Oral Naltrexone and Alprazolam in Combination with Environmental Enrichment Modifications

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The spontaneous occurrence of self-injurious behavior (SIB) can pose a substantial animal welfare concern in captive NHP colonies. SIB can have detrimental effects on the individual animal's wellbeing and health and may interfere with ongoing research studies. The underlying etiology of SIB is poorly understood, and few therapeutic interventions are published for the clinical management of this disorder. This case report describes a novel treatment protocol for SIB consisting of oral naltrexone and alprazolam in combination with environmental enrichment modifications. A 10-y-old, intact male, single-housed, rhesus macaque (*Macaca mulatta*) on a neuroscience research protocol presented with an acute onset of SIB. In total, 4 episodes of self-inflicted wounding, which required veterinary intervention and major wound care, occurred over 10 d. We hypothesized that the occurrence of SIB was mediated by endogenous opiates and therefore naltrexone, a pure opioid antagonist, was chosen for the acute treatment. Pharmacological treatment was initiated after the fourth incident of SIB with naltrexone (4.5 mg/kg PO BID). The initial dosing of naltrexone was reduced to 2 mg/kg PO SID after 2 d due to an onset of upper respiratory symptoms (i.e., intermittent cough). After the initial treatment course with naltrexone for 4 wk, the NHP showed intermittently selfinflicted behavior without tissue damage. We opted not to increase the dose of naltrexone due to the previously noted systemic side effects and added instead alprazolam at 0.25 mg PO BID to the treatment strategy. Environmental enrichment modifications were started in conjunction with drug treatment and consisted initially of removing the animal from the colony, and then relocating to a different room within the facility together with one other animal for visual and sensory support. Furthermore, the animal received daily puzzle feeders, forage boards, and ice treats. Naltrexone was discontinued after 16 mo and the NHP is currently receiving alprazolam only, which will be discontinued once the animal returns fully to the research study. The NHP did not suffer a relapse of SIB for more than 18 mo since the implementation of this treatment approach. This case study demonstrates the successful therapeutic management for SIB in a rhesus macaque and adds a novel therapeutic approach for this disorder to our arsenal of treatments.

P112 Advances in Patient Care for Preclinical Extracorporeal Life Support

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Extracorporeal life support (ECLS) reduces the functional workload requirements of the lungs, heart, or both for days to weeks in patients with reversible life threatening respiratory or cardiac disease. It is estimated that ECLS could have saved close to half of the seriously ill COVID-19 patients for whom mechanical ventilation was not effective if ECLS had been available. Supporting an ECLS patient however requires extensive knowledge and resources, and this is true also in the preclinical space when working with healthy animal models. The most common serious complications in the clinical setting are bleeding and thrombosis, both of which are also seen in preclinical studies performed on healthy sheep. From late 2020 to early 2021, the animal care and clinical laboratory teams made significant advances in supporting preclinical ECLS studies, reducing both mortality and other adverse outcomes significantly by implementing several interventions: 1) improving diagnostic ability to monitor anticoagulation status more accurately with the measurement of activated clotting time (ACT), blood heparin

concentration, partial thromboplastin time (PTT), activated partial thromboplastin time (aPTT), plasma free hemoglobin and red blood cell values; 2) refining ECLS circuit attachments and optimizing the kennel environment to allow for improved animal comfort while decreasing the risk for canulae or circuit kinking or migration and 3) refining nursing care and monitoring by the inclusion of improved AV system and standardized care protocols. One of the core improvements was supplementing periodic ACT tests with blood heparin concentration. After these refinements, mortality during 7 d in life ECLS studies was reduced from 50% (3/6 animals, study1) to none (0/3 animals, study2) Blood heparin concentration is also beneficial in the management of human patients on ECLS. Facilities performing ECLS studies can benefit from expanding anticoagulation evaluation from simple ACT measurements to a multimodal approach, with blood heparin concentration measurements being especially advantageous. In addition, skilled round-the-clock nursing care of the study animals is vital for success.

P113 Atypical Copper Toxicity in Research Sheep

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A 50-mo old Sire Max female research sheep housed indoors presented with inappetence and regurgitation that progressed to ruminal stasis, jaundice, and ataxia despite treatment. Investigated differentials included liver disease, renal disease, muscle wasting, ruminal imbalance, and study-related complications. Chronic copper toxicity was added to the differentials list after others were excluded. Elevated copper levels in liver were confirmed after euthanasia and necropsy. Subsequently livers from 15 other sheep were tested for copper at end of study and at humane end point. Eight of the longest residents (46 mo to 144 mo in age, in-house for 2 mo or longer) in the facility were found to have significantly increased copper levels. Three of these animals presented with inappetence and weight loss. All of the animals' feed was tested for copper, including hay, water, concentrates, mineral salts, treats (e.g., fig cookies, marshmallows, berry cereal bars), and water sources. Some rooms were found to have mildly higher than recommended copper levels in drinking water. The mineral salt was found to have a markedly increased copper content of 46-49 ppm, despite being labeled for sheep with no copper added. The results were verified by testing a second batch. The manufacturer of the mineral salt was contacted, and use of this product was discontinued in the laboratory. One of the symptomatic animals experienced sudden cardiovascular collapse and death during the investigation. Clinical signs in the 2 other symptomatic sheep resolved. All other sheep remained asymptomatic after removing the source of copper from their diet. Mineral mixes with over 20 ppm of copper are not recommended for sheep and should be clearly labeled according to the Association of American Feed Control Officials Animal Feed Labeling Guide. Close monitoring of laboratory sheep may help identify early signs of copper toxicity before hemolytic crisis. Verifying feed copper levels, even in products sold for sheep, is recommended.

P114 Effects of Capromorelin in Buprenorphine-induced Poor Appetite in New Zealand White Rabbits (*Oryctolagus cuniculus*)

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Poor appetite is a common clinical sign in rabbits associated with stress from handling and transport, surgical procedures, and treatment with analgesics such as opioids. In dogs, the medication capromorelin has been investigated and has shown promise as an appetite stimulant. Additionally, a recent study has shown that capromorelin increased food intake and fecal output in healthy New Zealand White rabbits. However, the effect of capromorelin in rabbits exhibiting poor appetite is unclear. In this crossover study, we evaluated the effectiveness of capromorelin as an appetite stimulant in 9 healthy New Zealand White rabbits with buprenorphine-induced poor appetite. All rabbits randomly rotated through 5 treatment groups, including subcutaneous buprenorphine twice a day with oral capromorelin once a day, subcutaneous buprenorphine twice a day with oral saline once a day, subcutaneous saline twice a day and oral capromorelin once a day, subcutaneous saline twice a day and oral saline once day, and untreated control. All treatments were given for 3 consecutive d. Food intake, fecal output, and water intake were measured daily for 7 d with a washout period of a minimum of 1 wk between each treatment. Fecal output was measured by collecting and weighing the feces. Data were analyzed via generalized linear mixed modeling. Posthoc analysis showed that buprenorphine-induced poor appetite rabbits treated with capromorelin returned to baseline food intake and fecal output a day earlier than the buprenorphine and oral saline treatment group. Furthermore, capromorelin increased fecal output but not food or water intake in untreated rabbits, and handling stress from injections and oral treatments did not affect food intake, fecal output, or water intake. This study supports the use of capromorelin as an effective treatment for rabbits with opioid-induced poor appetite with the caveat that it may take a few days of administration to see an effect. This delayed effectiveness suggests that it may be beneficial to start capromorelin before giving buprenorphine. Additionally, this study further supports the use of buprenorphine as a model for reversible poor appetite and ileus in rabbits.

P115 *Candida glabrata* Outbreak in a Colony of Immunodeficient Mice

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A 7-wk-old, male NOD.Cg-*Rag1*^{tm1Mom}*Ins2*^{Akita}*Il2rg*^{tm1Wjl}/Szj (NRG-Akita), experimentally naïve mouse presented with a severe head tilt, circling, and barrel rolling. The physical examination was otherwise within normal limits and the animal was euthanized. Two of the 3 remaining mice in the cage developed torticollis and occasional circling behavior. Eventually, mice in 4 other cages developed similar and progressive clinical signs. All affected mice were the same strain and part of the same study. All mice were housed in autoclaved individually ventilated cages with autoclaved food, water, bedding, and enrichment. Physical examination and modified SmithKline Beecham, Harwell, Imperial College, Royal London Hospital, phenotype assessment (SHIRPA) testing were completed on affected animals. Gait, coordination, and locomotor activity were consistently affected. Respiration rate, urination, defecation, limb position, grip strength, visual placing, limb and body tone, and tail elevation were not impacted. The affected animals were euthanized and diagnostics including blood work, full necropsies with histopathology, and bacterial and fungal cultures were performed. Bloodwork abnormalities included severe hyperglycemia (>700 mg/dL) and hyperglobulinemia (2.9 mg/dL). Gross necropsy findings included thymic hypoplasia, splenic hypoplasia, white splenic and renal nodules, and nephromegaly. Histopathology revealed marked mixed inflammation and yeast-like organisms in the inner ear and kidneys. Positive fungal culture results which led to mass spectrometry of fresh brain tissue identified *Candida glabrata*. Successful mitigation was achieved through culling and replacement of affected animals with no further spread in the colony. A thorough investigation did not reveal a definitive source of infection, which was likely accidental contamination from an animal handler or other fomite. Furthermore, the agent did not spread to other investigators' cages in the same room, further implicating a fomite originating from the lab. Although case reports implicate other *Candida* species in mice due to accidental contamination, herein we report *Candida glabrata* associated with an outbreak of neurologic signs and significant morbidity in immunodeficient mice.

P116 Characterization of *Helicobacter* Species in a Captive Colony of Olive Baboons (*Papio anubis*)

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Helicobacter spp. are gram-negative, helical bacteria with gastric or enterohepatic tropism that can be commensal, opportunistic, or pathogenic, depending on bacterial and host factors. While gastric *Helicobacter* has been widely studied in both humans and non-human primates (NHPs), the pathogenesis and treatment of enterohepatic *Helicobacter* species are less well-characterized. Few studies have evaluated naturally occurring *Helicobacter* infections in baboons, but both gastric and enterohepatic species have been described. In the current study, a captive-housed adult olive baboon (*Papio anubis*) developed regurgitation and hematemesis during an anesthetized procedure, after which *Helicobacter* was histopathologically identified from a gastric biopsy. Real-time PCR from feces detected *Helicobacter* spp. using a genus-wide assay, but available species-specific *Helicobacter* assays were negative. 16s rRNA sequencing closely matched several enterohepatic species, including *H. fennelliae*, *Helicobacter* sp. (MIT 03-7674c), rhesus monkey taxon 4 (MIT 99-10781), and *Helicobacter* sp. (Mainz). *H. fennelliae* is a human pathogen with no reports of natural infection in NHPs. Oral treatment with clarithromycin, amoxicillin, and omeprazole successfully cleared the infection based on follow-up fecal PCR; no further clinical signs occurred over the following 9 months. Subsequently, fecal PCR was performed on 11 adult baboons, individually housed in 2 shared rooms, for *Helicobacter* species commonly found in other NHPs. These animals were clinically healthy at the time of surveillance; 4 baboons had a prior history of acute gastrointestinal signs, but no chronic or persistent signs. *H. macacae* (10/11), *H. pylori* (3/11), and *H. suis* (1/11), as well as an organism like *Helicobacter* sp. MIT 99-5504 (previously found in rhesus macaque) (1/11), were identified. The *H. fennelliae*-like organism was identified in 8 baboons; 3 baboons were not evaluated for this species. Antibiotic treatment was performed in 8 baboons and cleared the common *Helicobacter* species in all cases, but the *H. fennelliae*-like organism persisted in all 8 animals. Further research is necessary to determine if *Helicobacter* species may be pathogenic in baboons.

P117 Analysis of Blood Lactate and Glucose Values to Treat Severe Diarrhea Diseases in Rhesus Macaques (*Macaca mulatta*)

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Shigella spp., *Yersinia* spp., and *Campylobacter jejuni* diarrhea cases in NHPs are challenging to differentiate from typically less severe *Campylobacter coli* on rapid blood analysis. Being able to differentiate diarrhea pathogens and begin the most effective antibiotic treatment early leads to a better prognosis for NHPs. I hypothesized that blood lactate and glucose values could be used in the evaluation and treatment of diarrhea cases by analyzing current averages of lactate and glucose between diarrhea organisms, patient ages, and animal housing during capture. Our outdoor rhesus macaques are housed in either shelter group housing or larger corrals. During daily health checks outdoor animals with dehydrated appearances are brought to hospital and triaged, with blood draws performed within 1 h after presentation. Data was compiled on outdoor housed animals over 8 mo of time for diarrhea cases using point-of-care blood analysis and fecal culture laboratory testing. Averages were obtained from 138 NHPs for lactate and glucose based on fecal organism cultured, age of affected animal, and location/relative size of social group housing. *Shigella flexneri* caused the highest average lactate in the sample set. Glucose averages for *C. jejuni* and *Shigella flexneri* were substantially higher than *C. coli*. *C. jejuni* had the highest average glucose and lowest average lactate. *Yersinia spp.* patients had the lowest glucose and lactate average, although the sample size was smaller. Overall affected infants between 180 d (weaning age) and 1 y had the highest lactate and glucose, while affected adults over 5 y of age had the lowest average lactate and glucose at sampling. *Shigella flexneri* infection was identified more frequently in patients of older age, while animals under 2 y old, cultured *C. coli*, *C. jejuni*, and *Yersinia spp*. more frequently than did adults. Increased glucose and lactate were observed in cases of *Shigella flexneri* captured from corral housing. Point-of-care lactate and glucose values are beneficial tools in determining the possible severity of GI pathogens in NHPs when including variables of patient age and environment.

P118 Phalanx Amputation Secondary to Necrosis in a Seba's Short-tailed Bat (*Carollia perspicillata*)

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An adult male Seba's short tailed bat (*Carollia perspicillata*) presented with depigmentation of its right distal wing membrane. Four weeks prior to presentation, an aluminum headpost was placed by the lab to facilitate optokinetic response and the vestibulocochlear reflex recordings. The bat recovered well from headpost placement and underwent training following standard procedures. On the fourth day of training, membrane depigmentation was reported by the lab. On exam, the membrane was completely depigmented with no other evidence of trauma or disease to the skin. No other abnormalities were appreciated, and the bat was behaviorally normal. Membrane depigmentation differentials include nutritional deficiencies, parasitic infection, trauma, and idiopathic change. Monitoring was elected as no other bats in the colony were affected and parasitic infection was ruled out via tape test and visual exam. After the initial presentation, the condition remained static for about 2 wk, and the bat was allowed to resume on study. Following a behavioral recording session, the affected membrane acutely necrosed, resulting in bone exposure of the distal half of the lateral distal phalanx (P3). At this time, joint swelling and sores were appreciated on multiple wing joints, as well as new membrane depigmentation on the contralateral wing tip. Amputation of the necrotic wing tip was performed, and the bone submitted for histological analyses. Histology was consistent with tissue and bone necrosis, and no infectious agents were identified. The patient received courses of meloxicam and TMS postoperatively, as well as topical manuka honey therapy to the joint sores. The amputation site and sores healed well, and the bat returned to experimental use with no complications. These findings were nonspecific but suggest a noninfectious cause, such as pressure necrosis, as the causative factor. This case describes an effective treatment strategy for phalanx necrosis in a bat, while still maintaining normal behavior and flight abilities.

P119 Modelling the Cost Impact of Creating Greater Preclinical Reproducibility through Digitization of Efficacy Study Monitoring

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The cost of developing a new drug from the initial phase of target-to-hit to the final phase of launching the drug is $873 million. Key factors that influence this cost are the probability of the drug making it to the next phase (probability of success); the time it takes for the drug to reach the next phase (cycle time); and the number of 'work in progress' drugs within a phase (WIPs). Any improvements to these key factors can significantly reduce the cost of producing a successful drug. We investigated the impact on the cost to produce a successful drug when digitizing existing analogue preclinical methodologies, using a 3D and thermal subcutaneous tumor measurement device (3D-TI) in efficacy studies. A previously published economic model

was used alongside statistical analysis of 3D-TI performance, using a data set of ~3,000 scans of subcutaneous tumors captured by 3D-TI with corresponding caliper measurements. Our previous work (in silico modelling demonstrates that user variability during tumor measurement can affect in vivo therapeutic efficacy outcomes) found that the 3D-TI device increased the probability of success within the efficacy branch of the preclinical phase on average by 9% over a caliper measurement methodology. As efficacy studies make up a significant portion of the preclinical phase, when input into the economic model this resulted in a cost saving of approximately $33 million. The more reproducible, automated 3D-TI methodology also helps to facilitate a shift to the 'quick win, fast fail paradigm', shifting the focus on drug developments to rapid testing within the preclinical phase, greatly reducing cost and increasing probability of success within the later phases. Within this paradigm, certainty of results is imperative, and 3D-TI offers an increase in outcome certainty. This investigation clearly demonstrates that by digitizing and creating more reproducible preclinical study methods, the impact on translatability, cost of development and cycle times can be impacted in a significant way.

P120 Granulosa Cell Tumor in a Rhesus Macaque (*Macaca mulatta*)

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A 6-y-old, 9.35 kg, nulliparous, female rhesus macaque (*Macaca mulatta*) housed in an outdoor specific-pathogen free breeding group presented for suspicion of simian retrovirus (SRV) following positive routine screening during semiannual health assessment. Physical examination revealed firm, movable masses in the mid-caudal abdomen. Radiography identified radiopaque masses in the right caudal abdomen and left caudal lung. Ultrasound indicated that the abdominal mass was hypoechoic and bilobed. PET/CT imaging highlighted metabolic activity in the lung mass. Primary differential diagnoses included tumors of reproductive or urinary tract origin with pulmonary metastasis and SRV-associated neoplastic disease. Euthanasia was elected, and necropsy revealed that the right ovary was replaced by a 3 x 3 x 4 cm multinodular mass with irregular red, grey, and white zones, several small areas of mineralization, and a 1.3 cm fluid-filled cyst; the left caudal lung lobe contained a 3 x 4 x 2.5 cm firm, grey mass. Histologic appearance of the masses in the right ovary and lung were consistent with malignant and metastatic granulosa cell tumors. Uterine mucosal hyperplasia was identified, likely due to excess hormone excretion by the tumors. Confirmatory testing indicated that the animal's samples were exogenous SRV positive by serology (MFIA and western blot) by 3 laboratories and negative by PCR. Cytopathic effects (virus-infected cells) were observed on co-cultures of Raji cells and bone marrow, lymph node, peripheral blood mononuclear cells, and spleen; however, SRV PCR was negative for all cultured samples. The tumors are acknowledged as contributing to a lack of offspring production in this animal but are currently considered an incidental finding since a relationship between false SRV positive results and granulosa cell tumors has not been positively identified.

P121 A Spontaneous Compound Odontoma in an Adult Sprague-Dawley Rat (*Rattus norvegicus*)

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Reports of compound odontomas in rats are lacking in the scientific literature. A 1 yr and 2-mo-old adult male Sprague-Dawley rat was found to have a hard mass associated with the caudal aspect of its left mandible. After 2 wk of observation, the rat was euthanized due to the mass growing significantly in size and the rat losing over 20% body weight. Initial differential diagnoses included osteosarcoma, ossifying fibroma, or tumor of dental origin (e.g., odontoma, ameloblastoma, cementoma). Grossly, the mass was well-circumscribed and 3.7 x 3 x 1.2 cm in size, hard, and heterogeneously colored white, tan, and red. The mass was associated only with the mandibular bone and not any surrounding subcutaneous tissue. On cut surface the mass was similarly colored and felt brittle. Histologically, the mass had numerous proto teeth embedded in ossified stroma. Each proto tooth had a central mesenchyme pulp surrounded by columnar odontoblasts and dentin matrix. The dentin was often bordered by enamel matrix, which itself was occasionally bordered by ameloblasts. These histologic findings are consistent with a compound odontoma, the first time such a tumor has been reported in a rat.

P122 Regurgitation in Two Laboratory Pigs

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Two laboratory pigs were reported to veterinary staff for vomiting and weight loss. The pigs were enrolled on 2 different studies and originated from two different vendors. The first pig was a 6-mo-old, female intact duroc, enrolled on a dermal wound study. The pig was sedated for study purposes multiple times. Following the sixth routine sedation, the pig began vomiting upon recovery. The second pig was a 6-mo-old, castrated male Yorkshire, enrolled on a cardiac imaging study. This pig recovered from the initial imaging, ate rations normally, and was returned to group housing. Three days later this pig was identified for weight loss (~4kg weight loss over 5 d), and further examination resulted in identification of vomiting. Differential diagnoses could include infection, foreign body, or inflammatory disease. Both pigs were treated with anti-nausea medications (famotidine and/or maropitant; 1mg/kg by mouth twice daily and 1mg/kg subcutaneously once daily, respectively) and gastric protectants (sucralfate; 1g by mouth twice daily). With treatment, symptoms improved but did not fully resolve. On follow-up evaluations the pigs were observed regurgitating as opposed to vomiting. Regurgitation is the passive ejection of food, water, or saliva associated with esophageal disease. Differential diagnoses could include hypomotility or a mechanical obstruction. With the identification of regurgitation, the treatment plan was altered to continue famotidine and offer more frequent, smaller meals. With the new treatment plan, the first pig was able to gain weight and remain on study, allowing this animal to meet the study endpoint. The second pig was unable to gain weight like conspecifics but was able to maintain weight. Due to ongoing symptoms and poor weight gain, this pig was unable to meet the planned study endpoint. Following completion of the study, both pigs underwent a necropsy to determine the cause of the regurgitation. Both pigs had developed a lower esophageal stricture impairing the ability of ingesta to pass into the stomach. We theorize vomiting and/or a gastric ulceration resulted in inflammation and subsequent scarring at the level of the lower esophageal sphincter. This scarring resulted in an esophageal stricture resulting in the clinical signs presented.

P123 Cardiac Rhabdomyomatosis and Clinically Silent Porcine Respiratory and Reproductive Syndrome in a Yorkshire Cross Piglet

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A 5-wk-old, clinically normal, Yorkshire cross piglet was submitted to necropsy at the end of an acute neuromodulation study using focused ultrasound treatment on the cerebral cortex. On gross exam, the lungs were mottled dark pink to red, slightly firmer than normal, and oozed small to moderate amounts of foamy, clear fluid consistent with possible interstitial pneumonia and pulmonary edema; all other organs appeared grossly normal. Histologically significant changes were identified in the heart, lungs, and several lymphoid tissues. In the heart, there were multifocal, variably sized (0.5-8mm), well-demarcated, unencapsulated, noninfiltrative intramuscular nodules expanding the right and left ventricular free walls and interventricular septum. These nodules were composed of large myoblastic cells containing abundant vacuolated and finely granular eosinophilic cytoplasm known as spider cells. A few small nodules also contained non-vacuolated cells resembling cardiac Purkinje fibers. These histologic findings were consistent with cardiac rhabdomyomatosis which is characterized by cells sharing features of both cardiac myofibers and Purkinje cells with glycogen vacuolization. This rare lesion is often congenital with familial predisposition reported in red wattle and red wattle-cross piglets. While most affected animals are asymptomatic, reports of sudden death in pigs and other species from suspected interference with myocardial conduction have been described. Other significant histologic changes included fibrinosuppurative and lymphohistiocytic interstitial pneumonia and moderate to marked lymphoid depletion and necrosis in several lymph nodes and the tonsils, suggestive of porcine respiratory and reproductive syndrome (PRRS) viral infection, which was confirmed in two clinically affected cage mates a week following this necropsy. As swine are commonly used as cardiovascular models, and as any of the lesions described herein could have potentially impacted cardiovascular or cardiopulmonary function, this case report emphasizes the importance for performing comprehensive analyses to investigate any potential asymptomatic and incidental lesions that could confound research results.

P124 Idiopathic Systemic Amyloidosis in Laboratory Society Finches (*Lonchura striata domestica*)

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Two adult society finches (*Lonchura striata domestica*) obtained from a commercial vendor and housed in quarantine at a biomedical research institution were evaluated for lethargy and abnormal respirations. On physical examination, the finches were found to be exhibiting increased respiratory effort and depressed attitude with delayed reactions to stimuli. Ancillary diagnostics for the quarantine group ruled out *Chlamydia psittaci* by negative PCR and a pooled fecal sample detected tapeworms. The finches received treatment with combined praziquantel and oxfendazole (30 mg/kg) in their drinking water, administered twice over 14-d. Differential diagnoses for the lethargy included shipping-related stress, subclinical dehydration, or infectious etiologies. Euthanasia was elected due to poor prognosis, and a necropsy was pursued to elucidate the causative disease. Necropsy was additionally pursued for 2 other adult society finches found as mortalities. Gross findings at necropsy included external feather loss and thickening of the liver surface. Histopathology of multiple organs revealed abnormal accumulation of amorphous eosinophilic material within tissue structures. Green birefringence of the accumulated material when stained with Congo red and under polarized light confirmed the presence of amyloid. The amyloidosis was multifocal, affecting the spleen, liver, kidneys, and pancreas of affected finches to varying degrees. Furthermore, histopathology revealed myocardial degeneration, renal tubular degeneration, and pneumonia, providing evidence of ongoing disease processes. Amyloidosis has been reported in captive zebra finches (*Taeniopygia guttata*) and in one breeding colony of society finches. Organ dysfunction represents a significant sequela that may follow amyloid accumulation. The inciting inflammatory events are unknown in these cases; however, the outcome is striking since it highlights an underreported disease of society finches. Although amyloidosis has been described in other avian species and in breeding society finches, amyloidosis should also be considered as a differential diagnosis for society finches used in biomedical research that present with nonspecific clinical signs.

P125 Presumptive Soft Tissue Injury Presenting as Paresis in a New Zealand White Rabbit

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A 2-y-old, female, New Zealand White rabbit was reported for lethargy and tetraparesis. One day prior to presentation, the animal was placed at full contact with another rabbit in a large floor pen for social housing. No external injuries nor signs of fighting were appreciated in either rabbit; apart from the paresis, physical exam was unremarkable. On neurologic exam, the animal was obtunded, proprioception was absent in all limbs, and deep pain sensation was intact. CBC and chemistry findings were nonspecific but revealed dehydration and low blood glucose. Unsedated spinal radiographs showed no spinal fractures. Due to a lack of torticollis and phacoclastic uveitis, *Encephalitozoon cuniculi* was ruled an unlikely diagnosis. Given facility exclusion criteria, combined with the lack of an obvious infectious cause for clinical signs, supportive care and a tapering antiinflammatory dose of prednisolone were prescribed to treat a suspected soft tissue injury. Four days after initial presentation and prednisolone treatment, the animal was still paretic in the hind limbs, but mentation had improved. Additional sedated spinal and pelvic radiographs ruled out hip dysplasia and vertebral disc pathology. Given the minimal response to prednisolone and to support normal posturing, hobbles were made using medical tape, preventing the hind limbs from splaying and giving the animal enough support for upright posture and ambulation in standard rabbit rack housing. The hobbles remained in place for 9 d and did not require replacement. Vitamin B was supplemented subcutaneously for the first 3 d and a nutritional mash was provided for the duration of hobble placement in case the hobbles impeded the ability to eat night feces. Localized hyperplasia formed on the lateral metatarsals at the site of the hobbles, which were subsequently discontinued. The animal was able to regain strength and muscle tone, indicating a presumptive diagnosis of soft tissue injury.

P126 Behavioral and Clinical Characterization of Mice Presenting with Atypical Ulcerative Dermatitis

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Ulcerative dermatitis (UD) is one of the most prevalent conditions in laboratory mice. As we have previously reported, mice at our institution commonly present with 2 primary types of UD: typical UD (tUD) and atypical UD (aUD). tUD lesions are partial thickness

lesions commonly confined to the neck or ears, whereas aUD lesions are full thickness skin lesions not confined to the neck or ears. aUD is refractory to first-line therapies for tUD, such as nail trims, and often necessitates removal from study. Mice with aUD are frequently observed during cage side evaluations performing excessive oral grooming at the site of the lesion rather than scratching which led us to refer to these cases as "lick-chews". To further characterize aUD, electronic medical records were retrospectively evaluated from a 12-mo period to determine if aUD lesions were correlated with specific anatomic locations and if the number of aUD cases was affected by seasonality. In addition, representative animals with tUD or aUD were video recorded for behavioral assessment and a clinical scoring system was revised to assess aUD lesion severity. The average mouse daily census was approximately 22,250 cages and there were 8,488 completed mouse health reports during this period. Forty-one percent of the health reports were classified by veterinary staff as UD. aUD accounted for 19% (644/3,453) of all UD cases. Lesions affecting the neck or ears accounted for only 7% (27/341) of aUD cases. In comparison, lesions affecting the neck or ears accounted for 69% (1,707/2,478) of tUD cases. There was no apparent seasonal pattern of aUD. Mice with aUD demonstrated higher frequencies of oral grooming at the site of the lesion whereas mice with tUD were more likely to exhibit higher frequencies of scratching at the site of the lesion. This data demonstrates that mice with aUD engage in different injurious behaviors than mice with tUD and that aUD lesions are not confined to the neck or ears. Mice with aUD should be distinguished from mice with tUD when evaluating potential etiologies and novel treatments for UD. The aUD scoring system should be further evaluated for implementation as a cage side tool for initial assessment of aUD lesions in mice and their response to potentially efficacious therapies.

P127 IPC-366 Is a Good Model in Balb/SCID Mice for study of Triple Negative Breast Cancer

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Inflammatory mammary cancer (IMC) is known for being a spontaneous and aggressive canine tumor. IPC-366 cell line has been established as a good in vitro model for studying this disease, due to its high malignancy. Previous authors demonstrated that steroid hormone production is involved in tumor progression; so the use of antiestrogenic compounds, such as anastrozole, can reduce the progression of the disease. Therefore, the aim of this study is to demonstrate that IPC-366 is a good in vivo model in Balb/SCID mice for studying IMC. For this purpose, a comparison of anastrozole efficacy in vitro and in vivo was performed using IPC-366 cells. In vitro studies consisted in a IPC-366 sensitivity assay to determine the half-maximal effective concentration of anastrozole which resulted in 1µM, and a subsequent cell viability assay was performed. In this assay, IPC-366 were seeded in 96 well-plates and treated with 1µM of anastrozole and media was collected. Parallel, a suspension of 10^6 IPC-366 cells was inoculated in the mammary fat pad of ten 6-8-wk-old female Balb/SCID mice. Mice were divided into 2 groups: control group, injected with saline solution; and experimental group, injected intraperitoneally with 3mg/kg/day of anastrozole for 10 d. Tumor volumes were measured periodically. At the end of experiment, blood samples were obtained intracardially, and immediately after were euthanized by cervical dislocation. At necropsy, tumors were collected and homogenized. Culture media, blood samples, and tumor homogenates were analyzed by enzyme immunoassay to determine estradiol and testosterone concentrations. Results revealed an intimate correlation between in vitro and in vivo results. Anastrozole reduced cell proliferation in vitro and tumor progression in vivo. Also, IPC-366 resulted in a rapid tumor progression in Balb/SCID mice. Regarding steroid hormone secretion, results showed that anastrozole treatment reduced estrogen and androgen secretion either in vitro or in vivo experiments, denoting that hormone secretion influence on treatment efficacy. In conclusion, in vitro studies resemble in vivo results, allowing us to assure not only that IPC-366 is a good model in Balb/SCID, but also animals could be reduced thanks to the results obtained in preliminary in vitro studies.

P128 Pharmacokinetics of Methylnaltrexone Bromide after Subcutaneous Administration in Rhesus Macaques (*Macaca mulatta*)

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Opioids are an integral component of the pain management plan for Rhesus macaques (*Macaca mulatta*) that undergo major surgeries or require analgesics. Opioids, while potent analgesics, have well known adverse effects in all species including bowel dysfunction with clinical signs of constipation, bloating, delayed gastric emptying, and firm stool. Methylnaltrexone bromide (MNTX), like the familiar drug naltrexone, is a selective mu and kappa opioid receptor antagonist. However, unlike naltrexone, methylnaltrexone bromide possesses an N-methyl-quaternary amine group preventing the molecule from crossing the blood brain barrier. Methylnaltrexone bromide can selectively antagonize the gastrointestinal opioid receptors without affecting central analgesia from a previously administered opioid. In the current study, 6 healthy adult Rhesus macaques were included in a methylnaltrexone bromide pharmokinetic analysis of serum and cerebrospinal fluid. We hypothesized that a 0.15 mg/kg subcutaneous dose of methylnaltrexone bromide in rhesus macaques would demonstrate a Tmax of 0.5 h, similar to humans, and that there will be no methylnaltrexone bromide yielded in the cerebrospinal fluid, due to its proposed mechanism of action and inability to cross the blood brain barrier. We achieved peak plasma concentration (113.88 ± 43.92 ng/mL) sooner than in human trials, at 0.25 h. And we demonstrated there is minimal cross over of methylnaltrexone bromide into the brain at concentration 0.35 ±0.21 ng/mL. These findings show that subcutaneous administration of methylnaltrexone bromide provides an option for a selective opioid reversal agent with minimal cross over into the brain and thus represents a potential solution to opioid induced gastrointestinal side effects.

P129 Galactorrhea and Dysmenorrhea in an Adult, Female Cynomolgus Macaque (*Macaca fascicularis*)

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A 14-y-old, 4.0 kg, female Cambodian cynomolgus macaque enrolled in a chronic addiction study presented with bilateral galactorrhea during a physical exam. Complete blood count, chemistry panel, and urinalysis were unremarkable. Cytologic evaluation confirmed normal mammary secretion and no indication of neoplasia or infection. Follow-up exam with radiographs and abdominal ultrasound were unremarkable and confirmed nonpregnant. Blood was collected to evaluate thyroxine (T4) and prolactin levels. Four additional female macaques including 2 age-matched were also evaluated. Thyroxine and prolactin levels were within normal limits; however, prolactin levels were highest for this animal. A 2-d video assessment revealed no abnormal neurologic behaviors, self-manipulation of the nipples or mammary glands, or over grooming by housemates. Five months later the animal presented with dysmenorrhea with normal timed menstrual cycles and no menorrhagia. CT scan of the abdomen showed an enlarged uterus that appeared to displace the colon laterally. An exploratory laparotomy was performed to evaluate for neoplasia, diverticulosis, endometriosis, ovarian, and uterine pathology. A 0.5 x 0.5 x 0.5 cm left ovarian cyst and bilateral fibrous adhesions of the omentum to

the ovaries were present. The uterus was mildly enlarged 7 x 5 x 4 cm with normal architecture. An ovariohysterectomy was performed and the remainder of the exploratory was unremarkable. Abdominal pain resolved and was attributed to the ovarian adhesions and cyst. Drug-induced galactorrhea is the most common cause of galactorrhea in humans after infancy and was ruled out because galactorrhea consistently persisted in the presence and absence of drug. Pituitary adenomas are the next most common cause of galactorrhea in humans and have been well documented in macaques. Diagnosis of a pituitary adenoma in this case is a challenge as necropsy diagnosis is not an option at this time, only prolactin level was evaluated, and imaging may not be definitive. The presence of a regular menstrual cycle and normal prolactin level are also abnormal findings with galactorrhea. Plan is to continue monitoring and MRI. Galactorrhea at this time diagnosed as idiopathic.

P130 Spontaneous Hypercholesterolemia in Common Marmoset (*Callithrix jacchus*): Possibility of a Familial Hypercholesterolemia

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Familial hypercholesterolemia (FH) is an inherited genetic disorder (autosomal dominant). It is known that several genetic mutations are related with FH and it is characterized by markedly elevated total cholesterol and low-density lipoprotein cholesterol (LDL-C) levels in serum. Humanized mice and genetically engineered mice have been established as animal models for FH study, but there are limits to clearly mimicking the phenotypes of human FH. In biomedical research, the demand for the common marmoset (*Callithrix jacchus*) as a laboratory animal has rapidly increased over the past decade. However, the insufficiency of disease models is a major weakness of marmoset as a laboratory animal. Spontaneous mutant disease model for marmoset is not well established. In our breeding colony, we found that several marmosets showed spontaneously elevated serum total cholesetrol and LDL-C levels. Furthermore, low body weight, jaundice, skin keratinization and low thyroxine level were observed. When the family tree was traced, while 6 out of 19 offsprings from first founder pair and 3 out of 14 from second founder pair showed hypercholesterolemia, all 21 offsprings from third founder pair showed normal serum cholesterol level. These results demonstrate that hypercholesterolemia in the marmosets is significantly associated with inherited disorder and it is similar to human FH. Analyzing the correlation between FH-related genes and the serum cholesterol level in our marmoset colony will be performed through our follow-up studies. It is expected that these finding would contribute to establish a useful spontaneous mutant disease model for biomedical research

P131 Using Industry Confirmation Standards via Digital Photography to Predict Early Onset of Lameness in Swine in Biomedical Research

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Digital photography has proven to be a useful prognostic tool within an ABSL 3-Ag setting. The advent of waterproof, high-quality digital cameras allow these cameras to be completely submersed within disinfection/sanitization fluids for recommended periods of time without harm to camera or digital media. The Animal Resource Branch (ARB) needed a diagnostic tool to visualize the root cause of lameness and decide the disposition of the animal in relation to the study the animal was assigned to. Animals which displayed early onset lameness could, by virtue of adequate veterinary care, eliminate themselves from biomedical research studies. Digital photography provided an objective foot and leg conformation record which enabled the ARB to rank animals for prospective lameness monitoring. Three photographs of each animal were recorded: 2 side views of the animal standing squarely, and a third view of the haunches (buttocks to floor). The joint angles for knee, hock, front and rear pasterns, and a rear stance position were measured. Twenty animals over a period of 1 y from 1 specific vendor were analyzed for likelihood of early onset lameness. Based upon the photographic record of each individual animal, the animals were ranked for use by likelihood of becoming lame sooner rather than later. Post euthanasia, veterinary record review provided dates of lameness observations for the individual, if applicable. Study results document a 66% correlation between joint angles analysis and the veterinary record documentation of lameness of study animals. This study, conducted within an ABSL 3-Ag setting with limited availability of diagnostic equipment, proved digital photography to be an indispensable tool.

P132 Age-related Ocular Changes in F344 Rats

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Two recently shipped 26-mo-old female F344 rats presented for evaluation of ocular abnormalities. On physical examination, both rats had rough coats, low body condition scores, hunched posture, and were hyperresponsive. Ocular examination revealed lenticular opacities in both eyes (OU), buphthalmia OU, corneal vascularization OU, and porphyrin staining OU. Fluorescein staining was negative OU on both animals. Treatment consisted of ibuprofen in the drinking water (20 mg/kg), subcutaneous sustained-release buprenorphine (1 mg/kg), topical triple antibiotic ointment OU, removal of porphyrin staining from the fur with saline, nail trimming, and supportive care (moistened chow, nutritional supplmement) provided on the cage floor. The rats were euthanized 1 wk later as part of the experimental study and the entire body was submitted for histopathology except spinal cord and brain, which were removed for study purposes. Findings included retinal degeneration, lens degeneration (cataract), corneal epithelial mineralization with keratitis, closed iridocorneal angle (glaucoma), and harderian gland dacryoadenitis. Both retinal and lens degeneration are common age-related, nonpainful ocular changes in rodents that can be accelerated by chronic exposure to high-intensity light. Corneal mineralization has a very high incidence in the F344 rat strain and is non-painful but can progress to painful conditions such as keratitis or corneal vascularization, which were seen in this case. Glaucomatous changes were likely secondary to cataracts and responsible for the buphthalmia observed. Inflammation of the Harderian gland was likely stress-related and explained the copious amount of periocular porphyrin staining. These cases provide a comprehensive review of common ocular findings in aged rats.

P133 Tiletamine/Zolazepam with Dexmedetomidine Effectively Anesthetizes Rats

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This study evaluates anesthesia induced by tiletamine/zolazepam with dexmedetomidine in rats. We hypothesized whether 7.5 mg/kg tiletamine/zolazepam with dexmedetomidine would provide better anesthesia than 2.5 or 5 mg/kg tiletamine/zolazepam with dexmedetomidine in a rat skin biopsy model. Adult Wistar rats (n=21) were subcutaneously administered with either 2.5 (T2.5), 5

(T5), or 7.5 (T7.5) mg/kg tiletamine/zolazepam combined with dexmedetomidine (0.25 mg/kg). After being anesthetized, rats were administered tramadol (12.5 mg/kg, SC) for analgesia. Heating pad, eye lubricant, prewarmed fluid (10 ml/kg/h, SC), and 100% O_2 were provided. When animals achieved a surgical anesthetic plane, skin biopsies performed, and anesthesia maintained for 45 min until atipamezole (1 mg/kg) was subcutaneously administered. Parameters monitored were: 1) anesthesia quality [onset of induction (time to lateral/sternal recumbency), onset of surgical plane (time to loss of paw withdrawal), duration of surgical plane (time from loss to return of paw withdrawal), onset of recovery (spontaneous movement); 2) physiological parameters (heart rate, respiratory rate, body temperature, and $\%SpO_2$). Results indicated: 1) onset of induction and duration of surgical plane did not differ among the 3 groups; 2) onset of recovery was longest in T7.5 (45 min) group. T2.5 (17%), T5 (17%), and T7.5 (58%) rats were re-dosed with atipamezole, which all rats were fully recovering; 2) physiological parameters did not differ in any group at any time point. T7.5 did not provide better anesthesia than T2.5 or T5 in this study. These results demonstrated that either 2.5 or 5 mg/kg tiletamine-zolazepam with dexmedetomidine effectively anesthetizes rats in a skin biopsy model.

Husbandry/Management

P200 Semiautomated Aquatic Live Feed Culture: A System Designed to Support Limited or Varying Production Needs

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The availability of nutritious, pathogen-free live feed of varied sizes for larvae remains a challenge when using aquatic species in research. Zebrafish facilities often rely on saltwater rotifers and *Artemia nauplii* to achieve optimal growth and survival. The maintenance of live feed cultures can be labor intensive and inconsistent culture maintenance can impact feed production. While semiautomated rotifer and artemia culture systems have been developed for use in larger aquaculture facilities, we aimed to design a low-cost, semiautomatic aquatic feed culture system for smaller production yields (1L daily). Using materials available at a local hardware store we designed a system with three 2.5L culture vessels that provide redundancy and scalability, as well as semiautomated feeding, harvest, and water exchange to minimize staff labor for rotifer culture. This system can maintain a rotifer culture density of as high as 1000 rotifers/ml which is comparable to larger culture systems and allows for optimization of live feed and larval culture practices for our zebrafish core facility. It can also be used in programs with small zebrafish facilities, or those that want to simultaneously maintain different species of live feed to support several aquatics species or fish life stages. It can be easily decontaminated between culture batches. While our focus has been on rotifer culture, this system can be adapted for use in phytoplankton or copepod culture, as well as hatching and rearing *A. nauplii*.

P201 The Great Retention: How to Keep the Best Employees Working in Your Facility

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Husbandry staff retention and development are vital to the successful operation of an animal research facility. High levels of employee turnover can negatively affect the morale of the team and service continuity for researchers, as well as increase the workload for the remaining employees. Critical turning points that put additional stress on an animal facility, such as the COVID 19 pandemic, can magnify the situation and further increase the sense of burnout among the remaining staff. To increase the likelihood of employee retention and encourage husbandry staff to pursue internal promotions, our leadership team has focused on 4 key points to meet these goals: provide opportunities for continuing education, emphasis on effective communication with the staff, ensure adequate recognition of employee contributions to the team, and create a psychologically safe culture where coaching and mentoring is used to minimize the need for formal disciplinary action. As a result of implementing these strategies, there has been a substantial increase in internal promotions and a decrease in resignations, having a significant operational impact.

P202 Determining Treat Preferences in Diabetic Yucatan Swine

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Determining safe and palatable treat options for diabetic swine in a research facility is vital in providing enrichment, as well as identifying a high-value reward for training purposes. Whether for daily husbandry or to support study objectives, treat training is a helpful method for rewarding laboratory animals. Treat rewards can be an additional portion of the animal's regular diet or can be a novel enrichment item. While sugary treats tend to be preferred by swine, diabetic animals pose a unique challenge. Diabetic swine require a treat that achieves motivation yet does not have major effects on the individual's blood glucose or overall health. This is especially important because small changes in diet can have a significant impact on study data by causing unpredictable alterations in blood glucose. To trial potential treats for diabetic Yucatan swine within our facility, a list of veterinary-approved food options was created consisting of low glycemic index fruits, vegetables, sugar-free yogurt, and peanuts. Seven diabetic adult Yucatan swine were presented with a selection of these treats 1 to 2 times a day over a 2-w period. Treat type, the amount given, the animal's interest level, and whether they consumed any of the treats were documented in each animal's medical record. Unique preferences and acclimation periods for individuals were noted. Despite varying degrees of interest in the treat items, initially, all individuals rarely consumed the treats. After repeated exposure to the same treats, they began to consume preferred treats. Testing determined that low glycemic index foods can be used for positive reinforcement and training in diabetic pigs. Preference testing can help determine each animal's ideal high-reward treat.

P203 Developing a Veterinary and Husbandry Plan for the Virginia Opossum (*Didelphis virginiana)*

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In 2017, an investigative team studying the kinematics of chewing submitted a protocol to use the Virginia opossum (*Didelphis virginiana)*, a species uncommonly found in current literature. We were challenged by a limited experience as only one team member had worked with them before at a different institution. To accommodate the investigator's study, the veterinary and husbandry teams worked together to determine a variety of factors including sourcing, housing, diet, enrichment, and medical care. Due to the lack of known commercial vendors of research opossums, the investigator decided to apply for a research permit to obtain wild-caught specimens. To house the normally solitary and nomadic species, we used nonhuman primate play cage-style housing with wood branches to promote species-specific behavior such as climbing

and exploration. Because opossums are nocturnal, they were housed in a room with a shifted light cycle to ensure they were awake during the lab's planned work time. To standardize the diet of a normally omnivorous scavenger, we provided them with 60 g of a commercial diet supplemented by daily enrichment items such as small pieces of cheese, ham, or fresh produce. We consulted wildlife rehabilitation protocols to develop reproducible intake procedures, inclusive of the topical application of moxidectin/imidacloprid antiparasitics on arrival. Animals were treated prophylactically with Vitamin K, since the history of potential rodenticide exposure was unknown. We have continued to refine our veterinary procedures with this species, including updated preoperative induction with alfaxalone (10 mg/kg) +/- midazolam (0.5 mg/kg) and providing long-acting opioid analgesia postoperatively instead of repeated injections of buprenorphine. We were able to provide stable husbandry and veterinary care for this unusual lab species through team collaboration and focused research on best practices.

P204 Comparison of Agents to Maintain Hydration of Yucatan Minipig Skin

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Yucatan minipigs are commonly used in biomedical research. These animals are prone to dry skin (xerosis) accompanied by pruritus. In previous work, housed minipigs were oiled twice weekly with lanolin to mitigate this condition. However, this resulted in excessive debris along the dorsum, sometimes accompanied by exfoliative lesions. In this study, alternatives (glycerol and coconut oil) were used to compare their abilities to hydrate skin appropriately. The agents were applied twice a week over a 4-w period in a crossover design so that each pig was treated with each agent for 1 wk with a 4-d washout period. The skin was assessed using a corneometer and both visual and tactile scoring at various timepoints. Results from visual and tactile scoring 24 and 48 h after each application showed that the ideal skin condition was achieved in the following order: glycerol (83.3% visual, 75% tactile), coconut oil (79.2%,64.6%), control (33.3%, 39.6%), followed by lanolin (29.2%,12.5%). No significant difference was found from the results of the corneometer; it was concluded that the device was inaccurate for this purpose. Lanolin pooled on the skin and left the pigs greasy days after the agent was applied while glycerol had the highest frequency of ideal scores. In conclusion, glycerol was found to be the best option for hydrating Yucatan minipig skin in the research setting.

P205 Scale Training Program for Yucatan Swine to Increase Weighing Efficiency and Reduce Stress Behaviors

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Laboratory swine are weighed for a variety of reasons to achieve research goals. Within our facility, the frequency of weight data collection is upon arrival, and typically weekly to biweekly thereafter depending on breed and study expectations. When swine are not trained to enter and remain still on the mobile scale, it can be difficult to record weights and swine may exhibit stress behaviors. To promote the efficient collection of weight data and improve animal welfare, we instituted a training plan. Our plan was designed to condition the pigs to the process of weighing and make it a positive experience while improving the efficiency of collecting the animals' weight data. This training plan was instituted for 8 adult Yucatan pigs. They were trained to sit, follow a lure, touch a target, acclimate to a scale, and then sit quietly on a scale while their weight was being collected. Using this training plan, we observed that swine were eager to get on the scale and participate in the weighing event. The time required to collect weight data was reduced because they sat still while the weight was being read. We determined that by following this training program, there were fewer stress behaviors observed, and the efficiency of collecting weight data was improved.

P206 Using a Time and Motion Study and the Importance of a Quality Assurance Position

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Research Services (RS) has increased personnel by 45% from 2011 to 2022. This expansion has contributed to inconsistencies in services delivered and how they researchers are billed. This prompted us to add a quality assurance (QA) position to our unit. This project focuses on time and motion and improving the overall billing process through QA. Charges were based on technicians completing self-evaluated time and motion studies (TMS) while simultaneously completing a requested task. Management would then take the evaluations and calculate the cost of each task. After noticing inconsistencies in the data, we decided to use the QA position to record the data while the technician performed the task. During this process, it was revealed that between 2011 and 2022, the fee per service experienced an average fluctuation of 79% in cost across technicians and billing periods. By focusing on 5 common services, we found the following discrepancies. On average, there was an increase in cost (i.e., undercharging) to wean (63.36%), wean/ tag/ tail (235%), tag (40%), gender determination and toeing (54%) and decrease in cost (i.e., overcharging) in body weight (-17%). This change was due to the QA focusing solely on the time, instead of another individual simultaneously focusing on time and the task. Furthermore, we discovered a broad misunderstanding in billing terminology due to a lack of definition and training. We will demonstrate how performing TMS with the QA can reduce the margin of error and prevent overcharging or undercharging of services. In addition, we will prove how redefining billing terminology and improving billing training will minimize inconsistencies in how services are billed, improving overall service level agreement quality.

P207 Refinement of Animal Transport Carts to Reduce Noise and Vibration Exposure

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At our institution it is common practice to transport rodents using plastic rolling carts, either after the arrival of a shipment of animals or to transport animals between facilities. Previous research has shown that animal transport with a hand-pushed cart exceeds recommended vibration levels. Therefore, an accelerometer and microphone were placed inside a typical plastic rodent shipping container and continuous noise and vibration levels were recorded during cart transport using the routine route taken between facilities. Noise and vibration levels were found to be significantly higher than currently recommended levels, which may affect breeding performance, immune function, cardiovascular parameters, and corticosterone levels. Therefore, multiple refinements were tested to mitigate noise and vibration exposure during transport with plastic rolling carts. Plastic wheels were replaced with rubber wheels and plate casters. Additionally, a 2 cm thick gel pad was placed underneath the shipping container. The baseline cart was compared to a cart with rubber wheels, a cart with a gel pad, and a cart with both rubber wheels and a gel pad. Each condition was tested 10 times on the predetermined route, and noise and vibration were measured continuously throughout the route. All 3 experimental conditions

showed statistically significant decreases in average and peak noise and vibration levels when compared to the baseline cart. The modification of rubber wheels with plate casters combined with a gel pad produced the greatest reduction in noise and vibration exposure: average noise exposure dropped from 84.4 dB SPL to 61.9 dB SPL, or a decrease of 26.7%, while average vibration exposure dropped from 1268.8 mg to 539.9 mg, or a decrease of 57.4%. Institutions may consider these rubber wheel and gel pad modifications to reduce the noise and vibration exposure of rodents during transport with rolling carts, which may reduce animal stress and research-related variables.

P208 Comparison of 3 Environmentally Friendly Enrichments in Singly Housed Sprague Dawley Rats (*Rattus norvegicus*)

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The majority of current enrichment literature is focused on mice; however, rats are often singly housed due to research objectives and would benefit from a species-specific enrichment program. Rats are a social species and single housing can have negative effects on their welfare. The objective of our study was to incorporate recycled disposable materials into 3 different enrichment items and determine which items were preferred. Fifty-one singly housed rats from a teaching colony were enrolled in the study and randomly assigned 1 item each week in addition to the colony's standard enrichment. Reusing disposable materials that were found in the research facility, as well as recycled outside materials that were autoclaved within the facility, staff created 3 types of paper/cardboard toys: a paper tootsie roll, a cardboard gift box, and a cardboard Christmas cracker. The staff then stuffed half of the toys with timothy hay and half the toys with shredded paper. The toy usage was measured daily with a ruler and the naturalistic nest scoring system was used to evaluate nest quality. This colony was routinely monitored for standard pathogens using dirty bedding sentinels. Based on chi squared analysis, rats preferred the cardboard gift box enrichment over the other two items; however, all items were used in addition to the standard enrichment. During the study period, no signs of injury or illness were noted in the daily health checks. Based on this study, we were able to safely incorporate novel enrichment items at minimal cost to the facility that greatly enhanced the welfare of singly housed rats.

P209 Best Practices for Cleaning a Vivarium

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Our department oversees the sanitation of the animal rooms in 10 buildings with 21 species, including 38,000 rodent cages. A comprehensive environmental monitoring program (EMP) was implemented to validate our sanitation processes throughout the department. The EMP exposed many challenges, particularly the effectiveness of our facility hygiene practices. ATP bioluminescence (Adenosine triphosphate) demonstrated the amount of organic material that remained after cleaning the floors was unacceptable. 48% of the floors failed with swab results of less than 500 relative light units (RLUs). To address this failure, we wanted to consolidate to one cleaner/disinfectant for all the surfaces in the animal rooms. The chemistry needed to show no behavioral aversion by the animals and have its cleaning and biocidal efficacy validated. We evaluated the cleaning effectiveness of 2 chemistries and 2 types of mops. An alkaline ph quaternary ammonium (quat) was compared to the chemistry of hydrogen peroxide (H_2O2). Cotton and microfiber mop heads were used. Four technicians received 1 chemistry, 1 mophead, and a 2-in-1 mop bucket. To create consistency, a specific protocol was followed for mopping. The behavior core tested potential odor aversion for each chemistry versus tap water using a 3-chamber assay. ATP samples were collected before and after to validate performance. The H_2O2 and microfiber mop exhibited a decrease in ATP ($P = 0.0039$) compared to the cotton mop. The quat and microfiber also trended towards significance ($P = 0.088$). Mice had a significant odor aversion to H_2O2 ($P < 0.0001$). Ergonomically, the microfiber weighed less than cotton. Considering aversion to the product, chemical residue buildup, efficiency, and cost, the quaternary ammonium was the chosen chemistry with the microfiber mop and 2-in-1 bucket system compared to the H_202 chemistry. Cleaning validation with ATP will be performed quarterly.

P210 Evaluation of Disinfection Methods for Aquatic Artificial Plants in Zebrafish Recirculating Support Systems

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The use of aquatic artificial plants as environmental enrichment for zebrafish in biomedical research facilities has been proven to improve overall animal wellbeing and reduce stress and anxiety. Despite the benefits they provide for zebrafish welfare, some research facilities are hesitant to begin implementing them into their routine husbandry practices due to concerns for disease transmission and a lack of guidance on the most effective disinfection practices between tanks. There have been few publications on ways to adequately disinfect aquatic artificial plants as they are commonly reused between tanks within the life support system. Investigating proper sanitation and disinfection methods for these enrichment items are crucial to preventing the spread of pathogens within the aquatic life support system. Two disinfection methods, a commercial grade laboratory glassware dishwasher and an ethylene oxide sterilizer (ETO), were evaluated using ATP detection and bacterial culture of aquatic artificial plants pre-and post- disinfection process. Plants were placed in the dirty sump of 2 separate recirculating life support systems (2,500-3,000 fish/system) for 2 wk before the start of the study. The commercial grade laboratory glassware dishwasher and ETO sterilizer reduced ATP levels by 100% and 97.19%, respectively. Both methods resulted in the complete eradication of live bacteria present pretreatment. This study demonstrates 2 effective methods for disinfecting artificial aquatic plants in zebrafish facilities.

P211 Assessment of A Gel-based Diet for Zebrafish: Water Quality, Feed Consumption, Growth Rate, and Reproductive Success

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Standard meal-style feeding of laboratory zebrafish requires considerable time, and ad libitum feeding is typically infeasible in aquatic environments. A commercial gel-based diet that mimics naturalistic grazing for zebrafish and reduces labor for personnel was recently developed. We hypothesized that adult zebrafish fed the new gel diet would have similar growth rates and reproductive success compared to those fed a standard commercial pelleted diet. Water quality safety was first assessed by measuring water quality parameters with both food types in static tanks for 24 h. Water quality remained safe for fish for 24 h in static conditions for the gel diet group, but not for the pellet diet group. Then 200, 23-wk old AB zebrafish of mixed sex were randomly placed in groups of 20 in 3.0 L recirculating tanks. Each tank was randomly assigned to either gel or pellet diet for 12 wk. The gel diet was fed as 1 or two 1 cm^3 cubes per 20 fish once daily, and the pellet diet was fed twice daily at 3% body weight per day. Fish were weighed and photographed every 2 wk with nose-to-caudal-peduncle body lengths measured using image analysis software. After 5 wk, a subset of randomly selected males or

females from each diet were bred. Viable eggs and embryos surviving 24 h were counted for each spawn. Data was analyzed using repeated measures ANOVA. Zebrafish consumed less of the gel diet than expected, requiring removal of excess feed to prevent tank fouling. By week 4, fish fed the gel diet had significantly shorter lengths and lower body weights. Gel diet fish generated fewer viable eggs and embryos than pellet diet fish. Low consumption of the gel diet may have led to slower growth rates and less reproductive success. Macro or micro-nutrient differences between the 2 diet types may have also contributed to altered growth and fecundity. Though the gel diet allowed for once daily feeding and could be more fish-safe in static conditions, the tested formulation was not a satisfactory alternative for the standard pellet diet in a research zebrafish colony.

P212 The Designing and Establishment of a Vivarium-integrated Zebrafish Aquatics Core

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Among the most expensive aspects of preclinical infrastructure is the establishment and maintenance of facility space. When institutional research interests include the establishment of an aquatics core for zebrafish, whether to incorporate this new capability into vivarium space or instead separate it from the vivarium as a self-standing entity may be influenced by whether research and/or animal program staff will be responsive to substantive zebrafish oversight. Our institution elected to design and construct a flexible, versatile aquatics core that functions well as an element in a multispecies vivarium. Specific needs of aquatics quarantine, housing, and use, aquatics equipment processing, and mechanical support were identified and established in a total of 1,346 net square feet (NSF) of vivarium space during a 2020 new construction. Quarantine was established in a separate 57 NSF room and provided using a standalone recirculating rack. Housing, use, and equipment processing occur together in an 805 NSF room, supported by a computerized central life support system based in an adjoining mechanical room of 484 NSF with pumps, ultraviolet lights, and biological filters. Housing is in reverse osmosis-purified and conditioned water, filling 1,380 tank sizes of either 3.5- or 8-L. Live artemia and 4 different diets are fed robotically, using up to 24 programmable feeding cycles. 144 genotyping drawer-like tanks facilitate genotyping. Fish are housed, used tanks are sanitized in a cabinet washer, artemia are cultivated, and genes are targeted all in the same large housing and use room. This versatile space was made flexible by incorporating the elements required for housing terrestrial mammal species, including installing ceiling thimble connections for exhausting racks used to support individually ventilated cages (IVC) for rodents, should institutional research interests change in the future. Its location integrated within the vivarium contributes to research and animal care staff learning and interest, zebrafish oversight, and future anticipations.

P213 The Associated Benefits with the Implementation of Solid Detergent in Cage Wash Areas

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Laboratory animal care facilities often have space limitations, storage constraints, and ergonomic issues related to handling and transferring liquid chemicals and disposal of large bulky chemical drums. Disposal of the drums can negatively impact the environment, and the pump-off of the remaining liquid chemicals within a drum can be a challenge. Shipping, delivery, and receipt of chemicals can also be problematic. Because of these challenges, a large multisite animal care facility evaluated the use of solid detergent in conjunction with automated washers. Various concerns that affect cagewash operations were considered and required strategic planning before the implementation of solid detergent use. The pilot project to use solid detergent in place of 30 gal detergent drums started in the fall of 2019 in one cagewash area with automated washers. Since 2019, solid detergent has replaced the use of 30 gal detergent drums in 6 different cagewash sites across campus. The operational space limitations combined with the storage and disposal constraints related to 30 gal detergent drums have significantly decreased. Storage of solid detergent cases (e.g., 1 case contains four 8-lb capsules) occupies far less space, freeing up operational space for other uses. Also, shipping costs and environmental waste has decreased. Ergonomic concerns are reduced for individuals who physically handle (i.e., package, ship, deliver, receive, move, and insert capsules into dispenser) solid detergent. Organizational team members have expressed gratitude over the change from the 30 gal detergent drums to solid detergent. The implementation of solid detergent use was a collaborative effort that required communication and insight from all members of the animal care team and chemical vendor to maintain production and operation. Overall, the use of solid detergent has positively impacted operational processes and has provided acceptable cleaning results validated and consistent with prior chemistry use.

P214 Wall of Thanks: An Inexpensive, Socially Distant, and Highly Successful Platform for Staff Recognition

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Laboratory animal caretakers deserve recognition for the hard work that they do. Recent staffing shortages and budget cuts related to COVID have made it difficult to motivate staff in a demotivating time. We used to have a popular digital Wall of Thanks on our website where staff could send a note of thanks that would be displayed for 1 mo. Sadly, it was taken down due to cybersecurity concerns. When a staff member suggested that we implement a platform for employees to recognize each other's accomplishments and small successes, we began discussing how to bring back the Wall of Thanks. We decided to use a whiteboard with colorful markers in each of our facilities. Instructions on the whiteboard indicate that it is for thanking our staff and can be done anonymously if preferred. When a board fills up monthly, we take a picture to save and erase it. The Wall of Thanks has been consistently in use since January 2021. The whiteboard version is better than the digital Wall of Thanks because it brightens the day of everyone who sees it. Our staff writes most comments, but researchers and others also use them. The intended audience for the Wall of Thanks was our animal care staff. Still, it has also been used to thank members of other departments (business office/facilities/veterinary staff) and has been unifying for our department. Staff members have reported feeling validated and that others noticed their efforts. Feeling appreciated by coworkers has been motivating for our staff to continue doing the hard work that they do. Managers have noticed star performers as their peers recognize them. As an unexpected benefit, managers were able to notice an area of concern when comments referenced a problem staff was experiencing. We encourage everyone to create their own Wall of Thanks and discuss the successes and benefits that can be realized from this simple, inexpensive tool.

P215 Reduced Labor and Improved Cleaning Efficacy with the Use of a Solid Enzymatic Detergent

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The Guide for the Care and Use of Laboratory Animals emphasizes the primary aim of environmental enrichment is to enhance animal wellbeing. Examples of enrichment include structural additions (e.g., perches, elevated shelves, visual barriers) and nonstructural or

manipulable accessories (e.g., igloos/shelters, foraging devices, and an assortment of toys). The challenge is establishing appropriate sanitation practices for such enrichment items and accessories that align with the regulatory guidance that ensures animal well-being and prevention of cross-contamination. Sanitation, as defined by the *Guide*, is "the maintenance of environmental conditions conducive to health and wellbeing and involves bedding change (as appropriate), cleaning, and disinfection." At a large multisite academic institution with both non-USDA and USDA species, it became apparent that excessively soiled enrichment items and accessories were not adequately cleaned and disinfected when sent through the automated washers. This problem created additional work for the staff to manually clean and resend enrichment items and accessories back through the automated washers multiple times. The problem has been minimized using a solid enzymatic detergent. Enrichment items and accessories are collected, placed in a tub filled with the enzymatic detergent solution, allowed to soak for 30 min before rinsing, and sent through the automated washer once. Assessing the effectiveness of sanitation is monitored by either visual inspection, microbiologic culture, or adenosine triphosphate (ATP) technology. Cagewash team members no longer spend time attempting to clean each enrichment item and/or accessory manually. The items are placed back into circulation more quickly with confidence that animal well-being is maintained with effective sanitation practices routinely evaluated.

P216 Approaches to Chinchilla Housing and Enrichment in the Laboratory Animal Setting

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The Chinchilla Consortium is a collective of 18 research institutions across the U.S. dedicated to developing and sharing best practices for the housing, husbandry, enrichment, and veterinary care of research chinchillas. With fewer than 1,500 chinchillas used annually in biomedical research across the country, few commercial caging systems are available, requiring many institutions to repurpose caging intended for housing other species. The caging used in the pet chinchilla and chinchilla fiber trade either does not meet the standards outlined by the *Guide for the Care and Use of Laboratory Animals* or is not designed to withstand the industrial sanitation practices commonly used in biomedical research facilities. Reusing caging systems commonly meant for rabbits and ferrets provides an economical alternative. However, it will often still require modifications due to chinchillas› unique husbandry, enrichment, and breeding requirements. Alternatively, if a program has it in its budget, consultation with a laboratory animal caging manufacturer can fabricate a specialized housing environment with built-in, detachable accessories to support the standard care and enrichment for this species. Regardless of the size of the biomedical research program, housing a new colony of chinchillas can pose challenges. The information presented offers guidance on housing considerations and enrichment options necessary for maintaining a chinchilla colony at any institution, including special considerations for a breeding colony and biocontainment.

P217 Out of Sight, Not Out of Mind: Investigation of Opaque Cage Walls on Intergroup Aggression in Rhesus Macaques

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Rhesus macaques (*Macaca mulatta*) at our primate breeding facility are group-housed in indoor/outdoor runs with chain link fencing, separated by an empty run to prevent intergroup fighting. To increase building capacity from 70% to 100%, the outdoor runs have undergone reconstruction to include solid metal walls, allowing groups to be housed in adjacent runs. To assess the impacts of the opaque walls on the monkeys' welfare, alopecia and wounding data were analyzed for 5 social groups (average group size: 11) at baseline and after the solid walls were installed. Alopecia scores were recorded at 3 time points: baseline (day monkeys were moved into runs with solid walls), and at 3- and 6-mo post move. No relationship between the study phase and alopecia severity was found. Wounding data was collected ad lib for 18 mo: a 6-mo baseline period, which corresponded with the breeding season (baseline), and two 6-mo periods following the monkeys' move into runs with solid walls (walls 1 and walls 2), where the latter also aligned with the breeding season. While overall wounding rates were low, wounding increased in all groups in walls 1 as compared to baseline (average wounding rate increase: 180%). Most wounds were minor (i.e., no veterinary intervention required). Minor wounding rate more than doubled from baseline to walls 1 (136% increase) and rose an additional 70% in the walls 2 period. The average daily wounding rate during the breeding season was higher when the monkeys were housed in runs with solid walls (walls 2 versus baseline), with wounding events that required veterinary intervention increasing by 75%. Without visual access to neighboring breeding groups, which are now housed closer than before, monkeys redirected stress/aggression towards groupmates. Going forward, colony management changes will be made to alleviate intragroup aggression: groups of juveniles will be housed in between breeding groups and metal shift doors between runs will be replaced with clear polycarbonate doors, giving the monkeys the opportunity to view neighbors. The same measures will be used to assess welfare proceeding with the intended modifications.

P218 New Era of Marmoset Research in South Korea

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The demand for nonhuman primates (NHP) in biomedical research has increased for several decades. Although macaque monkeys such as Cynomolgus and Rhesus have been widely used in NHP research, they have disadvantages as laboratory animals such as securing husbandry space, low reproductive efficiency, difficulties in handling, and risk of zoonotic infections. Recently, the common marmoset (*Callithrix jacchus*), has emerged as an attractive NHP to overcome the limits of macaque monkeys. Marmosets have several advantages of simple breeding, easy handling, fewer diseases, and small body size compared to macaque monkeys. For this reason, the United States, China, Japan, and Europe regard marmoset as a crucial strategic bioresource and are striving to secure marmoset and establish infrastructure. However, in Korea, the domestic colony and breeding system for marmosets, and related infrastructure are not well established. In 2021, National Bio-Resource Project was launched as multi-ministerial funding, and the Seoul National University

Hospital Marmoset Model Network Center (SNUH MMNC) project was selected to strategize the marmoset as a national bio-resource. First, SNUH MMNC project is carried out for 3 y, and the ultimate goals of the project are securing marmoset resources, standardizing marmoset breeding and quality management systems, and developing a marmoset disease model. To achieve these goals, the SNUH MMNC team is composed of laboratory animal medicine, microbiology, clinical disease, obstetric, and genetically engineering experts. Each expert will meet and communicate regularly and hold the SNUH MMNC symposium twice a year. Also, SNUH MMNC will organize a public relations team to promote the necessity and role of MMNC to the veterinary and biology major students and researchers. After the end of the funding, the marmoset colony will be maintained through the finance derived from its own production and sales revenue, and the marmoset resource bank will be operated to promote and encourage the researchers utilize the marmoset for research. We expect that these efforts will develop the quality of biomedical science of South Korea, and contribute the improvement of public health.

P219 Benefits of Increasing Cage Size on Reproductive Outcomes in Surrogate Mice

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We routinely recover valuable genetically engineered mouse strains by transferring embryos obtained from cryopreserved germplasm into surrogate females. The surrogates are singly housed or, more typically, pair-housed to promote aunting. One female from each cage serves as an ideal direct contact sentinel for health monitoring of pups to be distributed to clientele. We hypothesize that increasing cage floor space using large mouse cages would allow for more optimized reproductive indices (e.g., maternal and aunting behavior), which could increase the number of pups weaned. Moreover, this approach decreases the costs of housing and health monitoring as only 1 female per cage would be required for the latter. To this end, up to 4 surrogates were housed in large mouse cages (152.7 sq. in.) and the pregnancy rate was compared to that of pair-housed surrogates in small cages (78.1 sq. in.). Using small mouse cages, a pregnancy rate of approximately 55% was obtained from in vitro fertilization (IVF) and pronuclear injection (PNI) procedures. When using large cages, pregnancy rates using these techniques increased to approximately 63%. Moreover, the cost of housing 4 surrogates in 1 large cage resulted in 20% cost savings when compared to housing 2 surrogates in 2 small cages. Last, by using this strategy, only 1 of 4 surrogates was needed for health monitoring effectively reducing testing costs by 50%.

P220 A Fat-Tailed What?: Navigating Husbandry for the Fat-Tailed Dunnart (*Sminthopsis crassicaudata)*

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Ensuring proper husbandry for all animals used in research is essential for excellent animal welfare. Although fat-tailed dunnarts (*Sminthopsis crassicaudata*) are new to biomedical research in the United States, they are no exception to this rule. Dunnarts are carnivorous nocturnal marsupials whose nutritional needs, light cycles, enrichment, breeding conditions, and temperature needs vary from the typical mammal or rodent species familiar to laboratory animal technicians. Little literature on the husbandry and care of this exotic species in a biomedical research setting is available. To establish a baseline of husbandry and care for the dunnarts housed in our facility, data, and advice was compiled from multiple sources, including the wildlife center in Australia that supplied the dunnart founders. Using these resources, standard operating procedures regarding nutritional needs and environmental needs were created to suit dunnarts housed in a biomedical research setting. Fat-tailed dunnart husbandry and care require attention to detail, especially when making mince meatballs and feeding crickets. Because of this, SOPs include pictures for each step of specific tasks, in order to decrease the possibility of mistakes, along with visual tools for portioning food. From their arrival and the implementation of the husbandry SOPs, we have maintained a growing colony of fat-tailed dunnarts using multiple resources and our own experiences, to accommodate the physical and psychological needs of this novel species in a biomedical setting. We are still learning how to better our care for fat-tailed dunnarts, but the success of our current husbandry practices is evidenced by clinically healthy dunnarts that are breeding, using cage resources, and participating in behavioral assays. As we learn more, we continue to develop additional enrichment and husbandry practices to advance the care provided and improve the welfare of our fat-tailed dunnarts.

P221 Who's that Spiny Mouse? Identification Methods for *Acomys cahirinus*

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Permanent or semi-permanent identification methods for spiny mice (*Acomys cahirinus*) have not been described. Identification is uniquely challenging with this species due to their weak skin phenotype. Ear punches are unsuccessful due to this species' impressive and extensive regenerative abilities. Digit amputation may be considered, as the regenerative abilities of the species do not include regrowth of digits; however, this method causes unnecessary pain and distress when the sole purpose is identification. Traditional metal ear tags are removed easily due to weak skin, causing wounds along the ear margins. We looked at the feasibility, long-term success, and financial investments of 3 types of permanent and semi-permanent identification methods for spiny mice: microchips or implantable RFID; tattooing of the pinna, tarsal pedal area, and toe; and ear buttons. Limitations of these methods include appropriate location, time and equipment investment, and ease of cage-side animal identification. Ultimately, ear buttons were the solution chosen for the experimental cohorts. This method does require some modifications specific for spiny mice but was the most attractive method in terms of cost, ease of application, and long-term identification.

P222 Husbandry and Enrichment of African Striped Mice (*Rhabdomys pumilio*) in a Flexible Film Isolator Setting

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The African striped mouse (*Rhabdomys pumilio*) is an unusual rodent species used in developmental biology and behavioral ecology studies. Due to a lack of publications outlining husbandry practices of the African striped mice (ASM), particularly in a flexible film isolator setting, we were faced with unique operational challenges in developing a program for their care. In addition to applying gentle handling and restraint techniques, we also developed husbandry provisions such as enrichment, breeding, and feeding strategies. ASM are readily recognizable by the distinct pair of parallel racing stripes on their dorsal coat. They are larger in size than the laboratory mouse and known to overeat which can impact their reproductive biology. They are an active species and reach sexual maturity at approximately 6 wk of age. At our institution, animals were housed in isolators in rat cages (182.5 in^2) with a maximum of 4 adults per cage or 1 mating pair with litter during a quarantine period and treated with fenbendazole-medicated feed (150 ppm). ASM were fed daily, and quantities were limited to control their weight while body condition was monitored. Isolators housing the ASM were moved to a low traffic area and the age of breeders adjusted from 4-5 mo of age to 10-12 wk to optimize breeding. Typically, enrichment at our institution includes tissues, however, additional enrichment

provisions were added for the ASM including foraging, structural, and nesting resources. ASM require gentle restraint and manipulation of the tail must be avoided due to an increased risk of degloving injury. Furthermore, handling ASM by their scruff was difficult due to their active nature thus, plastic transfer tubes were also used. In brief, we successfully bred and raised ASM in flexible film isolators by optimizing conditions and the age of breeding, adding additional environmental enrichment, and careful species-specific considerations for handling and restraint.

P223 Innovative Disinfection System Using Gas Phase Nucleic Acid Digestion Technology to Support Laboratory Animal Management in Geriatrics Research

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Sterilization of animal testing facilities is performed not only on articles (breeding equipment, instruments, and laboratory equipment) but also on breeding rooms and laboratories to prevent the development of pathogenic microorganisms. Formaldehyde gas, hydrogen peroxide, and chlorine dioxide gas are used for nonheat-resistant sterilization. Some sterilization methods have been reported to caus corrosion and the accumulation of residues on breeding equipment (rubber products, gloves, etc.) and electronic equipment. Our facility has kept many naturally aged mice used in gerontology and geriatric research. Since the animals are kept for a long period of time, it is necessary to maintain a clean and appropriate rearing environment. Therefore, we evaluated a new system of combined gas equipment containing formaldehyde gas components with methanol as raw material, which allows decontamination with no corrosion and no residue. The method was performed in 2 facilities of different sizes: the disinfection room (DR, 10 m3) and the Infectious Diseases Laboratory (IL, 50 m3). The decontamination devices used were a gas generator and a gas decomposition device. To evaluate the sterilization of each room, chemical indicators (CI) (F-Sign) and biological indicators (BI) (*Bacillus atrophaeus* (ATCC #9372) incubated for 7 d) were placed in 4 locations in the DR and 8 locations in the IL. Gas decontamination was performed for 4.5 h in the DR and 7 h. in the IL. Different equipment and electronic devices were decontaminated in both rooms. CI results showed effective sterilization in both rooms. In addition, BI was negative from day 1 to day 7 (spores SAL<10-6). These results confirmed the adequate decontamination of both rooms. Furthermore, no residues or corrosion of instrumentation, electronic devices or experimental equipment were observed. Gas phase nucleic acid digestion method is effective for complete DNA degradation, and therefore for disinfection of COVID-19 (SARS-CoV-2) research laboratories and is expected to become a next generation sterilization solution.

P224 Evaluation of Rodent Chow Stability when Stored in Alternate Conditions than the *Guide* Parameters

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An essential aspect of animal programs is the storage and provision of food to various species. Guidance on storage conditions is described in the *Guide for the Care and Use of Laboratory Animals (Guide),* including nutrition, palatability, vermin-control, diet quality, and integrity of feed bags. Our institutional animal care program received a suggestion for improvement (SFI) during a site visit by AAALAC, indicating a review of feed storage areas with parameters falling outside of the *Guide* for temperature and humidity (<70 °F, under 50% humidity). Feed storage sites (n=5) were evaluated across animal facilities to document the variation in storage conditions inherent to an animal care program located in a Midwestern climate to address the suggestion. Our institution has a centralized feed storage facility (control site) from which bags of rodent diet are distributed to dedicated rodent facilities as needed. Rodent chow from the same vendor, type, lot, and expiration date was assessed over the warmest months of the year in Michigan (June-Sept 2021). Each experimental site continuously stored a vendor-packaged rodent diet (n=2 identical bags) for 3 m; 1 bag was assessed for nutrient stability at 1 m of storage, and one bag was assessed at 3 m. Temperatures within experimental storage rooms ranged from 87° to 65° F, and humidities ranged from 99% to 25% during the study; these ranges included a room designated for thermo-neutral studies and our standard storage areas. Outside ambient temperatures were recorded daily over the study months compared to internal storage locations. In comparison with baseline samples, a consultant nutritionist confirmed that nutrient values did not differ between rodent chow bags regardless of storage location and vacillation in temperature and humidity ranges temperatures. These outcomes were presented to the IACUC for discussion with the intent to discuss with forthcoming site visitors from AAALAC and validate that described alterations in environmental parameters are compatible with maintaining the integrity of rodent chow.

P225 Effects of Energy Intake Levels on Apparent Total Tract Digestibility of Essential Minerals in Dogs

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In studies using laboratory animals, feed and energy intake are the important factors that have an influence on the experimental results. Differences in energy intake can lead to differences in nutrient digestibility and availability in experimental animals. The aim of this study was to evaluate the apparent total tract digestibility (ATTD) of essential minerals in dogs fed different energy levels. A total of 12 spayed beagle dogs were divided into the control (n=5) and treatment (TRT, n=7) group. The dogs in the control group consumed energy equivalent to 132 kcal per metabolic body weight (kg), and the dogs in the TRT group consumed energy equivalent to 112 kcal per metabolic body weight (kg) during the 14-d experimental period. The energy intake level was individually adjusted by the feeding amount using the same experimental diet with 0.5% chromium oxide in both experiment groups. The ATTD was analyzed according to the indicator method presented by AAFCO (2021). The ATTD of essential minerals was observed to be high in the order of K, Na, Se, P, Fe, Ca, Mg, Mn, Cu, and Zn. The ATTD of potassium with the highest digestibility was 97.5%, and the ATTD of zinc with the lowest digestibility was negative. Interestingly, the ATTD for all minerals analyzed in this study was increased with lower energy intake (control) compared with those of TRT. Among essential minerals for dogs, the ATTD of Ca, P, Zn, Mg, Mn, and Se had significant differences between control and TRT groups ($P < 0.05$). The increase rate of ATTD in TRT compared to CON was found to be high in the order of Zn, Mn, Mg, Ca, P, and Se. Zinc with the highest increase rate was increased by 81.4% in TRT than control, and selenium with the lowest increase rate was increased by 11.1% in TRT than CON group ($P < 0.05$). The increase rate of ATTD in Mn, Mg, Ca, and P was 75.4, 68.9, 50.5, and 25.2 in TRT compared to control, respectively ($P < 0.05$). Our results demonstrated that the level of energy intake affects the availability of minerals in dogs. These results could provide significant information for nutritional control and management of experimental animals in the field of research using dogs as experimental animals.

P226 Does Plant Color or Foliage Concentration Influence Zebrafish Breeding and Egg Yield?

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Various plastic plants are used as environmental enrichment and harborage for zebrafish (*Danio rerio*) housed in the facility. The intent of this study was to determine if the color and foliage density of the plastic plants used would impact breeding success and egg yield. For this study, we used 2 cohorts of 5 breeding pairs of 4-mo-old fish. The evaluation of cohorts was separated by approximately 6 mo to assess the seasonal reproducibility of findings. Each study period included 2 mo of weekly collection of egg yield data under environmental conditions of varying plant color (red, blue, or green) and foliage density (pine-like or broad leaves). Based on these findings, we believe that environmental enrichment has the potential to significantly impact zebrafish husbandry and warrants further exploration.

P227 Comparison of Floor Cleaning and Disinfection Processes in a Laboratory Animal Facility

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Sanitation of the animal facility, including the floors, is essential for maintaining biosecurity and meeting regulatory requirements. However, best practices for method, frequency, and evaluation techniques used to assess floor cleaning and disinfection have not been established in laboratory animal facilities. Research done in human hospital settings suggests that traditional cotton string mop and bucket systems can spread microbial contaminants across floors. Therefore, we evaluated 2 mopping systems with 2 different disinfectants: quaternary ammonium compound (QUAT) and cotton string mop, QUAT and microfiber mop, hydrogen peroxide-based disinfectant (HPD) and cotton string mop, and HPD and microfiber mop. Each mop and detergent condition were used to clean 2 rooms housing mice twice a week for 2 w. The floors were swept and then sampled using RODAC plates and ATP swabs before and after mopping. The time to mop each room, time for the floor to dry, and amount of detergent used was recorded. The percent change between the precleaning and postcleaning samples was compared between each group using the Kruskal Wallis test. The QUAT and cotton string mop condition had a significantly lower reduction in CFU than the other 3 conditions. However, during the first mopping trial, only 1 of the conditions had statistically significant differences: the QUAT plus microfiber had a greater CFU reduction compared to the HPD plus cotton string. Additionally, both QUAT conditions had significantly lower reductions in ATP RLU measurements than the HPD conditions. A cost estimate was performed, and the microfiber cleaning process is estimated to be slightly more cost effective due to time saved in personnel hours. The results of this study can be used to select floor sanitation practices in laboratory animal facilities.

P228 Creation of Veterinary Medical Records within an Electronic Data Capture System

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Veterinary medical records are critical for documenting animal wellbeing and tracking animal health within a facility. While paper-based record systems are standard in many facilities, they are fraught with disadvantages when compared to an electronic-based system. In a large facility with animals housed in multiple buildings, it is increasingly difficult to track active veterinary cases from a single location (i.e., walking to the facility to find the paper record is required). Due to the poor accessibility to animal files, an electronic medical records system was necessary. We elected to leverage 2 inhouse software programs, an electronic data capture system (EDCS) and a common online survey creator. Although neither are designed to capture veterinary medical records, both are off-the-shelf electronic systems that we were able to adapt for record keeping. To create a veterinary health file, a new form was created within the EDCS to capture critical information, including assessments, time stamps, and treatment plans. In addition, an electronic notification system powered by the survey creator was created to allow operational staff to send real-time notifications of animal health issues that are then stored in a web-based collaborative platform for internal tracking by veterinary staff. After the first month of use, over 255 animal health notifications have been generated. The EDCS offers advantageous reporting options, including the ability to generate a complete animal history report, which allows visualization of any data collected on the animal, providing a thorough understanding of its medical and study history. It is possible to review the medical history at any location with network access, significantly decreasing the time compared to locating paper files. Furthermore, the collaborative platform allows real-time visualization of all active cases onsite, as well as the ability to visualize trends and anomalies. While not as efficient as a single, fit-for-purpose software, the approach has been overall successful at increasing the accessibility of veterinary medical records across the facility.

P229 Use of a Layered Cage Card System for Complex Projects

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Many rodent projects on our campus have competing special requests, procedures, and needs for documentation and communication, resulting in overlapping labeling requirements for cages. Reading through notations on cage cards became challenging due to the lack of space, handwriting, and organization of details. Frequently, cage cards had important information overlooked as it was hidden by a sticky note or written on the back of the card. To avoid these problems, we developed a system for tracking multiple special needs and treatments with a layered cage card system. The cage cards use checkboxes to decrease the need for writing notes and ensure they are easy to complete. The cage cards use a color-coded system to flag specific procedures to ensure they are easy to view. They are stocked in a central location in each facility to ensure consistency and ease of use for both researchers and animal care staff. Some versions have been laminated so they can be reused. This system helps facilitate compliance and excellent animal care by ensuring that postoperative care is documented and feed and/or water is refreshed on the required schedules. It also ensures the experimental details for each project are clear so that the animal care and veterinary staff are aware of the ongoing procedures with each cage of animals. The system also ensures that ongoing treatment or veterinary medical needs are clearly documented and not overlooked. We have used this same special cage card program across several species, including mice, rats, jerboas, and spiny mice.

P230 Spiny Mouse Husbandry: A Novel Housing Paradigm Solves Problems

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Spiny mice are commonly maintained in large harem-style breeding cages of up to 20 mice per cage. There are usually multiple breeding males and females that are maintained in the same enclosure with this housing paradigm. When initially using this housing style, we found 2 problems we needed to address. First, we saw a high incidence of aggression and fight wounds. It was challenging to identify the aggressors in the large density cages. Second, our

investigator needed to track birth dates and inject pups at a specific age. In the harem-style cages, it was challenging to identify parentage and track litter birth dates and ages of pups. We solved both problems by repurposing rat microisolator cages to house breeding pairs and trios and stock cages of same-sex spiny mice with similar environmental enrichment, bedding, and feeding paradigms. Manzanita branches are attached to the food hoppers to provide environmental enrichment. Additional enrichment includes a shepherd shack and wooden chew blocks. The seed mixture is provided in a food crock on the floor, and water is provided from a water pouch and valve system in the food/water hopper. After moving to this housing paradigm, we saw a reduction in reported fight wounds in the colony. The researcher also set up timed matings and performed injections at the specific time points needed. The spiny mice continue to breed well in this style of caging compared to the larger cages, and the containment in the microisolator cage allows for biohazard work. Microisolator rat caging has led to improvements in our husbandry care and has facilitated research needs.

P231 Initial Validation and Ongoing Quality Control of Process Equipment

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Initial validation and performance qualification of cleaning/sanitization equipment such as a cage, bottle, or rack washers rely upon assessing various measures of equipment performance, including temperature, pressure, detergent concentration, and spray jet patterns. Oftentimes, the performance of processing equipment is merely monitored by measuring water temperature and exposure time. Our process quality control (QC) groups enhanced our initial equipment validations and ongoing QC to minimize the risk of infection due to residual microbial burden or cross-contamination during the wash step(s) by adding ATP testing. Previously, the QC program consisted of data loggers to monitor time and temp during the cycles as well as riboflavin coating of materials to provide a visual evaluation of sanitation effectiveness. We developed an ATP testing methodology based on the residual amount of adenosine triphosphate (ATP) on the surface of sanitized caging components and compared it to standard colony forming units (CFUs). The goal was to establish an acceptable minimum ATP level, as detected in relative light units (RLU). We established baseline ranges for various materials (i.e., stainless steel, polysulfone) since different materials can have inherent luminescence (nonzero RLU values). We then performed 3 live challenge rounds by swabbing ATP and collected CFU data using a standard agar plate count pre and postsanitation to get a correlation between RLU and CFU limits. Results showed high levels of both RLU and CFU presanitation and zero to low levels post sanitation, with a correlation between ATP RLU counts and CFU. The ATP swabbing method is particularly useful for hard to clean surfaces such as corners, grooves, and irregularly shaped surfaces and identifies whether adjustments to the spray jets and/or water pressure are necessary for effective equipment sanitization. The use of ATP testing, in combination with the use of riboflavin testing and data loggers, provides a robust QC program, ensuring effective sanitation of cage components and minimizing biosecurity risks to our mouse colonies.

P232 Enrichment Impact on Breeding Metrics in a NOD SCID Gamma (NSG) Breeding Colony

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Breeder wellbeing is vital to the health of pups produced in a breeding colony, and thus enrichment impacts breeding metrics such as the number of litters, litter sizes, and pup weights, in addition to parent health. We have observed very limited cases of barbering that progress to wounding in some of our NSG breeders which we sought to curb via improved cage enrichment. Although current husbandry practices are designed to positively affect mouse wellbeing, extra measures of enrichment may have a more profound effect, particularly with respect to breeding metrics. From our NSG breeding colony, 24 cages of trio breeders were assigned to each of 5 different treatment groups: supplemental nesting, foraging treats, shelter, and nesting, and all 3. At 21 d, pups were each weighed and assessed according to the facility's standard growth curve. Breeder behavior was also monitored (including signs of aggression, excessive barbering, and pups found missing or dead). The average weight of pups, litter sizes, and total litters for every group were then compared to determine the best enrichment. Results showed that although litter sizes and total litters were similar, pups tended to have lower average weights in the groups with the foraging enrichment, but pups in other groups maintained a healthy weight. Possible reasons to explain this trend include the regular addition of forage materials requiring cages to be opened more often and distracting dams from nurturing their pups. We propose that supplying shelter that replicates the mouse's natural environment and supports thermoregulation in pups, is of greater benefit than foraging. However, foraging opportunities may in fact be of greater benefit to adults. The study held inconsistencies in the type of foraging treats as well as their administration, and additional data may show differences in diverse types of foraging options. Although the incidence is low, we continue to monitor for cases of aggressive behavior under different enrichment conditions.

P233 When Refinement Doesn't Work: Postmortem on a Novel Housing Attempt for Naked Mole-rats (*Heterocephalus glaber*)

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In October 2020, a founding colony of 3 naked mole-rats (*Heterocephalus glaber*) was established at our institution. By October 2021, 99 animals, for a total of 9 breeding colonies, had been added. This eusocial, poikilothermic species requires unique conditions to recapitulate its subterranean lifestyle. Polycarbonate rodent cages (11.5 x 7.5″) were connected by impact resistant polycarbonate tunnels (2.25″ w/a 2″ ID) in a variety of configurations based on colony size and density. Hand-tapped holes that aligned with small metal studs were created at each end of the tunnels to prevent disconnections. To safely and reliably provide the hot, moist conditions of their natural habitat, colonies were housed within a temperature-controlled unit (set points = 84 °F, 40-60% RH, 15-60 ACH) supplemented with humidifiers, as needed. We housed up to 3 colonies (50 animals) within a unit. Despite otherwise following SOPs from long-standing colonies, only 5 pups survived out of 9 litters (~10 pups each) in 16 mo. Efforts were made to supplement the diet and environment in a variety of ways, but the colonies were ultimately returned to open shelving. In the 3 mo since then, 13 pups from 2 litters have been born and survived. Due to the sensitivity of the naked mole-rat, vibrations and sound were measured within the units. Baseline vibrations at cage level ranged from 0-0.33 mm/s and 0-4.2 mm/s for duties within and around the unit. Sound at the cage level was 48-131 dBA. In open shelving, we can provide more complex configurations to larger colonies and more easily access each cage for health checks and husbandry, which decreases ancillary activity, noise, and disturbances to the animals and to adjacent colonies within the unit. We speculate that the vibrations and sound produced by the unit machinery, frequent opening and closing of the unit doors, and the tight space within which staff had to work, served as a source of chronic stress, resulting in nearly 100% cannibalization of litters. In the future, we recommend pilot studies in a well-established breeding colony to determine the impact of

changes in housing on reproduction and prevent lengthy delays in research.

P234 Use and Evaluation of Environmental Enrichment in Naturally Aged Mice Breeding

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Our facility has kept many naturally aged mice used in gerontology and geriatric researches. Social stress (fighting, barbering) and chronic stress-related inflammation resulted in increased risk for age-related diseases. On the other hand, changes in the characteristics of the environment (enrichment, size of the cage) have been shown to have a large effect on animal welfare. In this study, we evaluated the antistress effects of different types of cage enrichment. Male and female mice (n=10) were used per enrichment group, C57BL/6 (C57BL/6NCrSlc(B6N), C57BL/6J(B6J)) mice (4-wk-old) were kept over their lifetime. Four different types of enrichment, including small squares of nonwoven fibers; a nest manufactured from unprinted, uncoated white book publisher-grade pap; a swing; and a cardboard tunnel were tested. Mice without environmental enrichment were used as a control group. Physiological (body weight and temperature, food/water consumption and survival rates), behavioral (rotarod tests and alopecia rates), and biochemical (urinary corticosterone (CORT)) were performed from 3 mo-old to 18-mo-old mice every 3 mo. B6N male using the squares of nonwoven fibers (48.4±4.9g) and tunnel had body weight (49.3±7.0g) that was relatively higher than control groups (43.0±7.0g) at 15 MO. Rotarod test results showed that motor coordination in enrichment groups (176.4±44.5sec) were not lower than control (128.0±23.0sec) in both strains from 12 mo. The occurrence of hair loss tended to be higher in the experimental groups in both strains, but dermatitis and other skin disorder incidences were lower than control groups. The CORT were relatively lower in the 3-6-mo-old male B6 tunnel group (46.2±12.6ng/ml) than in the control group (76.1±5.2ng/ml). Results showed that enrichment reduced abnormal behaviors, incidence of skin problems, and stress levels in young mice. The survival rates were higher (10-20%) in enrichment groups than control groups. These results suggest that environmental enrichment was highly useful, and it is possible to create animal models of natural aging, or physiological aging, that are independent of the rearing environment among animals, such as fighting. In the future we will continue to investigate the different types and methods of environmental enrichment in mice.

P235 Comparison of Light Levels between 4 Different Primary Enclosures for Housing Laboratory Mice

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Light levels are evaluated on an annual basis in our rodent vivariums to assure they are compliant with the *Guide's* recommendation of 325 lux, 1 m above the floor which balances personnel and rodent needs. Recently we investigated intracage light levels across 3 different rodent vivariums, with 4 different primary enclosures as well as a positional variation to discern any potential impact on animal welfare. We hypothesized that all cages, regardless of relative distance from the light source, would present light levels below the *Guide's* recommendation that a carousel-style primary enclosure would experience lower light levels compared to double sided racks. Using a handheld light meter placed within an empty cage, we evaluated intracage light levels of 4 different primary enclosures (a carousel-style rack with wedge shaped plastic cages, and 3 different double-sided racks with shoebox style plastic cages) and 9 cage positions. Ten racks of each design were tested. Three styles had higher light levels in the front of the cage and a lower light level in the back of the cage; 1 double-sided rack had the opposite. Average light levels at the intracage level were reduced 48-90% from the front to the back of the cage, depending on the primary enclosure style. All cages experienced light levels below 325 lux, except 1 double-sided rack with an average of 325 lux in the second row and 330 lux in the middle row. Light levels saw a percent reduction of lux from the top to middle row on all double-sided racks at 0.11, 0.56, and 0.49%, while the carousel style rack saw a percent increase in the middle row of 0.21%. These findings suggest that all cages were experiencing light levels below 325 lux regardless of the relative distance from the light source. The carousel-style primary enclosure experienced lower light levels than the double-sided racks. All 4 primary enclosures created an intracage light gradient, providing a management strategy for mitigating inappropriate light stimulations.

P236 How I Learned to Quit Worrying and Love Cold Water

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The *Guide for the Care and Use of Laboratory Animals* provides guidelines for effective disinfection of caging materials with wash and rinse water between 143-180 °F or more. However, this guidance is based on studies that used only hot water for sanitization. More recent studies using a performance standard approach have demonstrated that a combination of detergent and lower temperatures can effectively sanitize rodent caging supplies while decreasing energy usage and cost. We were put in a position to test this ourselves when, in the middle of a major project to replace an aged rack washer, the boiler servicing the washer was found to be in significant disrepair. To continue moving forward with the project and minimize operational disruptions, we attempted to process our rodent caging supplies using a combination of our typical cage wash detergent and "cold" wash and rinse water at the warmest temperature we could achieve without a source of steam, 120 °F. Using our standard cycles, we verified via ATP testing that most of our caging supplies, including static and individually ventilated mouse and rat cages with wire bars and microisolator lids, water bottles, and tube and hut enrichment, achieved ATP levels below 45 RLUs, our threshold for appropriate sanitization. Out of 124 cage supplies tested in a 4-m period, all but 11 swabs demonstrated sufficient sanitization. We found that altering the physical orientation of most of these supplies in the presentation rack resulted in passing ATP values. Running wheels proved to be the most difficult to clean, regardless of how they were positioned on the presentation rack. However, doubling the length of the wash and rinse times has vastly improved our results with this equipment as well.

P237 High Room Humidity Does Not Alter the Moisture Levels of Unopened Laboratory Animal Diets

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According to the *Guide for the Care and Use of Laboratory Animals*, exposure to extremes in relative humidity hastens food deterioration. Therefore, the *Guide* recommends the storage of natural ingredient diets at less than 50% relative humidity. However, most modern laboratory animal diets are packaged in multilayered bags with at least 1 layer being moisture resistant. Because maintaining relative humidity <50% requires significant HVAC effort and energy consumption, we investigated whether high room relative humidity impacts moisture levels inside the feed bags, which could potentially impact feed quality. We sealed a commercially available logger with integrated sensors for temperature and humidity inside the bags of 5 different types of laboratory diets. We

then used a humidifier to increase the relative humidity of the room to well above *Guide* recommendations (60-90%) for 72 h. Both the room relative humidity and the relative humidity inside the bags of feed were recorded every 30 min. Before increasing room humidity (baseline), the relative humidity inside the bags of feed ranged from 65-70%. After increasing room humidity, the relative humidity inside the feed bags ranged from 66-70%. We were able to demonstrate that exposing bags of feed to high relative humidity does not alter the microenvironment inside the bags. These results suggest that the *Guide's* recommendations for humidity levels in feed storage rooms could be relaxed without impacting feed quality while saving on energy consumption in animal facilities.

P238 Comparing Cage Densities and Sanitization Frequencies to Evaluate the Effects on Animal Wellbeing

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The 8th edition of the *Guide for the Care and Use of Laboratory Animals* (*Guide*) provides guidelines for the amount of space required for rats and mice housed in groups as well as cage sanitization frequency. Any changes to these recommendations should be based on animal wellbeing and performance metrics and should be approved by the institution's IACUC. This study evaluates our hypothesis that internal standards for housing density and cage sanitization frequency for mice and rats, which differ from *Guide* recommendations, are not detrimental to animal wellbeing based on performance metrics assessed. We compared *Guide* sanitization recommendations (full cage change weekly) to internal standards that allow for a complete bedding change weekly with a monthly full cage change. Further, internal housing densities are increased compared to *Guide* recommendations. Juvenile male and female Sprague Dawley rats and BALB/c mice as well as breeding cohorts of each species are evaluated in 12 groups for 15 wk. The number of pups born and weaned, body weight, morbidity, mortality, cage ammonia, behavior, and cage cleanliness are assessed. No abnormal behavior is observed, and cages remain clean. Sporadic effects are reported in weight gain and ammonia during the study period but have not negatively impacted the overall animal growth or health status. Reproductive vigor was consistent across groups and no difference in litters born and weaned or weaned body weight was reported. These data indicate that animal well-being and performance in those housed via internal standards are comparable to *Guide* recommendations, supporting what has been well-documented that deviations from *Guide* recommendations do not have a negative impact on animal welfare within our institution.

P239 Commercial Supplemental Dietary Gel Product Reduces Rat Postoperative Weight Loss

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Anesthesia and surgery induce postoperative weight loss in rodents. Here, we investigated a commercial supplemental dietary gel to assess its impact on postoperative weight loss in rats. We hypothesized that a commercial supplemental diet gel would reduce postoperative weight loss in rats better than moistened chow. Sprague Dawley rats (n=10/group, male) were divided into 3 groups: 1) moistened chow; 2) 10 g diet gel; and 3) 20 g diet gel. Rats were exposed to moistened chow on day 3, day 2, and day 1. On day 0 (20-min skin biopsy surgery), rats were exposed to their assigned diets (moistened chow, 10 g diet gel, or 20 g diet gel). Rats were weighed daily from day 3 through day 7. Daily weight differences were calculated. Compared to day 0, 1) moistened chow rats' weights were decreased on days 1-7; 2) those receiving 10 g of diet gel had decreased weights only on day 1 but no different on days 2-7; and 3) the weights of those receiving 20 g of diet gel were not different on days 1-7. This study indicates that supplemental diet gels reduce postoperative weight loss in a rat skin biopsy model.

P240 Comparison of Plenum and Cage-level Filter Exhaust Dust PCR Testing to Soiled Bedding Sentinel Mice in Disposable Caging on an IVC Rack

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Soiled-bedded sentinels (SBS) for the detection of adventitious rodent pathogens have historically been the standard for colony health surveillance monitoring in our vivaria. With the advent of newer technologies that use dust collection filters tested by PCR, our institution compared SBS by traditional methods, and PCR testing of exhaust air dust (EAD) collected from a filter within the downstream vertical plenum and the cage-level exhaust air filter in disposable cages for health surveillance. Our hypothesis was that both methods of filter testing would identify more pathogens than SBS testing. Twenty-six individually ventilated mouse racks were sanitized and placed into rotation housing breeding animals in disposable cages. Rack plenum swabs were collected, and PCR was conducted to verify sanitation prior to housing animals. Racks received an EAD media kit in the exhaust plenum (n=24). SBS cages were placed on each side of the rack housing 2 mice per cage and received soiled bedding during each cage change procedure (n=41). At each cage change procedure, the filter for exhaust air was carefully removed from the cage bottom and saved in a sterile 50ml conical tube and pooled for submission (n=25). After 3 m, the SBS animals were tested via serology with bacterial and viral agents, and PCR for helicobacter species, pinworms, and ectoparasites. In addition, the EAD filter media and exhaust air cage-level filters were collected for PCR to evaluate all the same agents. Our results indicated that the cage-level exhaust air filter consistently detected more agents than the filter placed in the plenum, and the plenum filter detected more agents than the SBS animals. Our data suggest that both PCR methods of detection of rodent pathogens utilizing filter paper are superior to SBS in individually ventilated disposable rodent cages, but that the cage level filter may prevent some organisms from being detected on the plenum filter consistent with previous reports. This data has supported our institution moving away from SBS as a method of colony health surveillance.

Laboratory Investigations Posters

P300 T Cell-specific Deletion of Hematopoietic Protein-1 Alters Mature T Cell Distribution and T Cell Activation

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The severe primary immunodeficiency disorder and autoimmune disease seen in patients with loss-of-function mutations in the gene

encoding hematopoietic protein-1 (Hem1) warrant further investigation into the cell-specific role Hem1 plays in immune homeostasis. Hem1 is a hematopoietic cell specific component of the WAVE (WASP Family Verprolin homologous protein) regulatory complex which activates actin polymerization downstream of immune receptor signaling. Regulated actin polymerization is important for immune synapse formation, migration, and phagocytosis in immune cells. We hypothesized that T cell-specific deficiency of Hem1 would result in altered mature T cell populations in the periphery as well as disrupted T cell function secondary to disruption in f-actin polymerization. To study the effect of Hem1 deficiency on T cells, we created a conditional knockout mouse using the CreLoxP system by breeding Hem1 floxed mice to mice expressing Cre recombinase under control of the T cell-specific proximal promoter of the lymphocyte protein tyrosine kinase (B6. Cg-Tg(Lck-cre)548Jxm/J). Using flow cytometry, we evaluated different subpopulations of T cells, including ab, gd, Treg, and iNKT cells, in primary and secondary immune tissues of 10- to 12-wk-old mice. We found the peripheral populations of mature T cells were more affected than thymocytes with a decrease in the frequency of ab T cells ($P < 0.01$) and decreases in the frequency of CD4+ ($P < 0.05$) and CD8+ ($P < 0.001$) T cells. There were similarities between human patients and the mouse model in memory subsets with a decrease in naïve T cells and increases in effector memory T cells ($P < 0.01$). To determine if T cell activation was altered as it was in human patients, we performed an ex vivo stimulation of splenocytes and evaluated proliferation, activation marker expression, and intracellular cytokine expression. Preliminary data revealed decreased proliferation, CD69 (early activation marker) expression, and intracellular cytokine expression in CD4+ T cells only, similar to the findings in human patient T cells. These mouse models can be utilized to understand the disease process seen in human patients with Hem1 deficiency and be used as models for exploring potential therapies to treat the disease in humans.

P301 Ulcerative Dermatitis in C57BL/6J Mice: A Closer Look

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C57BL/6 mice are commonly affected by ulcerative dermatitis (UD), a disease of unknown etiology with poor response to treatment. To better characterize UD, 28 research naïve adult female C57Bl/6J mice, 14 with spontaneous skin lesions affecting the dorsal interscapular region and 14 with no clinical signs of dermatitis, were euthanized and skin samples collected from the affected and normal skin. The samples were fixed in 10% neutral-buffered formalin and processed routinely for light microscopy examination and selected samples were fixed in 2% glutaraldehyde and 1% paraformaldehyde and processed for transmission electron microscopy (TEM) examination. Very early lesions were characterized by an increase in dermal mast cells and focal areas of epidermal hyperplasia with or without hyperkeratosis. As the condition progressed, spongiosis of the epidermal basal cell layer was evident accompanied by a mixed inflammatory cell infiltrate in the dermis composed mainly of neutrophils and lymphocytes, followed by plasma cells and fewer eosinophils with or without epidermal surface erosion and/or scab formation. Dermal fibrosis increased with UD severity. Ultrastructurally, mast cell membranes were disrupted by the release of large number of electron dense granules; and the degranulated mast cells were filled with isolated and coalescing empty spaces as result of granule membrane fusion. Degranulating mast cells were commonly associated with small blood vessels and nerves. No parasitic, bacterial, mycotic, or viral agents were noted. Based on both routine light microscopy and ultrastructural findings, this study suggests initial UD lesions in C57Bl/6J mice are characterized by an increase in intact and degranulating dermal mast cells followed by epidermal hyperplasia. Ulceration appears to occur very quickly, probably as result of intense scratching due to the pruritogenic properties of the histamine released from mast cell granules. Histamine may also be responsible for the epidermal hyperplasia since histamine has been shown to stimulate keratinocyte proliferation. Consequently, treatments that prevent histamine release by skin mast cells may result in better outcomes if applied early in UD cases.

P302 Assessment of *Corynebacterium bovis* Growth in Tissue Culture Conditions

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A common concern in preclinical cancer research is the introduction of *Corynebacterium bovis* into immunodeficient mouse colonies through cancer cell lines. *C. bovis* is a known contaminant of patient-derived xenograft tumors passaged horizontally between immunodeficient mice. However, it is unclear if *C. bovis* can grow in the basal media used with mammalian tissue culture conditions. We hypothesized that *C. bovis* would not grow under tissue culture conditions or media, diminishing the risk of infection for tumor cell lines cultured in vitro. Three *C. bovis* isolates, CUAMC1, HAC, and ATCC-7715 were grown under ideal liquid culture conditions in heart-infused broth with 5% Tween 80 (HIBTW) at 32 °C, and rotary shaking at 250 rpm for 24 h. To determine if *C. bovis* can grow in tissue culture conditions, we selected 3 of the most common basal media used to grow human cancer cell lines including DME/F12 +10% fetal bovine serum (DME), DMEM/high glucose +10% FBS (DMEM), and RPMI 1640 +10% FBS (RPMI). One million CFU of each *C. bovis* isolate was cultured in each media using HIBTW as a positive control. Growth curves were generated using a Biotek Synergy automated incubator set to 37 °C and 5% CO_2 without shaking, and OD_{600} absorbance was recorded for each condition every hour for 72 h. These parameters reflect the most common conditions for tissue culture. In tissue culture conditions, *C. bovis* successfully grew in HIBTW. Unexpectedly, with the same conditions, all 3 isolates also grew in DME but failed to grow in DMEM and RPMI. After incubation, each condition was cultured on 5% blood agarose plates to evaluate cell viability. These results confirmed the propagation of *C. bovis* in DME, and suggest diminished viability in DMEM and RPMI after 72 h. Our data show that *C. bovis* growth in tissue culture conditions is possible and growth in tissue culture media is nuanced. These results highlight the importance of pathogen surveillance for tumor cell lines propagated in vitro and demonstrate the need for further investigation into *C. bovis* growth requirements.

P303 Impact of Infection with Mouse Kidney Parvovirus on the Adenine Diet Murine Model of Chronic Kidney Disease

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Mouse Kidney Parvovirus (MKPV) is a nephrotropic virus of the genus *Chaphamaparvovirus* that causes inclusion body nephropathy in immunocompromised mice and subclinical interstitial nephritis in immunocompetent mice. While this pathogen causes morbidity and mortality in immunocompromised colonies, the impact of this prevalent virus on study outcomes in immunocompetent mouse models is unclear. The aim of this study was to determine if MKPV infection influences hematology, serum chemistry, urinalysis, and histopathology endpoints which are routinely assessed in the

adenine diet model of chronic kidney disease (CKD) in C57BL/6NCrl (B6) mice. This model is an easily implemented, reproducible, and frequently used murine model of CKD. Female B6 mice (n = 30) were inoculated with 2.32×10^8 viral copies of MKPV via gavage and intranasal inoculation and confirmed to be infected via PCR of urine at 11 wk postinoculation. At 15 wk postinoculation, MKPV-infected mice and age and sex-matched uninfected control animals (n = 30) were fed a 0.2% adenine diet supplemented with casein to improve palatability. Serum chemistry, hematology, urinalysis, and histologic parameters were assessed at 0, 2, 4, 6, and 8 wk following initiation of the adenine diet. Infection with MKPV did not affect serum biomarkers of renal function including BUN, creatinine, and SDMA; urine parameters including specific gravity, urinary protein, and creatinine; or the hemogram. Semi-quantitative scoring of H&E-stained renal tissue demonstrated that MKPV-infected mice had significantly more foci of interstitial inflammation than uninfected mice, but other characteristics including the percentage of tubules displaying dilation, degeneration or necrosis, mineralization, intratubular inflammation, and the number of adenine crystals were unaffected. MKPV-infected mice had significantly less interstitial fibrosis at 8 wk after diet initiation as determined by quantification of Sirius red staining on whole kidney sections. There was no significant difference in the degree of macrophage infiltration as assessed by immunohistochemical staining for F4/80. These results indicate that MKPV should be excluded in mice enrolled in studies of CKD using the adenine diet model where histologic outcomes are evaluated.

P304 Monitoring of Commercial Transgenic Models Identifies the Presence and Frequency of De-novo Copy-number Variants

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Transgenic animals generated by pronuclear microinjection have significantly contributed to understanding mammalian gene function since the 1970s. The methodology to make transgenic animals results in random integration of transgenes into the host genome. Linear DNA tends to integrate in several copies per site, with limited integrations per founder. These concatemers can range from one to hundreds of tandem copies per site. There are several transgene integrities challenges that can impact phenotypes, namely multiple integration sites, alteration of a host genetic element, and transgene mutation and copy-number changes. We have adopted a plethora of special and routine testing techniques to analyze our commercial transgenic models, including transgene mapping, copy-number testing by quantitative real-time PCR, and expression analysis. For 3 of our highest-volume models, we used a combination of these techniques to test the hypothesis that transgene variability with potential phenotypic consequences happens in our breeding colonies. In the first model, we have observed copy-number deviations from the expected 3 copies at a frequency of roughly 1 in 1,000 animals. For consistent performance, it is important that the transgene has 3 copies. The second model contains a transgene composed of copies from 2 co-injected constructs. We identified variant alleles that reduce the copy-number of both constructs and a variant allele that results in complete loss of 1 and severe reduction of copies of the other construct, suggesting a complex internal structure. A reduction of transgene expression coincides with the change in copy-number. In the third model, a transgene copy-number variant was identified that results in expression variability. Interestingly, the low copy-number allele showed higher expression than the high copy-number allele. We developed genotyping tests that identified animals carrying either allele. Transgene mapping data confirmed that there are 2 integration sites. In this study, we identified variability in transgene integration site and copy-number which has the potential to affect phenotypes and highlights the importance of maintaining transgene integrity. Consequently, we implemented a routine transgene monitoring program that informs breeding decisions.

P305 Size Matters: Evaluating Chamber Size and Flow Rate during Carbon Dioxide Euthanasia of Mice and Rats

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The American Veterinary Medical Association (AVMA) updated euthanasia guidance using carbon dioxide to allow flow rates of 30 to 70% for rodent euthanasia. The mouse and rat are the most common animal models used in research and appropriate euthanasia methods are an integral part of achieving the 3Rs. Across laboratories, the carbon dioxide flow rate should be within the guidelines, however, the size of the euthanasia chamber may vary significantly and the correlation between chamber size and flow rate during euthanasia has not been previously studied. We hypothesized that the chamber size and flow rate may affect the behavior of mice or rats during the euthanasia process. Two chamber sizes (small: 9.5 × 22 × 9.5cm and large: 21 × 22.2 × 14.6 cm) with corresponding carbon dioxide flow rates were evaluated. Male (24) and female (24) Sprague–Dawley rats and CD-1 mice were euthanized individually in each chamber with approximate flow rates of 30%, 50%, or 70%. Behavior during the euthanasia process was video recorded and evaluated by a single individual. The total length of time animals exhibited ataxia, the time to reach unconsciousness, the number of times animals pawed at the face, and the number of rears were recorded. At a 30% flow rate, there were significantly longer times to unconsciousness for both male (P = 0.0012) and female (P = 0.0137) mice in large chambers compared to small chambers. Female rats in large chambers with a 30% flow rate had statistically significant (P = 0.0384) shorter times exhibiting ataxia and fewer rears than in small chambers. At a 70% flow rate, there were statistically significant (P = 0.0244) longer times to unconsciousness for male mice in large chambers compared to male mice in small chambers. Male rats in large chambers with 70% flow rate had a significantly fewer (P = 0.0252) number of rears than male rats in small chambers with 70% flow rate. This data suggests that a larger chamber may be associated with less distress during euthanasia than small chambers as evidenced by shorter times exhibiting ataxia and fewer rears despite a longer time to unconsciousness.

P306 Development of a 'Dogicized' Mouse Model for Canine Cancer Research

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Evaluation of novel immunotherapies for neoplastic diseases requires robust preclinical models that accurately predict clinical outcomes. Canines can model spontaneous tumors with an intact immune system that closely approximates disease biology in humans. Keeping with the One Health philosophy and employing 3Rs principles by favoring mice for preliminary work before moving into a clinical dog model, we seek to develop a 'dogicized mouse' with a canine immune system. This model could enable immunotherapy research that may benefit canines, translate to humans, or provide a canine PDX platform. We hypothesized we could achieve engraftment with protocols similar to those for humanized mice. Mixed breed dogs from 2 to 11 y were used as donors; recipient groups were n = 5 per sex. Adult NSG mice of both sexes were given sublethal total body irradiation followed by IV injection of canine bone marrow (cells treated with anti-canine-CD3); subsequently, peripheral blood was monitored by flow cytometry for canine CD45, CD4, CD8, and CD3. Next, we performed similar experiments using the NSG-$(K^bD^b)^{null}(IA)^{null}$ mouse (NSG-DKO, lacking murine MHC molecules) without preconditioning, and with IV injection of peripheral blood mononuclear cells (PBMCs). We also compared fresh cells to previously frozen cells. We determined that a dose of 1×10^7 bone marrow cells was ideal for survival and engraftment lasting a minimum of 10 wk in NSGs. In NSG-DKOs, the optimal

dose of PBMCs was 5×10^6 cells. The dogicized model in the NSG is slower to engraft, with canine CD45 populations increasing sharply after 8 wk, whereas human CD45 cells rise more gradually. With the NSG-DKO model, CD45 cells are detected at 2 wk and gradually taper. Both fresh and frozen cells engraft. Mice receiving PBMCs from younger dogs show higher CD45 populations than those from older dogs, which may be an important consideration for modeling canine cancer patients that skew older.

P307 Determining the Role of Innate Lymphoid Cells in *Brucelllosis*

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Brucellosis is a globally significant zoonotic disease that causes serious agricultural and public health problems worldwide. Affected humans can develop mild flu-like symptoms as well as more severe complications including arthritis and neurobrucellosis. Prior to our studies, no mouse model for neurobrucellosis had been described. Innate lymphoid cells (ILCs) are a group of tissue-resident innate immune cells that include the cytotoxic natural killer cells (NK cells) and three groups of helper-like ILCs (ILC1, 2, and 3). The purpose of our study was to uncover the role that ILCs play in the pathogenesis of Brucellosis following intranasal or footpad infection with 1 x105 Brucella melitensis. To examine the role this particular cell type, we utilized 6- to 12-week-old age- and sex-matched Rag2 and Rag2/IL-2RγC-/- mice on a C57BL/6 background (n=24). Throughout disease progression, joint swelling and development of neurologic signs were monitored weekly for up to 8 weeks and following euthanasia, bacterial burdens and histology of various infected organs were examined. When compared to mice with intact ILC populations, mice lacking all ILCs exhibited significantly higher bacterial burdens in the joint, brain, blood, and spleen, as well as worsened swelling in the tarsus on histologic analysis. These mice lacking ILCs also showed a significantly higher likelihood of developing neurologic signs (head tilt, circling, diminished proprioception, and lethargy) and on examination of brain histology, severe inflammation in the meninges was noted while none was appreciated in the control group. Mice lacking only NK cells showed an IFN-g dependent increase in bacterial colonization within the joint. Additionally, the absence of individual helper ILC groups did not play a significant role in bacterial colonization, possibly indicating a synergistic protective role of these ILC subsets. Collectively, our findings indicate that ILCs play an important role in the prevention of bacterial colonization and development of focal complications throughout Brucella infection. Our studies also describe utilizing Rag2/IL-2RγC-/- mice as the first described mouse model for neurobrucellosis.

P308 Sperm Specificity of Gynogenesis in the Amazon Molly (*Poecilia formosa*)

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In humans, 8 to 12% of heterosexual couples world-wide experience unwanted infertility. Often male factor infertility is a cause, but the best diagnostic test (semen analysis) is only 89.6% sensitive. A lack of effective diagnostics for infertility results in 15 to 30% of couples being diagnosed with unexplained infertility after diagnostic workup. We propose to explore the Amazon Molly (*Poecilia formosa*) as a novel animal model for factors contributing to unexplained infertility, separate from the genetic contribution of sperm. *P. formosa* is an all-female live-bearing fish that reproduces exclusively by gynogenesis, a rare form of asexual reproduction where sperm is required to trigger embryogenesis, but male genes are not incorporated into the genome of the embryo. Sperm is provided by males of several species, including *P. mexicana.* If there is a difference in fecundity of females bred with different populations of males, then further study of population differences in fertilization can lead to the identification of sperm and egg compatibility characteristics which may translate to human infertility. In an experiment, 12 nulliparous *P. formosa* females of one lineage were group-housed in 10-gallon tanks in 2 groups of 6 females with two *P. mexicana* males each from either a sympatric population or an allopatric population. Females were dissected for embryos when the first offspring were born or at 9 wk after pairing if no offspring were born. All females in the sympatric pairing produced offspring or embryos, and only 1 female in the allopatric pairing produced offspring or embryos. Statistical tests were conducted at $\gamma = 0.05$. On a Mann-Whitney U test of size-corrected numbers of embryos and offspring, females in the sympatric group produced significantly more offspring or embryos than females in the allopatric group. The difference between sympatric and allopatric groups implies that there are behavioral, mechanical, or epigenetic factors affecting fertilization. These findings suggest that *P. formosa* is a good model for studying fertilization factors apart from the genetic contribution of sperm.

P309 Imaging Resting-state Functional Connectivity in the Mouse: Balancing the Effects of Anesthesia and Motion to Create a High-throughput Neuroimaging Protocol

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Optical functional neuroimaging of resting-state functional connectivity (FC) in mice could allow preclinical development of translational biomarkers of neurologic injury. However, brain hemodynamics and neurovascular coupling are affected by anesthesia. Our aim was to develop methods to improve animal restraint to minimize both artifacts and corruption by brain activations. We performed optical neuroimaging of intrinsic signals through intact-skull cranial windows. Mice were restrained with a custom-designed head fixation device that attached to the windows as well as a commercially available sling that we modified to further prevent motion. Twelve C57Bl/6J mice (8 male and 4 female mice, 8 wk of age) were acclimated to head fixation and physical restraint over 3 to 5 d with treats provided for positive reinforcement. Neuroimaging was performed using 1) ketamine (100 mg/kg)/Xylazine (10 mg/kg) anesthesia IP with stereotaxic ear bars, 2) ketamine/xylazine anesthesia with the new head fixation plate, 3) isoflurane (0.5%) anesthesia with the head fixation plate, and 4) awake with the head fixation plate. Scan quality was assessed using framewise displacement (FD), quality control measures, and DVARS (Derivative VARiance over pixels). Mice tolerated greater than 30 min of head fixation and restraint after 4 to 5 d, except for one mouse that tolerated 20 min. Both FD and DVARS were improved with the new head fixation plate compared to prior ear bars. While ketamine/xylazine anesthesia resulted in the cleanest, most segregated networks, this protocol was harsher on mice, prolonging recovery. Awake imaging was corrupted with large, intermittent hemodynamic spikes (likely motor activations) that affected the FC networks. Sedated imaging with 0.5% isoflurane resulted in fewer motor activations and intermediate network quality. Head-fixed mice placed into restraint slings with clamps to reduce sling motion can tolerate awake and minimally-sedated imaging. FC networks are not only affected by anesthesia, but also by motor activations present in awake mice. Sedation using 0.5% isoflurane may be an effective compromise allowing repeated imaging sessions while reducing anesthetic exposure. Future work will involve temporal censoring to remove residual high variance time points to capture a better representation of the resting-state.

P310 Development and Qualification of Adeno Associated Virus (AAV) Neutralizing Antibody (NAb) Titer Assays for Prescreening in NHPs

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Adeno-associated virus-based vectors are a leading delivery method in the development of gene therapies. Pre-existing immunity to AAVs is common and can interfere with vector distribution, altering transduction and transgene expression in these studies. Cell based AAV NAb detection assays have emerged as the preferred method for NAb prescreening of NHPs prior to selection for studies. A diversity of AAV serotypes have been identified and differences in tropism are expanding gene therapy studies from predominantly utilizing AAV2 and AAV9 to employing a wider range of serotypes. Consequently, development of NAb detection assays for a multitude of AAV serotypes is necessary. AAV NAb screening and titer assays were developed and qualified for serotypes AAV2, AAV8, AAV9 and AAVrh.74. Transduction of each AAV serotype was optimized by testing a range of viral titers, serially diluted down from a multiplicity of infection (MOI) of 50,000, as well as varying infection times from 1 to 3 d in multiple cell types including HeLa and HEK-293T cells. Significant variability in the potency of lots of viral vectors from different vendors was observed. Optimized assays underwent multi-tech, multiday qualification studies with a total of 6 runs performed to assess diagnostic sensitivity, specificity, and reproducibility of in vitro AAV NAb assays for screening and titer determination of NAb in sera. Cells incubated with 1/10, 1/20 and 1/40 dilutions of 8 known positive and negative samples were infected with serotype-specific AAV-CAG-luciferase providing a luminescent readout of infection. To assess titer assays, high titer samples were serially diluted 2-fold from 1/40 to 1/5,120 and measured by 2 techs. Qualification of the AAV NAb assays showed high sensitivity (> 95%), specificity (>99%), and reproducibility. Selectivity of the screening assays was very high with minimal cross-reactivity observed by other AAV serotype antibodies (AAV1, AAV2, AAV5, AAV6, AAV8 and AAV9). Comparative testing between internal and external labs showed good agreement in measured NAb titers for identical samples. In conclusion, cell-based AAV NAb assays were validated and are highly sensitive and specific for routine screening for the presence of neutralizing antibodies in NHPs.

P311 Can Hypoimmune Pancreatic Islet Cells Be the Key to a Better Future for T1D Patients?

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Type 1 diabetes (T1D) affects more than 1.6 million Americans according to the American Diabetes Association. T1D develops when the body's pancreatic islets malfunction or are damaged, thus failing to produce adequate insulin levels. Allogeneic donor islet transplantation as a treatment for T1D has limited success due to the morbidity of potent immunosuppression (IS). Few patients remain insulin independent 4 y beyond transplantation due to gradual loss of islet function caused by general immune rejection. We sought to engineer mouse primary islet cells (mPI) that avoid host immune detection and rejection without immunosuppression, and that could be transplanted intramuscularly. We used our Hypoimmune engineering strategy knocking-out the function of MHC class I and II and overexpressing CD47 to evade both adaptive and innate immune cell killing. Islet cells from male C57/Bl6 mice were engineered to overexpress CD47 (HIP mPI). Control islet cells (Wt mPI) from male C57/Bl6 mice were unmanipulated but cultured in the same way. Male allogeneic Balb/C mice were made diabetic using streptozotocin and blood glucose levels were monitored every 4 d via later tail vein collection. A total of 600 firefly luciferase+ HIP mPI or Wt mPI were transplanted intramuscularly and monitored over a 30-d period. Bioluminescence imaging showed survival of all HIP mPI (n = 4) but rapid rejection of Wt mPI (n = 4). Glucose levels (measured 4 h after fasting) gradually decreased after HIP mPI transplantation and remained stable around 200 mg/dl after 3 d and beyond. No effect on glucose levels were seen with Wt mPI transplantation. At the end of the 30-d period, the mice (n = 8) were euthanized, and tissues were evaluated to verify no immune activation via ELIspot and XCelligence assays. These preclinical findings suggest that HIP islet cells transplanted intramuscularly may be capable of persisting and functioning in diabetic patients without IS. Furthermore, this strategy could be expanded into pluripotent stem cell-derived islet cell therapies.

P312 CLEFMA Treatment in a Murine Model of Intestinal Ischemia-Reperfusion Injury

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Intestinal ischemic-reperfusion injury (IRI) occurs when vascular blood flow is interrupted in the mesenteric vessels, followed by reperfusion when blood flow is re-established to the ischemic tissue. Restoring vascular circulation worsens the intestinal injury and causes systemic effects by engendering oxidative stress and an inflammatory cascade. The American Gastroenterological Association reports mortality rates as high as 93% in patients experiencing acute intestinal ischemia. Conventional surgical treatment corrects vascular occlusion but is limited in success due to the reperfusion injury and gut barrier dysfunction that follows. There are no pharmacotherapeutic options currently available for patients experiencing an IRI event. 4-[3,5-Bis(2-chlorobenzylidene)-4-oxo-piperidine-1-yl]-4-oxo-2-butenoic acid (CLEFMA) is a synthetically created curcumin analog that has demonstrated suppression of pro-inflammatory cytokines, chemokines, and reactive oxygen species. This study investigated the ability of CLEFMA to reduce the severity of injury to the intestinal tissue after IRI in a mouse (CD1) surgical model. Animals were anesthetized with isoflurane with medical grade air and euthanized with CO_2. The study was designed with 3 treatment groups (n = 6 per group). In the CLEFMA and vehicle treatment groups, intestinal ischemia was surgically induced through a laparotomy with vascular clips for 75 min followed by 24 h of reperfusion. Animals in the CLEFMA group received 0.4 mg/kg of CLEFMA IP, while animals in the vehicle group received 100 ml of polyethylene glycol 400 IP. Animals in the third (sham) group experienced laparotomy with intestinal manipulation without vascular clip application. Histopathology scoring (Chiu) and TNF-γ were evaluated to compare the 3 treatment groups. This study found a statistically significant difference in histopathology scoring (P = 0.01) and serum TNF-γ concentrations (P = 0.01) when comparing the sham group to the CLEFMA and vehicle group. However, there was no statistically significant difference in evaluated data between the CLEFMA group compared to the vehicle group. This study failed to show reduced severity in intestinal tissue damage after an IRI event with CLEFMA treatment. Further investigation into the application of CLEFMA in IRI models is warranted given the promising anti-inflammatory and gut barrier protection demonstrated in previous studies.

P313 Ear Slicing of PD 7 C57BL/6 Mice as a Method for Permanent Identification and Genotyping

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No current standard techniques exist for the simultaneous identification and genotyping of mice between the ages of 7 and 14 d of age. Genotyping and identification of < 7-d old pups are limited to toe clipping with strong justification required for its approval in study protocols as defined by the *Guide for the Care and Use of Laboratory Animals*. Developing a technique to genotype and identify mice at 7 d of age would improve breeding efficiency, reduce housing costs, and provide a minimally invasive and reliable identification and genotyping option. We hypothesized that ear slicing 7-day-old mice with different ear slice combinations would provide a permanent method of identification and successful genotyping results. In a preliminary study with C57BL/6Crl mice of both sexes, 2 were ear sliced at 7 days of age, 2 at 10 days of age, and 1 without (control). The entire dorsal edge of the pinna (horizontal), or outer, lateral edge (vertical) were sliced with sharp scissors. Mice had left horizontal, right horizontal, left vertical, right vertical, or no ear slice. Vocalization during ear slice, dam acceptance, and outward ear appearance were assessed. Ear slice pictures were taken daily until 24 d of age. The sliced segment of ear (3 mm × 0.08 mm) was collected and sent for genotyping through a commercial automated genotyping company. All genotyping samples yielded 100% successful genotyping of C57BL/6Crl strain. The DNA quantity (cT values via TaqMan qPCR assay) and DNA quality (Mini Mouse Universal Genotyping Array) were excellent. A survey of the ear slice images taken between the ages of 7 and 24 d was presented to 16 participants and over 80% of participants were able to accurately identify the ear slices. Vocalization was minimal to absent during the slicing sessions, and no dam acceptance issues were noted. The right horizontal ear slice was the most difficult to correctly identify; as the ear pinna grew, it shifted the horizontal ear slice to appear more vertical. A larger study followed, in which fifty C57BL/6Crl mice of both sexes were ear sliced at 7 d of age and followed until day 60. The horizontal ear slice was modified to correct the appearance of the horizontal ear as the pinna grew. The studies highlight the potential for simultaneous permanent identification and genotyping of 7-d-old mice using a combination of ear slicing patterns.

P314 Evaluating the Efficacy of a Single Dose of Oxfendazole Against Oxyuriasis in 2 Lizard Species

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Lizards remain popular as pets and exhibit animals at zoological institutions, as well as research models for clinical and basic science research. Unfortunately, these animals are commonly infected with one or more endoparasites, regardless of their source (for example, wild-caught, captive-bred). One of the most common nematodes identified in captive reptiles is oxyurids. Oxyurids complete their entire life cycle in the gastrointestinal tract. Anecdotal reports suggest that these parasites are difficult to eliminate; however, there is limited evidence-based research confirming these reports. The purpose of this study was to determine if a single dose of a benzimidazole followed by weekly cage cleaning via a cage washer could eliminate oxyurids from 2 species of lizards. We hypothesized that a single dose of oxfendazole (25 mg/kg, per os) and cage cleaning would be sufficient to eliminate oxyurids from leopard geckos (*Eublepharis macularius*) and blue-tongued skinks (*Tiliqua scincoides*). Ten geckos and 8 skinks purchased from captive breeders and confirmed positive for oxyurids using a modified McMaster's were recruited for the study. After the treatment, feces were collected at 2, 4, and 8 wk posttreatment and screened for oxyurids using the modified McMaster's. Most geckos (9/10, 90%) and skinks (6/8, 75%) were oxyurid negative at 8 wk. Results suggest that a single dose of oxfendazole can clear oxyurids in the majority of animals; however, the treatment is not 100% effective. Animals should be monitored and provided additional treatment to prevent these parasites from becoming established in a collection.

P315 Effects of Dietary Tree Exudates On Fecal Calprotectin and Microbiome Composition In a Group of Captive Housed Common Marmosets (*Callithrix jacchus*)

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The common marmoset (*Callithrix jacchus*) is used in a wide variety of research including gene therapy, aging, neuroscience, and immunology. Marmosets in captivity have significantly higher rates of obesity, chronic lymphocytic enteritis, and metabolic bone disease compared to wild populations. While many factors are associated with the development of these conditions, diet is likely a major player. In the wild, most of a common marmoset's time is spent foraging for *Tapirira guianensis* tree sap, making them obligate exudativores. This study sought to assess the effect of the addition of tree exudates to the diet on the captive marmoset microbiome and gastrointestinal health. Changes in microbiome were examined via Illumina sequencing with 16S rRNA libraries targeting the V4 region and the effect on gastrointestinal health was assessed via ELISA for fecal calprotectin (an inflammatory protein often used as a marker for IBD in humans). Thirty-nine animals were randomly assigned to 1 of 3 types of supplements: acacia (a sap isolate used widely in captive marmoset feeding), mastic (a sap more similar to *T. guianensis*), and yogurt as a vehicle/control. Each animal received supplementation of 7g acacia, 54.33 mg mastic, or 1 tsp yogurt each morning, M through F, for 4 wk. Fresh fecal samples were collected from the pan beneath the enclosure in the afternoons, 3 times per week for 6 wk with 1 wk each for pre- and postsupplement feeding. The pretreatment samples served as a baseline. Acacia supplementation resulted in an increase in Actinobacteria and a decrease in Alphaproteobacteria as well as a decrease in species richness; mastic supplementation and yogurt control groups did not demonstrate similar changes. The addition of tree exudates to the diet did not affect calprotectin values. These data are intriguing because the microbiome of marmosets in the wild is similarly enriched in Actinobacteria, lower in Alphaproteobacteria, and less rich, implying that the addition of acacia gum to the diet may drive a shift in the captive marmoset microbiome towards that seen in wild marmosets. Further research on this topic is needed to determine if regular supplementation with acacia gum can decrease the incidence of gastrointestinal disease in captive common marmosets.

P316 Assessment of Individual Renal Function Using 99mTc-MAG3 Scintigraphy in Rabbits with Complete Unilateral Ureteral Obstruction

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Urinary tract obstruction is a condition that can progress to renal dysfunction. Blood and urine tests can measure overall renal function but are not able to measure individual kidney function. In the present study, 99mTc-labeled mercaptoacetyltriglycine (99mTc-MAG3)

renal scintigraphy was used to evaluate its utility in identifying unilateral ureter obstruction. Twelve healthy male New Zealand white rabbits (age: 12 wk, body weight: 3.1 tp 3.2 kg) were divided randomly into 2 groups of 6 animals: saline-injected control and 99mTc-MAG3-injected group. Complete double ligation of the right ureter was induced to achieve unilateral ureter obstruction in rabbits. Changes in renal function were investigated for 4 wk postobstruction by obtaining planar images of 99mTc-MAG3 activity after being injected via ear using noninvasive scintigraphy in conjunction with histologic and hematologic examinations. The scintigraphic images were recorded every 2 s for 1 min, then every 1 min for 25 min. Blood samples were taken to measure blood urea nitrogen (BUN) and creatinine levels at intervals during 4 wk of scintigraphy and commencing on day 1 of surgical intervention. Renal uptakes of 99mTc-MAG3 were remarkably and rapidly reduced in the ureter-obstructed kidneys. During the experimental period, serial scintigraphic images showed a progressive increase in the size of the ureter-obstructed right kidney whereas the left stayed consistent within the normal size. BUN and creatinine levels were significantly higher at week one of the ureter obstruction, in comparison to the respective levels before the obstruction. Histopathologic examination showed flattening and atrophy of tubules, enlargement of interstitial tissue, accumulation of extracellular matrices, and infiltration of inflammatory cells in the obstructed kidney. These results suggest that 99mTc-MAG3 scintigraphy is a sensitive, noninvasive method for assessing individual renal function in rabbits with complete unilateral ureteral obstruction.

P317 Establishment of a Mouse Model to Evaluate mRNA Vaccine Safety

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FDA granted emergency use authorization (EUA) for the world's first mRNA vaccine, developed by Pfizer-BioNTech, in December 2020. As a result, many vaccinated people were protected from the fatality of COVID-19, but some people suffered from various side effects of the mRNA vaccine. The EUA was immediately decided to control COVID-19 pandemic and the deregulation of preclinical safety assessment for mRNA vaccine was inevitable. In preclinical phase, efficacy assessment of several mRNA vaccine candidates has been performed by using COVID-19 mouse infection model. However, the guideline of safety assessment for mRNA vaccine in mice has not yet been established. Therefore, it is necessary to identify mRNA vaccine-induced toxicity and clinical symptoms. In this study, we evaluated the clinical and serologic changes induced by the intramuscular injection of 4 types of mRNA vaccines (100 µg/head) with different compositions (C2/LNP90, C2LNP128, C3LNP90, and C3LNP128) in 6-wk-old male and female ICR mice. Five mice per group, a total of 25 male and female mice, respectively, were used in this study. mRNA vaccines were injected twice at an interval of 2 wk and necropsy was carried out 2 d after secondary injection. CBC, blood chemistry analysis, and visual evaluation of whole-body tissues were performed. The results showed that the body weight was decreased for 2 d after the first injection in C2/LNP128 and C3/LNP128-injected mice compared to vehicle-injected mice, but it was almost recovered at 14 d postinjection (dpi). The endpoint blood and serum analysis demonstrated that C2/LNP128 and C3/LNP128 decreased the number of lymphocyte, monocyte, and reticulocyte carrying the abnormal level of liver function indicator such as albumin, AST, ALT, and total protein. Additionally, C2/LNP128 decreased the number of platelets and C3LNP128 decreased the number of red blood cells, respectively. Spleen and inguinal lymph nodes were enlarged in all experimental groups compared to the control group. Notably, C2/LNP128 and C3/LNP128 induced severe edema in the injection site, the femoral muscle, that was significantly enlarged. Although more detailed analyses should be carried out, these results suggest that the safety assessment of mRNA vaccines must be systematically established with multiple aspects of toxicology and laboratory animal medicine.

P318 Comparison of Anesthetic Regimens for Mechanical Ventilation in Mice

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For clinically relevant models of critical care, mechanical ventilation may be necessary for extended periods of time. However, choice of anesthetic and inspired oxygen concentration may affect basic physiology and cellular function. To optimize the development of murine models, anesthetic regimens were compared for efficacy and baseline effects on inflammation. Male, C57BL/6 mice (25 to 30g) were randomized for anesthesia with either intraperitoneal pentobarbital (PENTO; 60 mg/kg, redosing 15 to 30 mg/kg) or isoflurane (ISO; average maintenance = 1.2 to 1.4%). After tracheotomy, mechanical ventilation with 21% or 100% oxygen (tidal volume = 7 mL/kg, 100 breaths/min) was initiated. Body temperature was regulated by an infrared warming pad and maintenance fluids were given every hour. Pulse, oxygen saturation (SpO_2), and temperature were monitored continuously. After 6 h, the mice were euthanized for harvest of blood and bronchoalveolar lavage fluid (BALF). Overall, there were no differences in survival rates or physiologic stability among groups. The isoflurane and pentobarbital dosing were not impacted by inspired O_2 level. Pulse rates were higher in the ISO groups and significantly different between the ISO:100% O_2 and PENTO:100% O_2 groups. The hourly, mean SpO_2 was > 94% in all groups, with the ISO:100% O_2 group significantly increased over PENTO:100% O_2. Although complete blood counts and plasma IL-6 levels trended toward lower mean values with 100% compared to 21% O_2, significant differences were evident only in monocyte counts. Likewise, mean BALF cell counts were slightly lower, but not significantly different, with 100% compared to 21% O_2. Indicators of pulmonary epithelial injury were higher in the 100% O_2 groups and significantly increased with PENTO:100% O_2 versus ISO:21% O_2. Overall, both pentobarbital and isoflurane provided suitable anesthesia for 6 h of mechanical ventilation with either 21% or 100% oxygen in healthy mice. The level of inspired oxygen had some impact on baseline biomarkers of inflammation and epithelial injury. Further investigation of cell function and disease states is warranted to optimize anesthetic regimens for murine models using prolonged mechanical ventilation

P319 Comparison of 2 Types of Bedding on Breeding Fecundity in C57Bl/6J Mice

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One of the only options for generating complex transgenic laboratory mice is through in-house breeding and management strategies. One consideration in the management of these colonies is how the animals' environment may affect reproductive success. Several aspects of the microenvironment can be controlled or manipulated, including cage type, bedding, enrichment, diet, and temperature and humidity. This study seeks to evaluate reproductive outcomes for C57Bl/6J mice that were randomly assigned to 1 of 2 different bedding types: paper-based or corn-cob bedding. Our hypothesis was there would be no significant difference in reproductive outcomes between the 2 bedding types. A total of 10 males and 10 females were paired at 45 d of age. Animals remained together and

were allowed to breed for 15 consecutive weeks. Cages had daily checks for the presence of pups and a pup count was performed at 7 d of age. Weaning occurred at 20 or 21 d of age, at which time a final pup count, pup weight, and sex were recorded. All litters born and pups weaned in the 15-wk timeframe were used for data analysis. Fischer's Exact Test was used to compare cannibalization between the 2 groups and the results showed no statistical difference between the animals on corn cob versus paper-based bedding ($P > 0.05$). An unpaired *T*-test was used to compare both litter size and weight of weaned pups between the 2 groups, both resulting in no significant difference for either outcome. The study did find that 100% of the animals that produced a total of four litters within the 15-wk timeframe were housed on corn cob bedding. In addition, all pups counted at day 7 survived to weaning age in both groups. We concluded that both bedding types produced similar success regarding breeding fecundity in C57Bl/6J mice.

P320 E-cigarette Condensates Cause Rapid Impairment of Cellular Metabolism and Ciliary Function in Precision-cut Lung Slices

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Common models for e-cigarette (e-cig) toxicity often lack standardization of delivered dose for product comparisons and evaluation of functional responses of the intact lung. Precision-cut lung slices (PCLS) are viable excerpts of the lung, retaining all resident cell types and structures of the lung, thus enabling the investigation of unique functional and cellular endpoints. Here, we evaluated cellular energy metabolism, and ciliary beating frequency (CBF) responses of PCLS to e-cig condensates prepared from vehicle, nicotine, menthol, or nicotine + menthol e-liquids. Condensates were analyzed for glycerin, toxic metal, nicotine, and menthol content for quality control. PCLS (300 µm) were prepared from adult C57BL/6 mice and exposed to e-cig condensates at doses normalized to glycerin. Using a WST-1 assay, we previously showed that cellular metabolism was suppressed after exposure of PCLS to menthol-containing condensates. To further elucidate, we used a Seahorse XF Analyzer to assess glycolysis and mitochondrial oxidative respiration. Responses were monitored over a total of 1-h exposure to e-cig condensates. We observed a reduced basal respiratory rate within 6 min and decreased glycolytic reserve after 1 h. Glycolysis, measured as extracellular acidification rate, was significantly reduced within 30 min of exposure to 300 mM nicotine and menthol-containing condensates. Maximal oxygen consumption rate decreased by 90% relative to media controls after 1 h exposure to 300 mM for menthol-containing condensates, but not for nicotine. Using video microscopy, CBF was recorded before and after exposure of PCLS to e-cig condensates. At 300 mM, menthol-containing condensates caused a reduction of CBF by 75% compared to baseline within 21 min, while ciliary responses to nicotine condensate were more variable and less pronounced at the same dose. Together, these data demonstrate that the presence of nicotine or menthol in e-cig condensates are principal drivers of acute metabolic and functional impairment. NIH P30ES005022 and T32ES007148.

P321 Measurement of Subcutaneous Tumour Height Achieves Greater Volumetric Accuracy

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Subcutaneous tumor xenografts in mice are used as a model for testing new therapies in vivo. Therapeutic efficacy is commonly assessed by monitoring changes in tumor volume over time, by comparing tumor growth curves between control and treated groups of rodents. The current method of choice for capturing tumor volume is manual measurement with calipers. Tumor length and width measurements are taken by placing caliper blades on the outside of the tumor mass. Tumor height is difficult to capture using calipers, as the tumor base is obstructed by the mouse body, leading to larger instrumental error (0.5 mm for height vs 0.1 mm for length and width), thus, methods to estimate tumor volume from length and width alone are used. We aimed to investigate the potential accuracy improvements of using 3D and thermal imaging (3D-TI) to capture length, width, and height. We used our anonymized global database of tumor scans taken by 3D-TI users to first define the average relationship between tumor height and width, then aimed to validate our findings by comparing LWH and LWW tumor volumes to the true volumes of excised tumors. Subcutaneous tumors from 148 rodents across 12 cell lines, 6 strains, and 13 studies were measured in situ (274 for callipers and 299 with 3D-TI) and compared to their true volumes determined after excision. It was found that 3D-TI using LWH was just -1.4% away from excised weights on average compared to +50.5% with callipers, making 3D-§ TI LWH volume measurement over 30× closer to excised weights on average. These results indicate capture of tumor height is essential to the accurate monitoring of tumor volume and therapeutic efficacy. By incorporating 3D-TI imaging, scientists can gain a greater understanding of efficacy, reducing the likelihood of obtaining an incorrect result, better separating dose groups, and managing welfare more precisely.

P322 Optimizing Measurement Methods of Subcutaneous Rodent Leg Tumors

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Radioligands used to visualize tumors in preclinical mouse studies also accumulate in the liver and kidneys in the same region as inoculated flank tumors, so it is common to inoculate tumors in the forelimb shoulder or leg instead so that signals will not overlap. Such tumors are judged more difficult to measure using traditional calipers, as tumors quickly become larger than the leg. We aimed to establish whether a 3D and thermal imaging device (3D-TI) could improve accuracy and reduce the variability of leg tumor measurements in comparison to calipers, as previously shown for flank tumors. Two cohorts of mice were inoculated with the same tumor cell line in both forelimbs (n = 12 and 14). Repeat measurements were taken by 3 users, using calipers and 3D-TI on the same day, 3 times per week for approximately a month for each cohort. When measuring Cohort 1 it was noted that tumors lower down the leg were more difficult to scan and measure. Therefore, Cohort 2 tumors were inoculated as high up the leg in the shoulder area as possible. From Cohort 1 to Cohort 2, variability assessed by coefficient of variation decreased by 30% with calipers (0.268 to 0.188) and by 17% with 3D-TI (0.188 to 0.156). In comparison to previous flank tumor data, leg tumor measurement was more variable for both devices, however, 3D-TI decreased variability in comparison to calipers by 30% and 17% in each cohort (114 and 149 paired repeats respectively, $P < 0.0005$). 3D-TI also decreased user bias and standardized measurement across the 3 users. The average difference from the group mean for one user decreased from 15.4% with calipers to just 0.12% with 3D-TI. We conclude that variability of rodent leg tumor measurement can be decreased in 2 ways: by ensuring that the tumor is inoculated high up in the rodent shoulder, away from the knee joint, and by using 3D-TI instead of calipers to take tumor measurements

P323 Adjusting Active Scavenging to Reduce Exposure to Waste Anesthetic Gas in a Research Facility

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Exposure to waste anesthetic gas (WAG) leaking out of a nose cone/induction chamber is an occupational hazard to veterinary and research staff, leading to acute symptoms of headaches, dizziness, and potentially nervous/reproductive system harm. California Occupational Safety and Health Administration (Cal/OSHA) has created a 2 ppm Permissible Exposure Limit (PEL) for an 8-h time-weighted average. Exposure can happen if users do not weigh/date activated charcoal canisters and flush the induction chamber prior to removing an animal from it. PEL can be measured by a charcoal monitor badge. This study helped determine a cost-effective method to reduce PEL with available utilities, using a direct reading instrument calibrated to detect isoflurane. Various engineering controls remove/adsorb WAG onto a charcoal bed. Vacuum lines are a form of active scavenging used in some labs without a way to determine appropriate flow rate because of a lack of meters and guidance on which rate to use. We installed an adjustable flow meter/rotameter to the vacuum line to measure volumetric flow rate in L/min. The line was attached to the nose cone similarly to a passive charcoal canister. O_2 nose cone flow rate was 1.5–2 L/min. Given the established protocols of inhalatory anesthesia, a cage of 5 C57BL/6 mice sufficed to test the system. Anesthesia was induced at 5% isoflurane in a vented induction chamber. Once an animal was anesthetized, it was removed and placed in a nose cone. Ophthalmic ointment was applied to both eyes. Anesthetic depth was assessed every 5› by loss of toe pinch reflexes. Isoflurane was set to 3% for maintenance. Vacuum was first set to 1.5 L/min and adjusted accordingly to maintain anesthesia depth. Isoflurane measurements were made with a device that uses infrared spectrophotometry and absorbance. The study complied with Cal/OSHA standards, which require evaluating engineering controls to lower PEL if it is exceeded. We sought to determine vacuum flow range to keep exposure to PEL< 2 ppm ensuring animals remained anesthetized. Measurements were made in the breathing zone of where an employee would be working at ~6–8 in. away from/12–18 in. above an animal. Mice began to regain reflexes above 1.8 L/min. Since the flow rate of the active scavenging unit may impact the ability to keep the animal properly anesthetized, we concluded that a house vacuum system can be used in the absence of other ducted engineering controls to successfully control PEL <2 ppm when the vacuum is set at 1.5–1.8 L/min using an adjustable rotameter.

P324 The Neurobehavioral Effects of Buprenorphine and Meloxicam on a Blast-induced Traumatic Brain Injury Model in the Rat

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Studies have shown that opioids and nonsteroidal antiinflammatory drugs (NSAIDs) may be neuroprotective after traumatic brain injury in rodents, but only limited studies have been performed in a blast-induced traumatic brain injury (bTBI) model. We examined the neurobehavioral changes in 48 male, Sprague–Dawley rats given a single, subcutaneous, preblast dose of meloxicam (1.4 mg/kg), buprenorphine, or no pain-relieving medication before being anesthetized and exposed to tightly coupled, duplicate air-pressure blasts in an advanced blast simulator. Rescue analgesics are typically not needed, as these experiments are conducted without analgesia, however, additional doses of buprenorphine or meloxicam or combinations of both were considered for rescue analgesia. Rats were randomized into 1 of 4 treatment groups: sham, repeated blast (BB), repeated blast pretreated with buprenorphine (BB+BUP), and repeated blast pretreated with meloxicam (BB+MEL), with each group containing 12 rats. The rats were pair-housed before and after blast injury and participated in 3 different behavioral assays (rotating pole test, novel object recognition test, and open field exploration test) at multiple time points up to 28 d postblast injury. A 16.7% mortality rate was recorded in the rats treated with buprenorphine, which might be attributed to the physiologically depressive side effects of buprenorphine in combination with isoflurane anesthesia and acute brain injury. Rats given buprenorphine, but not meloxicam, took more time to recover from the isoflurane anesthesia given just before the blast, $P < 0.01$. We found that treatment with meloxicam protected repeated blast-exposed rats from vestibulomotor dysfunctions up to d 14, $P < 0.05$, but by d 28 the protective effects had receded, $P < 0.01$. Open field exploratory behavior results showed that blast-exposed rats treated with meloxicam engaged in significantly more locomotor activities and possibly less responses thought to reflect anxiety and depressive-like behaviors than any of the other groups. In addition, rats treated with analgesics gained more weight than their untreated counterparts, $P < 0.01$, suggesting that analgesics alleviated at least some of the pain that may be associated with diminished eating and weight gain over time. These results suggest that meloxicam and, to a lesser extent buprenorphine alter a variety of neurobehavioral functions in a rat bTBI model and, because of their impact on these neurobehavioral changes, may be less than ideal analgesic agents for preclinical studies evaluating these neurobehavioral responses after TBI.

P325 Efficacy of High-dose Meloxicam for Surgical Pain in Female CD1 Mice

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Mice are one of the most commonly used species in biomedical research and are often used for surgical models. Meloxicam is a valuable non-steroidal anti-inflammatory drug for its analgesic effects in postoperative patients, but the effective dosages are not well understood for mouse surgical procedures. This study examined the analgesic efficacy of high-dose meloxicam in female CD1 mice using a laparotomy model. Mice were randomly assigned to 1) surgery and high-dose meloxicam ($n = 8$), 2) surgery and low-dose meloxicam (n = 10), 3) surgery and saline (n = 8), 4) anesthesia-only and high-dose meloxicam (n = 8), 5) anesthesia-only and low-dose meloxicam (n = 10), or 6) anesthesia-only and saline (n = 12). High-dose at 10 mg/kg (2.5 mg/mL), low-dose at 2.5mg/kg (0.5 mg/mL), or saline were given subcutaneously every 12 h. Pain was assessed at 3, 6, 12, 24, and 48 h postoperatively via an automated tracking analyzer and blinded observations of orbital tightening, rearing, wound licking, grooming, distance traveled, arched posture, and von Frey. The surgery and high-dose group demonstrated fewer pain behaviors than the surgery and low-dose or saline groups with lower mean cumulative orbital tightening scores 3 to 6 h postoperative and less hunched posture at all postoperative time points. Surgical mice were frequently less mobile than the anesthesia-only mice 6 to 12 h postoperatively. Surgical mice also frequently reared less, traveled less, and tolerated less abdominal pressure via von Frey compared to anesthesia-only mice. While surgical mice receiving 10 mg/kg every 12 h had fewer pain behaviors than surgical mice with a dose of 2.5 mg/kg, there was incomplete analgesia compared to the anesthesia-only groups. These findings suggest that 10 mg/kg meloxicam every 12 h improves analgesia compared to 2.5 mg/kg; however, it is not completely mitigated.

P326 Optimization of the Timing and the D-Luciferin Dose for Bioluminescence Imaging Using Tumor-bearing PBMC-humanized Mice

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In vivo bioluminescence imaging is invaluable in preclinical immuno-oncology research as it allows noninvasive and repeated measurements to follow the spread or recession of cancers. While a single, fixed time point after D-luciferin injection is widely used for high throughput measurements, the time for injected D-luciferin to

be metabolized in vivo by luciferase-expressing cancer cells and emit peak signal varies by tumor burden/location and D-luciferin injection dose/route. Particularly, the tumor burden is subject to change with the treatment of immunotherapy and with the engraftment of human peripheral blood mononuclear cells (PBMCs). We performed bioluminescence imaging sequentially post D-luciferin injection and tested two D-luciferin concentrations to pinpoint the most efficient and effective use of the compound. In the first study, we humanized 20 female NSG-MHC I/II DKO (DKO) mice with 10-15x10^6 human PBMCs and inoculated them with either 0.5x10^6 MDA-MB-213-Luc-2a-GFP cells or 2x10^6 Raji-Luc-GFP cells. We measured bioluminescence 5, 8, 10, 12, 15, 18, and 20 minutes after injecting 150mg/kg of D-luciferin intraperitoneally. We repeated the measurement every 2-3 days over nine days to capture the signal peak time depending on varying levels of tumor burden. MDA-inoculated mice showed tumor growth over time and the time to reach peak signal was 10-12 minutes. Raji-inoculated mice showed tumor shrinkage over time and the time to peak signal increased as tumor burden decreased, ranging from 10-20 minutes post-injection. Following the previous study, we injected 20 female PBMC-humanized DKO mice with 4x10^6 or 2x10^6 Raji-Luc-GFP cells and measured bioluminescence under the same time conditions, but with half of the mice receiving 150mg/kg of D-luciferin and the rest receiving 100mg/kg.150mg/kg D-luciferin yielded higher signals than 100mg/kg D-luciferin in both tumor burden conditions. The data suggest that the best results for bioluminescence imaging are achieved by taking images at least 10 minutes post-dose of D-luciferin and using manufacturer-recommended dosage concentration consistently across the treatment group and study period.

P327 Safety and Tolerability Assessment of Commonly Used Vehicle Formulations on Strain, Age, and Gender of Mice

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A critical part of preclinical studies is selecting a vehicle that will have minimal to no effect in animals with repeated administration for the intended route of administration while ensuring solubility/stability and maximizing bioavailability of the test compound. Previous studies on vehicle tolerability have occurred, however, there is little to no information regarding safety related to different strains, ages, or gender. In this study, we aim to fill this gap by analyzing 4 vehicles with components commonly used with test compounds that are poorly soluble in water in order to evaluate the safety and tolerability in 3 different mouse strains. Vehicles were dosed by oral gavage at 10 ml/kg once daily for 14 d. Vehicles tested were: Vehicle 1 (0.5% Hydroxypropyl Methylcellulose, 0.5% Tween80, Water), Vehicle 2 (0.2% Tween80, 10% PEG400, 10% Kolliphor HS 15, Saline), Vehicle 3 (5% N-methyl-2-pyrrolidone, 10% PEG400, 10% Kolliphor HS 15, Saline), Vehicle 4 (15-30% Labrasol, Saline), and control (Saline). Three strains of male and female mice were tested with 6–8 mice per gender: C57BL/6j (85 wk and 8 wk), CD1 (8 wk) and B6C3F1 (8 wk). Body weight, food consumption, and toxicology assessment using the Modified Irwin test were recorded daily. At the end of the study, necropsies were performed, primarily focusing on the stomach, liver, kidney, spleen, lungs, and reproductive organs. Mice dosed with Vehicle 4 showed a rapid decline in body condition after 4 d of dosing, therefore the concentration of Labrasol was decreased from 30% to 15% in all groups. Significant observations were found in body weight dependent upon vehicle, day, strain, age, and gender. In CD1, food consumption was lower for all vehicles compared to control. In the 85-wk C57BL/6j group food consumption was lower only in the Vehicle 4 treated group compared to control. Minimal toxic effects, as determined by the modified Irwin test, were observed in all groups. Necropsy results showed differences between strain, age, and gender. Treatment with Vehicle 4 in C57BL/6j and B6C3F1 strains resulted in abnormal observations of the spleen and liver, primarily affecting size and color. This study confirms that the assessment of strain, gender, and age of interest prior to a routine preclinical study is a key step for ensuring the study results are valid and not compromised by the vehicle formulation.

P328 The Use of Electropenetrography to Examine *Aedes aegypti* Probing and Ingestion in a Murine Model

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Mosquitos are among the most important arthropod vectors of human and animal virus transmission worldwide. A detailed understanding of mosquito ingestion behaviors is crucial in developing novel interventions to interrupt the transmission of important viruses. The present study used an alternating or direct current (AC-DC) electropenetrography (EPG) to observe the ingestion behaviors of adult *Aedes aegpyti* mosquitos on a mouse host in real-time. EPG allows us to observe, record, and quantify probing and ingestion behaviors of arthropods with penetrating mouthparts. EPG recordings were performed on mosquitos actively feeding on anesthetized female CD1 mice (n = 5) inside a biocontainment glovebox surrounded by a Faraday cage. Waveforms produced in this system were similar to previous publications with mosquitos feeding on human hands. We were able to visualize and differentiate mosquitoes entering the skin, cannulation of the blood vessel, and ingestion of blood based on the frequency and resistance of the waveforms recorded. The optimal settings for EPG recordings of *Ae. aegypti* probing on mice were 100 millivolts of alternating current with 10^7 Ohms of resistance. Understanding the bite dynamics at the time of ingestion may help characterize vector pathogen host interaction. The results from this study demonstrate the novel use of EPG in a murine model and will facilitate future studies with arbovirus infected mosquitos and hosts.

P329 Fetal Sex Differences May Obscure Impacts of Experimental Treatments in a Rat Model of Perinatal Nicotine Exposure And Infection

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Animal models of perinatal infection are essential to the advancement of maternal, fetal, and perinatal health. Chorioamnionitis (a maternal inflammatory response, MIR, affecting fetal tissues) and umbilical vasculitis (a fetal inflammatory response, FIR) are often seen with *Ureaplasma* spp. infection in humans. Such intrauterine infection/inflammation is associated with adverse pregnancy and neonatal outcomes. We use experimentally induced *Mycoplasma pulmonis* (MP) infection in rats to model the intrauterine infection and inflammation seen in humans. Additionally, nicotine (NIC) exposure persists as a public health issue, and study of its impacts on fetal health and development remains relevant. We looked at low and medium NIC exposure impacts on intrauterine MP infection/inflammation. Based on neutrophil infiltration and tissue damage, MIR and FIR were scored as none/mild (low) or moderate/severe (high). Our study identified multiple distinct patterns of MIR/FIR severity in fetuses (high MIR/high FIR; high MIR/low FIR, and low MIR/ low FIR) within treatment groups (low or medium NIC) and infection status [MP culture negative (-) or positive (+)] of intrauterine sites. We hypothesized that fetal sex may be a factor contributing to these varied patterns of MIR/FIR severity. To determine how fetal sex contributed, we extracted genomic DNA from the placenta of Sprague–Dawley rat fetuses necropsied at gestation day 18. We performed PCR for the presence of the sex-determining region of the Y chromosome (SRY) gene specific to male fetuses. We then re-analyzed histologic inflammatory response scores from various

uterine, placental, and umbilical sites using 2-way ANOVA with fetal sex and MP culture status as factors. We found multiple instances where fetal sex differences resulted in divergent inflammatory patterns. Specifically, in the low NIC group, female sex interacted with culture status to impact inflammatory responses of the umbilical vessels (high FIR in MP(-) fetuses). At the medium NIC dose, female sex impacted MIR [low MIR in MP(-) fetuses], while male sex impacted FIR [high FIR in PL(-) fetuses and low FIR in MP(+) fetuses]. We conclude that the sex of the fetus is an important consideration when evaluating inflammatory response data from intrauterine sites.

P330 Comparing PBMC Engraftment Rates Between Different Blood Collection Methods in Humanized Mice

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Retro-orbital (RO) bleeding and cardiac puncture (CP) are widely used techniques for blood collection in laboratory mice. Blood samples collected via RO or CP can be used to check whether engraftment of human peripheral blood mononuclear cells (PBMCs) is successful in mice. Preliminary work from our lab has found that blood collected via CP from mice that were engrafted with PBMCs showed higher CD45 events and other white blood cell populations than blood collected from RO. These results could suggest bleeding methods can affect blood parameters. Here we aim to expand these findings and investigate whether white blood cell populations measured from blood collected via RO versus CP differ, and if performing RO bleeds immediately before collecting blood via CP has any effect on absolute cell numbers. In this study, we humanized 32 7-wk-old female NSG-MHC I/II DKO (DKO) mice by irradiating the mice and then IV injecting PBMCs. PBMC engraftment was checked from blood samples collected on study days 6 and 11. On both days, blood was withdrawn from 2 different groups of animals: 1 group of mice had 100 µl of blood collected via RO just prior to euthanasia using CO_2. Following euthanasia, 200 µl of blood was collected from these same mice via CP and stored in a separate tube. The second group of animals was euthanized using CO_2, and 200 µl of blood was collected only via CP. Tetracaine was applied to all mice among RO groups. The blood samples were then stained with hCD19, hCD3, hCD14, hCD16, hCD45, and mCD45, and analyzed with a flow cytometer. We found that blood collected via RO resulted in lower absolute cell numbers as compared to both groups of blood collected via CP. Additionally, RO bleeding prior to CP blood collection has no effect on the number of cell populations measured. With these findings, we conclude that different methods of blood withdrawal are not interchangeable and that researchers should be mindful of the type of blood collection they use throughout their studies.

331 Pharmacokinetics of Single-dose Oral Gabapentin in the Guinea Pig (*Cavia porcellus*)

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Gabapentin is widely used in human and veterinary medicine to treat neuropathic pain, epilepsy, and anxiety. Gabapentin's wide margin of safety, relatively low cost, and ease of access (as it is not a controlled substance) make it an attractive medication for the previously mentioned conditions. Guinea pigs, as prey animals, are especially predisposed to experience stress, and pharmacological methods to reduce stress are crucial to improving animal health and welfare. To evaluate the pharmacokinetics of gabapentin in guinea pigs, 6 male and 6 female 10-wk-old Dunkin Hartley guinea pigs (weight: 464 to 616 g) were dosed orally with 40 mg/kg. After a 1-wk washout period, guinea pigs were dosed again at the same dose. The same guinea pigs, at 17 wk of age (weight: 648 to 966 g), were orally dosed with 20 mg/kg gabapentin with repeated dosing after a 1-wk washout period. Guinea pigs were anesthetized with isoflurane anesthesia and blood was collected from the jugular vein at 0, 0.5, 1,3, 6, 12, 24, 48, and 72 h postadministration for both the 20 and 40 mg/kg doses. Plasma concentrations were determined using liquid chromatography-tandem mass spectrometry and noncompartmental pharmacokinetic analysis was performed. Peak plasma concentrations were 4,770.1 ng/mL and 13,103.5 ng/mL and time to maximum concentrations were 0.5 h and 1 h for 20 mg/kg and 40 mg/kg, at 20 and 40 mg/kg respectively. These results suggest linear absorption of gabapentin in the guinea pig model. No severe adverse events were observed in any of the animals. However, mild to moderate sedative effects were observed between the 0.5- to 3-h time points, but these observations were difficult to interpret as animals were also undergoing anesthesia. Subjectively, guineapigs also reached an adequate plane of anesthesia for jugular blood collections faster during the 0.5- to 3-h time points. The results of this study suggest that gabapentin at doses of 20 to 40 mg/kg can safely be used in a guinea pig model. Future work to better understand the pharmacokinetics and pharmacodynamics is necessary to provide better dosing information in this species.

P332 The Onset of Action for 3 Formulations of Buprenorphine in an Incisional Pain Model in Mice.

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Providing effective and continuous analgesic care is imperative to prevent pain and distress in laboratory animals. The onset of analgesic effect for long-acting buprenorphine formulations has not been previously evaluated. This study aimed to investigate the onset of buprenorphine hydrochloride (Bup-HCL), sustained-release buprenorphine (Bup-SR), and extended-release buprenorphine (Bup-XR). We hypothesized that the onset of Bup-HCL would be shorter than that of Bup-SR and Bup-XR.In part 1, adult male C57BL/6J mice (n = 48) were randomly assigned to 1 of 4 treatment groups 1) Saline, 1 ml/kg SC, once; 2) Bup-HCL, 0.1 mg/kg SC; 3) Bup-SR, 1 mg/kg, SC; and 4) Bup-XR, 3.25 mg/kg, SC. After drug administration, an incisional paw pain model was performed. At day -1 and then at 20, 40, 60, 90, and 120 min following drug administration, thermal hypersensitivity (thermal latency using a hot plate assay) was measured. In part 2, the buprenorphine plasma concentration of adult male C57BL/6J mice (n = 30) was measured at 20, 40, 60, 90, and 120 min following drug administration. Results for the incisional paw pain model indicated that for the Bup-HCL, Bup-SR, and Bup-XR groups, 1) compared to their day-1 values, thermal latency did not differ at any time point; 2) compared to the saline group at the same time point, thermal latency was significantly increased. Buprenorphine plasma concentrations for all treatment groups were above the expected therapeutic level (1 ng/mL) as early as 20 min. Hyperactivity was the only clinical observation noted in the Bup-SR (83%) and Bup-XR (75%) treated mice. Results indicated that the onset of Bup-HCL was not faster than that of Bup-SR or Bup-XR. In conclusion, Bup-HCL, Bup-SR, and Bup-XR are all effective at attenuating thermal hypersensitivity at 20 min for the incisional paw pain model in mice.

P333 Deficient B Cell Responses Correspond with Cerebrospinal Fluid Leak Inflammation and Encephalitis in SIV-infected Macaques (*M. mulatta* and *M. nemestrina*)

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Simian immunodeficiency virus (SIV) infection in rhesus and pigtail macaques (*Macaca mulatta* and *M. nemestrina*) are both widely used models for AIDS pathogenesis. The course of infection in these species physiologically resembles HIV infection in humans, and the immune response and disease progression similarly vary widely between individuals. We hypothesized that differences in the adaptive immune response to SIV drive inappropriate innate activation which leads to poor clinical outcomes, including central nervous system (CNS) disease. Here we demonstrate that deficient B cell responses are associated with the development of SIV encephalitis (SIVE) in infected macaques.SIV-specific antibody responses were assessed by Western blot and ELISA using plasma and CSF samples from 42 d postinfection and the terminal time point (35 to 490 d) in 47 macaques, 26 of which developed SIVE. Cytokine production was measured using an MSD U-PLEX assay. Immunohistochemistry (IHC) of encephalitic brains was performed using antibodies against Iba-1, IL-18, IL-1ɣ, and ASC and counterstaining with hematoxylin.While SIV-specific antibody responses vary between individuals and over time, there was a marked deficiency in the development of antibody responses in animals that subsequently developed encephalitis (n = 29 pigtail, 18 rhesus). Macaques with encephalitis showed nonspecific cytokine elevations in the CNS, with greater increases in inflammasome-associated proteins. Similarly, IHC of the brain from encephalitic macaques shows prototypical SIVE with giant cells and macrophage infiltration as well as increased levels of inflammasome proteins ASC and IL-18. These findings demonstrate that crosstalk between the adaptive and innate immune systems is an important determinant of pathological outcomes of SIV infection. In particular, the development of appropriate B cell responses after inoculation closely correlates with protection from SIVE. Understanding the role of B cells in protection from SIV pathogenesis may help address ongoing cognitive dysfunction and chronic inflammatory pathology in people with HIV.

P334 Expression Analysis of the *Tmppe* Gene in the Intron 1 of the *Glb1* Gene by Generating Knockout Mice

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In 1997, we generated knockout (KO) mice of the *Glb1* gene and have been using them as a mouse model for GM1 gangliosidosis. The *Tmppe* gene, whose coding sequence is in the intron 1 of the Glb1 gene, shares the first exon with the Glb1 gene and is probably driven by the same promoter, but little is known about the nature of the *Tmppe* gene. Therefore, we separately generated KO mice of the *Tmppe* and *Glb1* genes and examined the interaction between their gene expression. Tmppe-KO and Glb1-KO mice were generated by genome editing using the TAKE method. The entire coding region of *Tmppe* was deleted by 2 guide RNAs flanking the coding region of the gene without perturbing the *Glb1* exons. On the other hand, the *Glb1* gene was knocked out by removing its exon 7 with 2 guide RNAs. After introducing Cas9 protein and guide RNA complexes into C57BL/6N pronuclear embryos by electroporation, pups were obtained by embryo transfer. Genetically modified individuals were selected from the pups and their offspring by PCR. The cerebrum, liver, and kidney from Tmppe-KO, Glb1-KO, and wild-type mice were subjected to Western Blot analysis (WB). For *Tmppe*, 44 2-cell stage embryos obtained by electroporation of 70 pronuclear stage embryos yielded 5 pups after embryo transfer; for *Glb1*, 52 2-cell stage embryos obtained by electroporation of 86 pronuclear stage embryos yielded 10 pups after embryo transfer. Genotyping PCR results showed that 3 of the former and 4 of the latter had gene deletion. Tmppe-KO mice were apparently normal with no effect on viability or fertility. TMPPE and GLB1 expressions in Tmppe-KO, Glb1-KO, and wild-type mice analyzed by WB with the 3 organs indicated that loss of TMPPE did not affect GLB1 expression, while loss of GLB1 did not affect TMPPE expression. These results suggest that the *Glb1* and *Tmppe* genes do not affect each other in terms of expression. The organ specificities of expression of the 2 genes were different even though they may share their promoters, suggesting the existence of regulatory mechanisms other than promoters.

P335 Spontaneous Tumors in NSG Mice

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NOD.Cg-*Prkdc*^{scid}Il2rg^{tm1Wjl} (NSG) mice are profoundly immunocompromised. NSG mice lack T, B, functional NK cells, and both alleles of the IL2 receptor common gamma chain. Compared to other immunodeficient mouse strains, NSG mice show excellent human tumor take rates. This makes this strain of mouse an ideal host for patient-derived xenograft (PDX) and human immune cell engraftment ("humanized" mouse) models. NSG mice were also described to be lymphoma-resistant, providing a major advantage compared with other immunodeficient mouse strains, such as SCID and NOD-SCID mice, in which spontaneous lymphomagenesis is frequent. However, little is known about the incidence, pathological type, host gender, and age factors in the development of nonlymphoma spontaneous tumors in NSG mice. Previous studies have reported the development of spontaneous tumors in aged female NSG mice, and murine tumors could spontaneously occur in NSG hosts with PDXs. Our aims were to create a database of spontaneous tumors among the retired NSG breeders in our mice colony and analyze the tumor incidence, and histopathologic tumor types. We necropsied 386 retired NSG breeders (191 males, 195 females; average ages of 338 and 337 d, respectively), and found that 36 mice had developed tumors, yielding an incidence of 9.33% (2.62% in males; 15.90% in females). Based on histopathology, the 5 tumors detected in male NSG mice (with average ages of 375 d) were 1 nick myoepithelioma, 1 hepatocellular carcinoma, 1 lung AB adenoma, and 2 leukemias involving multiple organs. The 31 tumors detected in the female NSG mice (with average age of 388 d) were 22 mammary adeno/adeno-squamous carcinomas, 5 neck/subcutaneous myoepitheliomas, and 4 other tumor types (1 leukemia, 1 hepatocellular carcinoma, 1 renal carcinoma, and 1 subcutaneous epidermal inclusion cyst). We did not detect any thymic lymphoma in our retired NSG breeders. To our knowledge, this is the first report of spontaneous tumors in aging male and female NSG mice in a defined-flora mice colony. In conclusion, spontaneous tumors are rare in nonaging NSG mice, supporting the use of this mouse model to study PDX with minimal risk of confounding murine tumors occurrence in pathogen-confined conditions.

P336 Dermatitis Factors, Bacteria, and Antibiotic Therapy in the Laboratory Mouse (*Mus musculus*)

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Dermatitis is common in laboratory mice and hinders research, but etiologies and effective therapies are elusive. Dermatitis represents more than ɣ of clinical cases in our research mice and often becomes ulcerative, alters behavior, and necessitates euthanasia with loss of research data. We hypothesized that bacterial culture and sensitivity of dermatitis lesions would guide effective antibiotic therapies and that identification of factors associated with dermatitis could lead to better management of this important condition. Fifty mice with dermatitis were skin-swabbed and received our standard treatment of Vetericyn and toenail trim. Gender, age, strain, number in cage, room and cage temperature and relative humidity (RH), bedding, food, cage location, and lesion location were recorded. Swabs were submitted for culture and sensitivity. Cases with bacterial growth were treated according to sensitivity results with 0.25 mg/ml (50 mg/kg/d) enrofloxacin (n = 3), 0.8 mg/ml (160 mg/kg/d) sulfatrim

(n = 2), 0.25 mg/ml (50 mg/kg/d) gentamycin (n = 5), or 0.8 mg/ml (160 mg/kg/d) ampicillin (n = 2) in drinking water for 14 d. Controls with bacterial growth (n = 9) or no growth (n = 23) received no antibiotic treatment. Lesions were photographed and scored on days 0, 7, 14, and 21 based on size and severity. Cases were 70% females, 30% males, aged 309 ± 169 (mean ± SD) d, representing several strains, primarily C57/B6 background, with 2.8 ± 1.3 housed per cage. Room environments were 73.6 ± 1.2 °F (23.1 ± 0.7 °C) and 42.5 ± 10% RH, and cages were 75.0 ± 3.7 °F (23.9 ± 2.1 °C) and 55.1 ± 11.7% RH. Cage location was 62% toward the room and 38% toward the wall, with 24% high, 26 52% middle, and 24% low on the rack. Most dermatitis was on dorsal neck, shoulder, limb, and dorsal flank, with an initial lesion score of 2.2 ± 0.7. Bacteria were cultured in 48% of cases, some with multiple species, including 81% *Staphylococcus* (primarily *S. xylosus*), 24% *Corynebacterium*, 19% *Streptococcus*, 10% *Enterococcus*, and 1% each of *Micrococcus*, *Jeotgalicoccus*, *Acinetobacter*, *Tatumella*, and *Pseudomonas*. Lesion scores did not correlate well with mouse or environmental factors or bacteria, but mean lesion scores decreased in all treatment groups between days 0 and 21. Our study contributes information regarding factors and methods that may aid in managing this perplexing condition in research mice.

P337 Lateral Tail Vein Cannulation Methodology for IV PET Radiotracer Injections in Mice

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Positron emission tomography (PET) is a clinically validated and widely used non-invasive imaging technique in drug development. A challenging and user-dependent step of PET imaging is lateral tail vein injections. Variability in tail injections has been reported to cause variability in PET data. We have optimized and validated a technique to minimize variability using ^{18}F-fluorodeoxyglucose (FDG, a glucose analog). A group of 8 FVB mice was anesthetized using 3% isoflurane in an induction box. Once absence of toe pinch reflex, mice were transferred to an imaging shuttle bed with anesthesia (2% isoflurane). The animal was placed in a lateral resting position with either the left or right lateral tail vein facing the injector. The tail was inserted with a 29G butterfly needle attached to PE-10 tubing flushed with 10U/µL heparinized saline into the tail vein and aspirated to confirm placement. A drop of surgical glue was applied between the needle and skin to fix the needle. Following cannulation, the animal was transferred to a warmed imaging bed inside the PET scanner and respiration maintained at 40–60 bpm via a respiratory monitoring system. A 60-min dynamic ^{18}F-FDG PET acquisition followed with 10-s, 30-s, 60-s, 5-min, and 10-min frames to verify delivery of ^{18}F-FDG tracer to the vascular compartment via a slow 20-sec manual push. PET images were reconstructed with a 3D OSEM algorithm with a co-registered 100um ISRA reconstructed CT scan. A 3D Region of Interest (ROI) was drawn around the whole body using Amira software to measure and calculate whole-body radioactivity. Injection accuracy was calculated with the formula: [dose] – [whole body radioactivity] / [dose] = % of unaccounted for radioactivity. Of the 8 mice evaluated, 5 mice had 0% unaccounted radioactivity, indicating complete intravenous injection with no residual activity in the tail. The remaining 3 mice had minimal unaccounted radioactivity of 0.88, 4.28, and 8.13 %. The current procedure demonstrates complete intravenous delivery of ^{18}F-FDG with minimal residual activity. This technique for intravenous delivery of ^{18}F-FDG PET tracer is reproduceable, robust, and results in minimal perivascular infiltration.

P338 Expression Patterns and Tropism of AAV Serotypes in Ferret Primary Visual Cortex

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Adenovirus associated vectors (AAVs) are regularly used to introduce genetically encoded calcium indicators (GECIs) into cortical neurons in a range of species, to allow measurements of neural activity using 2-photon imaging. Several AAV serotypes are available and sometimes used interchangeably. However, the efficiency and tropism of AAVs vary with serotype and host species. For the domestic ferret (*Mustela putorius furo*) these expression patterns have not been rigorously assessed despite their common use for neuronal imaging studies. Here, we examine the properties of GCaMP6f expression for 3 AAV serotypes in the ferret primary visual cortex (V1). For each of 3 AAV serotypes (AAV1, AAV5, and AAVDJ), 3 to 4 adult ferrets were sedated with intramuscular ketamine and atropine, had an intratracheal tube placed and maintained on an isoflurane vaporizer at 1 to 3%. Anesthetized ferrets were injected in V1 in 1 or both hemispheres to yield a total of 5 injection sites per serotype. Each serotype expressed GCaMP6 under the same promotor sequence (hSyn). After use in in vivo neural activity assays, each animal was euthanized by intravenous injection of an overdose of pentobarbital sodium and phenytoin sodium followed by intercardinal profusion of PBS (pH 7.4), the 4% paraformaldehyde (pH 7.4). Fixed brain sections were fluorescently labeled for interneurons (parvalbumin) and neural nuclei (NeuN). After manually identifying regions of expression in V1, the number of cells and area of expression were compared. We found minimal differences between the serotypes in expression area size, density of transduced cells, or in tropism for interneurons in V1. To assess viral transport to connected brain regions, we also quantified expression area and density in the lateral geniculate nucleus (LGN) for each corresponding hemisphere. Here we found significantly fewer transduced cells for AAVDJ and a significant correlation between V1 expression area and number of LGN cells transduced for AAV1 and AAV5. Any of the studied serotypes efficiently transduce local neurons without significant tropism for interneurons, however, AAVDJ has significantly less transport to the LGN. This provides guidance for selecting vectors for use in systems neuroscience projects in the ferret model system.

P339 Screening of NHPs for Possible Changes in Seroconversion for AAV Neutralizing Antibodies Over Time

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Adeno-associated viruses (AAVs) are harmless viruses ubiquitous in nature and are critical components in the development of gene therapies. After the first FDA approval in December 2017, AAV vectors have emerged as a leading delivery method for gene therapies across a range of therapeutic indications and dosing routes. Most of the 200+ gene therapy trials employing viral vectors are based on AAV vectors. A big challenge in these studies is immunogenicity as pre-existing immunity may interfere with vector distribution and consequently alter transduction and transgene expression both in humans and NHPs. Specifically, the presence of AAV neutralizing antibodies (NAb) in patients and research animals can undermine the potential efficacy of the drug being tested which biases the outcome of the study. Prescreening of NHPs for AAV NAbs prior to studies is routinely done; however, it is unclear how close to a study start date NHPs should be screened to avoid them seroconverting. We conducted a time-course study of AAV NAb prevalence in NHP sera to find out how far in advance NHPs can be screened for NAbs based on housing conditions. In this study, 100 cynomolgus NHPs were sourced from a single Asian supplier. NHPs were housed in separate rooms but were not always kept with the same roommates and occasionally were grouped with cohorts from other suppliers. Sera were collected from NHPs at 3 time points starting with 100 animals at the first time point (0 mo postarrival) falling to 87 at 4 mo and 65 at 7 mo postarrival. Out of these, 20

serum samples (12 males and 8 females) were tested by luminescence-based AAV2, AAV8, AAV9, and AAVrh74 NAb screening assays at 1/10, 1/20, and 1/40 sample dilutions. Samples were reported as positive if there was ɣ50% inhibition in RLU signal. While the majority of NHPs showed no significant change in AAV NAb titer over the course of the study, 2 animals showed an increase in NAb titer greater than 4-fold. Results from these studies suggest that routine prescreening for AAV NAbs in study animals can be done 3 to 4 mo in advance without any significant seroconversion with proper housing conditions.

P340 Adenovirus-mediated Gene Transfer in Rat Tenocytes for Nonsurgical Treatment of Tendon Injuries

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Tendon injuries are prevalent in human and veterinary medicine, yet few nonsurgical treatments exist. While oral anti-inflammatory medications and intra-articular injections provide temporary relief, they do not repair the damaged tissue. Therefore, alternatives that strengthen tendons could improve clinical outcomes. Recently, human in vitro and rat in vivo studies have demonstrated gene transfer therapy's role in strengthening tendons and preventing degenerative tearing. Tendons are primarily made up of fibroblast-like cells, or tenocytes. Proof-of-concept experiments in tenocytes with the expression of fluorophores, for example, GFP (green fluorescent protein), are the first step needed to achieve the goal of gene transfer treatment for tendon injury. To demonstrate this, tenocytes were isolated and grown in vitro from the Achilles, Supraspinatus, Patella, and flexor tendons of 2 male, F344 rats. Tenocytes were then transduced with Ad-GFP [recombinant adenovirus containing the green fluorescent protein gene] at various multiplicities of infection (MOI) and imaged by fluorescent microscopy at 24 h, 48 h, 72 h, 96 h, 7 d, 14 d, and 21 d postinfection (dpi). Early time points revealed dose-dependent GFP fluorescence. Cell death was observed at 7 dpi in the 2 highest MOI groups. By 21 dpi, tenocytes with an MOI of 100 and 250 pfu/cell were viable and fluorescent. This preliminary data supports successful expression following adenovirus-mediated gene transfer in rat tenocytes. Future directions aim to transfer genes of interest important for tenocyte regeneration in vivo, leading to valuable therapies in both humans and animals without invasive surgical intervention.

P341 Peripheral Tissues Respond to Gonadotropins to Produce Dehydroepiandrosterone and Estradiol, and to a Lesser Extent Testosterone, in Male Fischer Rats

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Circulating sex steroids are derived from the gonads, and to a lesser extent from peripheral tissues like the adrenals, brain, and adipose tissue. Sex steroid production by the gonads is mediated by luteinizing hormone (LH) signaling through the LHCG receptor. Gonadectomy results in an abrupt loss of circulating gonadal hormones but increases in gonadotropins due to the loss of gonadal hormone feedback on the hypothalamus and pituitary. In this study, we examined the contribution of peripheral tissue sex steroid production relative to gonadal sex steroid production in male Fisher rats. Intact and castrated rats (n = 4/group) were injected on alternate days for 10 d with human chorionic gonadotropin (hCG; 0.1 mL of 500 IU/mL hCG/animal/injection, SC, in the back of neck), the fetal equivalent of LH that also binds the LHCGR (Chorulon, Intervet Inc., Merck Animal Health, NJ). Blood was collected (between 8 a.m. and 10 a.m.) through the saphenous vein of the animal under anesthesia (2 L/min of oxygen with 3% isoflurane for 10-15 min) at 0, 1, 2, 3, 4, 5, 7 and 9 wk post-hCG injection using 2-mL EDTA K2 blood collection tubes and spun down at 2,500*g* for 10 minutes at 4 ɣC to separate the plasma. Plasma sex steroids were then assayed by electrochemiluminescence (Mesoscale Diagnostics, LLC). Castration led to a rapid decline in testosterone (T; precastration: 7.3 ± 2.2 ng/mL vs. postcastration: 0.58 ± 0.12 ng/mL, $n = 8$; $P < 0.01$), but not dehydroepiandrosterone (DHEA; 24.3 ± 4.2 ng/mL vs. 27.2 ± 4.5 ng/mL, $n = 8$; $P > 0.05$), or estradiol (E_2; 0.035 ± 0.01 ng/mL vs. 0.037 ± 0.01 ng/mL, $n = 8$; $P > 0.05$). hCG did not increase circulating DHEA, T, or E_2 in castrated rats. hCG increased DHEA, T, and E_2 by 5.6-, >3-, and 2.3-fold, respectively, in intact rats above baseline. These results indicate that the testes contribute 92% of circulating T, but do not contribute to circulating DHEA and E_2; and that elevated gonadotropin concentrations post-castration are sufficient to maintain DHEA and E_2 production from peripheral tissues, likely the adrenal gland. In conclusion, this data indicates peripheral tissues respond to gonadotropins to produce DHEA and E_2, and to a lesser extent, T.

P342 Testing and Validation of the Microbial Environment of the NASA Rodent Spaceflight Habitat Water Delivery System

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Sterilized, deionized water within a closed, self-sufficient system has been used in NASA spaceflight rodent studies for several decades. Within the specialized spaceflight rodent habitat, water is delivered through a compression spring-loaded bag system to maintain positive pressure. Refill of the drinking water occurs every 30 d by direct transfer of potable water from aboard the International Space Station (ISS). This enables long-term use without a weekly water change out, which meets spaceflight requirements but contrasts with the general guidelines for the sanitation of water delivery systems. Even though the water is iodinated to minimize microbial growth, rodents are fed a special diet of high moisture nutrient-rich food bars based on the AIN-93 diet that may contribute to microbial growth in this water system. We designed a ground study to assess the quality of drinking water that is given to rodents throughout a mission. We conducted the ground test using 20 female C57BL/6J mice housed in this specialized habitat to mimic the timeline of a 90-d mission as well as the environmental conditions (temperature, humidity, and pCO_2) within the ISS. We used a novel sampling method to test water at 2-wk intervals for the 90 d, and also after each 30-d refill of the water delivery system. Mice in standard vivarium cages with water bottles were also included for comparison to the habitat. The results showed that overall microbial load remained close to zero for the duration, while total organic compound concentrations increased from 1,370µg/L to 10,650µg/L over the course of 90 d but remained below the level of concern. Inorganic ions and pH were also found to be at acceptable levels. Overall, we conclude that this system is effective in delivering clean, potable water to rodents for the 90-d duration of current missions to the ISS.

P343 Characterizing Infant Rhesus Monkey Development of Vocalization Suppression during Presence of Potential Threat

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When presented with potential threats, nonhuman primates (NHPs) respond by inhibiting ongoing behaviors. This response is appropriate for avoiding detection by indirect threats and ensuring safety. When behavioral inhibition is extreme, it is no longer adaptive and interferes with safety seeking and effective anxiety regulation. Behavioral inhibition in NHPs is characterized by the No Eye Contact (NEC) condition of the Human Intruder Paradigm (HIP). During NEC, an NHP is placed in a novel cage while a stranger presents its profile to the monkey. In juveniles, this triggers behavioral inhibition, partially observed through suppression of vocalizations. While this response is innate, it's not observed in newborns. Instead, the predominant stressor in NEC is separation from their caregiver. Infant NHPs respond to separation by expressing many vocalizations. This means facilitating retrieval so caregivers can provide them with protection. In this study, we assess how vocalization expression during NEC changes over the first year of postnatal development. Thirty-five NHPs (24 female. 11 male) underwent 30 min of NEC at 5 ages during the first year of postnatal development. Monkeys were approximately 11, 43, 84, 168, and 365 d old at the 5 time points assessed. At 11 d, NHPs expressed an average of 1,034 vocalizations during NEC. Across 1 y we found a significant decrease in the number of vocals, totaling 134 vocalizations by 1 y old ($P < 3\times10^{-15}$). We also observed changes in the types of vocalizations expressed. The proportion of vocals that were coo-calls (retrieval calls) increased across the first year of life ($P < 2.5\times10^{-10}$). Meanwhile, the proportion of vocals that were shriek-calls (intense distress signals) decreased during this time ($P < 1.5\times10^{-7}$). Our findings suggest early developmental trajectories of NEC-induced vocalizations reflect a shift in NHP expression of separation-related behaviors to behavioral inhibition. Further investigations of these trajectories will enhance our understanding of the development of appropriate anxiety responses.

P344 Analysis of Water-based Foam as a Depopulation Method for Adult Cattle

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Current options for euthanasia and depopulation of adult cattle outlined in the AVMA Euthanasia Guidelines and AVMA Depopulation Guidelines are limited and pose logistic constraints when used on a large scale. Previous outbreaks of foot and mouth disease as well as supply chain/staffing challenges associated with the COVID-19 pandemic have highlighted the need for a rapid depopulation method for livestock that is humane, minimizes psychological impacts on personnel, and addresses carcass disposal challenges. Water-based foam (WBF) is an effective depopulation method in poultry and swine, but no prior research has been conducted on cattle. We hypothesized that WBF would be an effective option for depopulation of cattle based on previously reported efficacy in swine and poultry. Additionally, the necessary equipment is readily available, easy to use, and works in multiple types of enclosed settings. Using a 64.5 m^3 modified rendering trailer in a field setting, 6 anesthetized and 6 conscious cattle were immersed in medium-expansion WBF in an initial pilot trial to investigate the efficacy of the foam in our setup. A second large-scale trial of 72 cattle across 4 replicates ($n = 18$) was conducted based on the small-scale results. A subset of animals ($n = 60$) was implanted with subcutaneous telemetry devices that recorded activity and electrocardiograms. In all trials, animals were loaded onto the trailer and foam was delivered into the trailer with a 15-min foam dwell period. Average (± SD) time to completely fill the trailer was 84.8 ± 10.0s. No animal vocalizations were heard during foam application or the dwell period, and a 100% mortality rate was observed after 15 min of foam immersion. Time to cessation of movement as determined by activity data was 151.8 ± 76.4 s. ECG data supported that WBF is a rapid method of causing death in adult cattle. As an effective depopulation method in various agricultural populations, WBF is a useful tool for producers with multispecies operations. WBF also has potential operational and efficiency advantages over current methods, such as captive bolting and IV barbiturates. This study highlights the role laboratory animal veterinarians play in researching and assessing methods of causing death in species other than rodents.

P345 The Combination of Tiletamine/Zolazepam, Ketamine, and Dexmedetomidine Effectively Anesthetizes Pigs

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This study investigated anesthesia effects of tiletamine/zolazepam and ketamine with either dexmedetomidine or xylazine in pigs. We hypothesized whether the combination with dexmedetomidine would provide safe and reliable anesthesia comparable to the combination with xylazine in pigs. A randomized crossover experiment was performed on 6 healthy intact male miniature pigs during a 45-min radiographic examination. Pigs were assigned into 2 groups: 1) TKD: 5 mg/kg tiletamine/zolazepam, 2.5 mg/kg ketamine, 12.5 ug/kg dexmedetomidine; 2) TKX: 5 mg/kg tiletamine/zolazepam, 2.5 mg/kg ketamine, 12.5 mg/kg xylazine. TKD abd TKX were administered at 0.05 ml/kg (intramuscular injection, IM). At 45 min, atipamezole was administered. The following parameters were monitored: 1) duration parameters (time to sternal recumbency, lateral recumbency, loss of palpebral reflex, return of palpebral reflex, and return to sternal recumbency); 2) physiological parameters (heart rate, arterial blood pressure, and $\%SpO_2$). Statistical analysis revealed that duration and physiological parameters in both groups were within normal range and did not differ at any time point. These results indicate that TKD (0.05 ml/kg IM) provides safe and reliable pig anesthesia comparable to TKX during radiographic examination.

P346 The Secondary Structures of the 18S rRNA and 28S rRNA for a Novel Mouse Fur Mite, *Radfordia affinis*, and *Myocoptes musculinus*

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Mouse fur mites, including *Myobia murismusculi* (aka *Myobia musculi*, MOB) and *Radfordia affinis* (RDA) in superfamily Myobioidea, and *Myocoptes musculinus* (COP) in superfamily Sarcoptoidea are the most prevalent ectoparasites in contemporary laboratory mice. Recently, a novel fur mite was identified from naturally infested mice obtained from research colonies in Taiwan, characterized morphologically and molecularly, and designated as *Myobia muris* (MOM). Besides the comparison of the primary sequences of ribosomal RNA (rRNA) gene traditionally applied in phylogenetic relationship study, the predicted secondary structures of rRNA are shown to be a new and more reliable data applied in the phylogeny and evolution studies. In this study, followed by rRNA gene sequencing of RDA and MOM, the secondary structures of the 18S rRNA and 28S rRNA gene sequences of these mouse fur mites were predicted and compared. For these mouse fur mites, the 9 variable

regions (V through V9) of the 18S rRNA and the 13 domains (D1 to D6, D7a, D7b, D8-D12) of the 28S rRNA were also existed in the predicted secondary structures. The predicted secondary structures of the 28S rRNA revealed that a few domains (D2, D3, D7a, and D10) of the 28S rRNA presented more remarkable differences in composition (additional loops or helices) and length among different mouse fur mite species. These variations in the predicted secondary structures of the 28S rRNA could be the possible choice to be applied in differentiating other MOM-like organisms from other fur mite species in the future. In this study, the secondary structure prediction of the 18S rRNA and 28S rRNA of MOM, RDA and COP was first completed and the 28S rRNA presented more remarkable differences than the 18S rRNA did among MOM, RDA and COP.

P347 Effects of Instillation Volume on Survival, Bacterial Distribution, and Infection Kinetics in Murine Pulmonary Infection Model

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Intranasal installation of substances is a common route of administration used in murine models. The volumes of the substances instilled range depending on the study, generally with no explanation for the volume administered. Female, BALB/c mice, 5 to 6 wk of age, and group-housed under specific pathogen-free conditions were used for all experiments. All animals were rendered transiently neutropenic by 150 mg/kg and 100 mg/kg cyclophosphamide pretreatment IP at days -4 and -1, respectively. On day 0, mice were anesthetized by inhalation of isoflurane to facilitate intranasal administration. Animals were randomly assigned to 1 of 6 experimental groups: (1) 3.0×10^6 CFU in 25 µl, (2) 3.0×10^6 CFU in 50 µl, (3) 5.0×10^6 CFU in 25 µl, (4) 5.0×10^6 CFU in 50 µl, (5) 25 µl, or (6) 50 µl. In the first set of experiments, survival was the primary outcome. The second set of experiments focused on bacteria distribution within the respiratory tract at various time points post bacteria instillation: 4, 24, 48, 72, 96, and 120 h. All surviving animals were euthanized at 192 h post bacterial instillation. At each time point, randomly selected animals from each group were euthanized and the upper (trachea) and lower (lungs) respiratory tract carefully dissected and processed to determine CFU/gram of tissue. The results obtained in this work showed a significant effect of the instillation volume on survival. In these studies, we observed 100% survival with both the 3.0×10^6 CFU in 25 µl and the 5.0×10^6 CFU in 25 µl groups. However, survival decreased to 87.5% for the 3.0×10^6 CFU in 50 µl group and to 37.5% in the 5.0×10^6 CFU in 50 µl group. The bacterial distribution within the respiratory tract and infection kinetic studies have been initiated and results will be available to report at the time of the conference. Our results provide important information about the often overlooked effect of the volume of pulmonary instillation on survival, bacterial distribution and infection kinetics in a mouse challenge model. This information will help inform the development and refinement of pulmonary challenge models, and may have a significant impact on the comparison of novel therapeutic efficacy in the drug discovery process.

P348 Molecular Hydrogen Improves Oxidative Stress-induced Damage to Mouse Sperm Mitochondrial Function

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Oxidative stress caused by the imbalance between reactive oxygen species (ROS) and biological antioxidant system leads to an increase in damaged human sperm and subsequent male infertility. Because molecular hydrogen (H2) acts as therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals, we hypothesized that H2 treatment is beneficial for the prevention of oxidative stress-induced impairment in mice sperm. To investigate the effect of H2 on injured sperm, they were collected from the epididymis tail of B6D2F1/Crlj mice (12 to 15 wk old, 3 males) and suspended in TYH medium. One hour after preincubation, it was made into a sperm suspension. Hydrogen peroxide diluted with water was added to the sperm suspension to a concentration of 0.3 mM. In addition, they were incubated for an additional 20 min with or without H2 saturated culture. To assess sperm damage, the number of untreated motile sperm was compared to the number of motile sperm in the damaged sperm. The H2 saturated culture solution was added thereto, and the functional recovery effect of H2 was observed. To further investigate the effect of H2 on ROS-dependent mitochondrial damage, we stained sperm with Nonyl Acridine Orange, mitochondrial cardiolipin binding dye, and MitoTracker Red, mitochondrial membrane potential-dependent dye, and observed them with a super resolution microscope. Fresh sperm (motility rate: 82.4%) were treated with hydrogen peroxide, resulting in damaged sperm with a low motility rate (14.6%). H2-treatment significantly restored their motility rate (63.9%) accompanied by improvement of intrasperm ATP content. The fertilization rate of damaged sperm was markedly improved from 37.6% to 59.2% by H2 treatment. It was cleaved as the percentage of fertilized eggs cleavage 24 h after insemination. Transfer of 2-cell stage embryos obtained from H2-treated damaged sperm showed normal ontogeny (94.6%). Because of the rapid diffusion and high membrane permeability, H2 can reach and react with intrasperm ROS, including hydroxyl radical, possibly in mitochondria, and improve low sperm motility. Our results strongly suggest that H2 is a new promising tool for male infertility treatment.

Platform Sessions

PS1 Venezuelan Equine Encephalitis Virus DNA Vaccine Delivered by Needle-free Jet Injection Shows Immunogenicity and Protection in Macaques Against Viral Infection

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Venezuelan equine encephalitis virus (VEEV) is a mosquito-borne alphavirus that causes fever, lymphopenia, central nervous system infection, and mortality in humans. VEEV is a public health concern in endemic areas, has caused laboratory-associated infections, and is considered a possible biological weapon. There are no approved vaccines and those used experimentally cause unwanted side effects and can fail to induce a detectable immune response. A candidate VEEV DNA vaccine (pWRG/VEE) administered to animals and humans using electroporation (EP) showed induction of high-titer neutralizing antibodies (nAb) and protective efficacy. Use of EP, however, is cumbersome and impractical for field use. In this study, a needle-free disposable syringe jet injector (DSJI) was tested as an alternative to administer the pWRG/VEE vaccine intradermally (ID-DSJI) or intramuscularly (IM-DSJI) to male and female cynomolgus macaques. Animals were separated into 3 groups ($n = 6$/group): IM-DSJI (2 mg), ID-DSJI (0.4 mg), and control empty pWRG vector. Macaques were vaccinated twice at 0 and 4 wk and blood was collected at 0, 4, 6, and 8 wk for nAb and T cell responses. Eight weeks after the 2nd vaccine, macaques were exposed to VEEV (6.43×107 PFU average) then monitored daily and blood was drawn on days 1, 2, 3, 4, 5, 6, 8, 10, 14, 21, and 27 postchallenge for hematological and viral load analyses, then euthanized for necropsy

and histopathology on day 28. IM-DSJI induced high anti-VEEV IgG and IgA binding and neutralizing antibodies and IFNg T cell responses, while the ID group elicited lower levels of binding ($P < 0.05$ at 8 wk) and neutralizing antibody and poor T cell responses ($P < 0.05$ at 4 wk, $P < 0.01$ at 8 wk) compared to the IM group. After the challenge, all pWRG/VEE-vaccinated animals remained aviremic, most maintained normal lymphocyte levels, and fewer ID-DSJI animals developed fever (via telemetry) and showed evidence of encephalitis than IM-DSJI animals (ID group total pathology score of 15 compared to 28 in IM group). DSJI elicited comparable levels of nAb to previous EP studies. These results show that DSJI vaccination elicits similar immunogenicity and protection against VEEV compared to EP, indicating feasibility for use as a VEEV DNA vaccine delivery method.

PS2 Standardization of a Sentinel-free Environmental PCR Sampling Method for Detection of Rodent Infectious Agents in Pooled Soiled Bedding

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The majority of prevalent and commonly excluded rodent pathogens go undetected or are poorly detected in soiled bedding sentinels (SBS). Alternative PCR-based methods demonstrating equal or greater sensitivity to SBS have been developed, which reduce or replace SBS. A recent approach is to directly sample dust from pooled soiled bedding with a contact media (filter or swab). This is achieved by manual agitation of the media in the soiled bedding or by swabbing through the contents of the bedding container. The goal of this investigation was to evaluate different exposure schedules and contact media types. Two 3- to 4-wk-old female SOPF CD-1 contact sentinels were placed in each of 2 cages of 3, 6- to 10-wk-old and one cage of 3, older-than-14-wk-old female pet shop quality mice. During weekly cage changes, soiled bedding from all pet shop mice was mixed and diluted to ~17% with bedding from naïve SOPF CD-1 mouse production isolators for contact media (8 types) and soiled bedding sentinel exposure (3, 3- to 4-wk-old female SOPF CD-1 mice). All media and treatment schedule variables were performed in triplicate. Sampling schedules for filters varied from monthly to weekly exposure to bedding as well as monthly-pooled filters versus a single filter used throughout a 3-mo period. Adhesive and flocked swabs of bedding containers were collected weekly and pooled by replicate. Using all available diagnostic methodologies, 42, 33, and 11 different infectious agents were detected among pet shop mice, contact sentinels, and soiled bedding sentinels respectively. The 2 optimal media and sampling schedules detected 28 and 29 agents among triplicate samples by PCR. The total number of positive PCR infectious agent assays (PPIAA) combined among media and sampling schedule triplicates ranged from 20 to 76. The most sensitive media and treatment schedule incorporated weekly exposure using either one filter continuously over the 3-mo period (76 PPIAA), separate monthly filters pooled (72 PPIAA), or weekly swabbing with an adhesive swab (68 PPIAA). Although the optimal conditions determined in this study were more effective than SBS, the greater number of agents detected by direct evaluation of pet shop mice underscores that no method is perfect.

PS3 Comparison of Analgesic Efficacy in Attenuating Spontaneous Pain Behaviors in Rats (*Rattus norvegicus*)

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Significant analgesic shortages and price increases have occurred since the COVID-19 pandemic, having direct implications on available treatment options thus impacting animal wellbeing, repeatability, and biomedical research. Traditionally, multimodal treatments (e.g., systemic with local analgesic) and opioids (e.g., buprenorphine) are recommended for mitigating major postoperative pain in rodents, although, in extenuating circumstances, other analgesics like nonsteroidal anti-inflammatory drugs (NSAIDs) (e.g., meloxicam) must be used instead. However, the analgesic efficiency in attenuating spontaneous pain behaviors across treatment options has not been extensively evaluated in the rat laparotomy pain model. Here, we investigated whether extended-release buprenorphine (XR) was more effective at mitigating spontaneous pain behaviors in postoperative rats compared to other analgesic regimens. We hypothesized that XR would have the greatest analgesic effects in minimizing spontaneous pain behaviors and male XR rats would maintain longer therapeutic concentrations than other experimental groups. Sprague–Dawley rats (male $n = 33$; female $n = 40$) were randomly assigned by sex to 1 of 6 experimental groups: XR (0.65 mg/kg), buprenorphine (0.01 mg/kg), meloxicam (2 mg/kg), meloxicam with local bupivacaine (2 mg/kg and < 2 mg/kg, respectively), saline (5 mL/kg), and unoperated control. After subcutaneous drug administration, a 3-cm midline incision was made, penetrating the peritoneal cavity under isoflurane anesthesia. Spontaneous pain behaviors were evaluated using a subjective pain score, the open field arena test, and the burrowing test at baseline, 2, 24, 48, 72, and 168 hr postlaparotomy. Clinical observations were recorded daily and a gross necropsy was performed. Blood was collected for pharmacokinetic analysis from a separate cohort receiving identical analgesic doses (male $n = 17$; female $n = 23$). All groups displayed spontaneous pain behaviors as measured 2 hr postlaparotomy despite preemptive analgesic treatment ($P < 0.02$) and having therapeutic drug plasma concentrations. This study provides support that a multidimensional approach is warranted when investigating the analgesic efficacy and suggests more sensitive, objective outcome measures of spontaneous pain are needed.

PS4 Assessing the Potential Costs of Delaying Maternal Separation in Purpose-bred, Group-housed Rhesus Macaques (*Macaca mulatta*)

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UK guidelines for institutions breeding rhesus macaques (*Macaca mulatta*) for use in research advise that infants should be kept in their breeding group for as long as possible and maternal separation (herein, weaning) should not occur before 10 to 14 mo of age. These weaning age guidelines have been identified as a promising candidate for refinement. A common justification for early weaning outside of the EU/USA is to increase productivity and reduce injuries. However, no study systematically assessing these assumed costs exists. To assess the perceived costs, we analyzed the Medical Research Council's Centre for Macaques (CFM) colony records from 800 monkeys (female = 436) from 2004 to 2021. The CFM breeding groups consist of one adult male, 2 to 13 females, and their offspring. Prior to supply, offspring will be separated from their group and placed in same-sex 'weaning' groups. Furthermore, between November 2020 and November 2021, video data were collected from breeding groups to assess levels of aggression directed from the breeding male to other members of the group. With these data we used generalized mixed-effect models to test 3 predictions: older juveniles would receive the most injuries, interbirth intervals would positively correlate with weaning age, and the adult male would disproportionately direct overt aggression towards older juveniles. We also examined other factors that could be associated with injury

rate and productivity in breeding groups such as introduction of a new male to the group, age of the eldest female, and relatedness of females in the group. We found females over 2.5-y-old were the most likely to be injured, accounting for 80% of injuries in the data, although there were low incidences of overt aggression from the video data. Furthermore, we found no association between interbirth interval and offspring weaning age. The biggest risk factor for injury was the introduction of a new male into a breeding group. However, this also produced a large increase in the proportion of females that got pregnant. These results suggest the perceived costs of delaying weaning in breeding colonies have been overstated and that other factors relating to colony management are more likely to affect injuries and productivity in breeding groups.

PS5 The Relationship Between Functional and Structural Abnormality of the Retina in Wistar Han Rats

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The electroretinogram (ERG) is a measure of the electrical activity of retinal neurons in response to a light stimulus. The full-field ERG is indispensable for retinal research, and it is commonly used to quantify retinal function in humans as well as in many preclinical species including albino rodents. Optical coherence tomography (OCT) devices allow noninvasive detection of retinal structures, including longitudinal quantitative measurements of retinal thickness. Based on literature reports and our previous experience, a certain percentage of Wistar Han (WH) rats often show absences of ERG response to flash stimuli. The correlation between the ERG and OCT read out was studied in 117, 8-wk-old, WH rats [60 male and 57 female, Crl:WI(Han)]. Baseline ERG measurements demonstrated that 19 of 117 (16%) rats (38 of 234 retinas, respectively) had no response to ERG flash stimuli. Among these 19 rats, 12 (24 retinas) were male and 5 (10 retinas) were female. Baseline OCT measurements of all 234 rat retinas demonstrated total retinal thickness (TRT) in the same range, regardless of normal or abnormal ERG response. All rats were subsequently examined by ERG and OCT 3 mo later. The average TRT loss for all 234 retinas over 3 mo was 23.30 ± 10.34 µm. Twenty-eight of 38 (73.68%) retinas with abnormal ERG response had above-average TRT loss (36.42 ± 7.78 µm) and 10 of 38 (26.32%) retinas exhibited below-average TRT loss (20.77 ± 2.82 µm). On other hand, of the 196 retinas with normal ERG response, 52 of 196 (27.04%) retinas had above-average TRT loss (32.87 ± 10.83 µm) and 144 retinas (73.35%) had below-average TRT loss (17.43 ± 6.66 µm). The biology variation mathematics calculation model indicated that the incidence of excessive retinal loss in rats with abnormal ERG response was significantly higher than in rats with normal ERG response ($P < 0.005$). In summary, the occurrence of abnormal ERG response in WH rats was much higher in males than in females; WH rats with abnormal ERG response were much more likely to develop severe TRT loss than those with normal ERG response. Therefore, eliminating rats that have no response to ERG stimuli during prestudy should be considered, particularly in longitudinal ophthalmic research.

PS6 Use of Carbon Monoxide for the Euthanasia of Neonatal Rats and Mice

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The euthanasia of neonatal laboratory rats and mice can be challenging due to the unique physiological characteristics of these animals in the weeks following parturition. Previous work with neonatal rats and mice has shown that the commonly used anesthetic gases of carbon dioxide and isoflurane do not effectively euthanize these animals. In this study, we proposed to evaluate the use of carbon monoxide to induce euthanasia. This gas was selected because the stability of carboxyhemoglobin suggests that it might be a good candidate to induce irreversible euthanasia. Neonatal mice and rats aged postnatal day (PND) 0 to PND 12 of multiple strains and sexes were evaluated for the irreversibility of the euthanasia process after continuous exposure to the gas for as long as 30 min. An exposure dwell time was determined to be successful if 40 neonates euthanized with 8% carbon monoxide in room air did not recover after removal from the gas. An exposure time was determined to fail if any animals recovered after removal from the gas, and the exposure time was then extended for at least 30 s. For both rats and mice, carbon monoxide did not result in reliable or irreversible euthanasia of nonhaired neonates (PND 0 to 6) exposed to the gas for as long as 30 min. However, irreversible euthanasia was induced in both rats and mice in haired neonates (PND 7 to 12) following exposure times ranging from 5 to 23 min. This irreversible euthanasia was achieved with dwell times comparable to the use of carbon dioxide for neonatal rats, but the dwell time for irreversible euthanasia of neonatal mice was much shorter than that reported for carbon dioxide in this species. The findings from this study suggest that carbon monoxide may be a potential refinement for the euthanasia process of haired neonatal animals (aged PND7 and older).

PS7 Inducing Pseudopregnancy in Female Mice Without the Need for Vasectomized Males Prior to Artificial Insemination

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Recipient female mice must be induced to a pseudopregnant state for successful maintenance of pregnancy after artificial insemination or embryo transfer. Traditionally, female mice are paired overnight with vasectomized males and the presence of a copulation plug is assessed the following morning. To increase the efficiency of producing pseudopregnant females, we have standardized a cervical manipulation technique. The blunt end of a small plastic rod is inserted vaginally to contact the cervix and is vibrated for 30 s by contact with a trimmer. The procedure is quick and does not require anesthesia or analgesia. The technique entirely replaces the need for vasectomized males and increases the reliability and predictability of producing pseudopregnant females. Vaginal cytology was compared for female CD1 and C57Bl/6 mice after 1) mating with vasectomized males and 2) cervical manipulation. First, by comparing the type of cells obtained from a daily vaginal swab (leukocyte, epithelial, cornified epithelial), a cytology profile was created for animals from both strains (n=20) correlating to the stage of the estrous cycle. While the percentage of cell types found during a particular stage of estrous was variable between females, the trends of cell types observed over the 4–5 d estrous cycle were similar. Next, a cytology profile was created for each strain using pseudopregnant females that had either mated with vasectomized males or had been induced by cervical manipulation. We have determined that the cytology profile of

pseudopregnancy induced by cervical manipulation is indistinguishable from the profile of pseudopregnancy induced by mating. Both cytology profiles mimic the first 10–12 d of a cytologic pregnancy profile. For CD1 mice, efficiency of pseudopregnancy induction using cervical manipulation was 83% for females in estrus (*n*=76) with this technique but only 38% of females in estrus were plugged by vasectomized males. Artificial insemination recipients receiving cervical manipulation (*n*=76) had a pregnancy rate of 72% and an average litter size of 8.3 pups. Therefore, induction of pseudopregnancy by cervical manipulation is an efficient and convenient alternative to mating with a vasectomized male when performing assisted reproductive techniques. Use of cervical manipulation for assisted reproduction techniques provides 3Rs benefits by reducing the number of animals needed and eliminating the need for surgically altered males.

PS8 Frequency, Breeding Effects, and Heritability of Vaginal Septa in C57BL/6J & BALB/cJ Laboratory Mice

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Vaginal septa are bands of connective tissue that divide the vaginal opening and can impair breeding and increase risk of dystocia in laboratory mice. We studied incidence, breeding effects, and heritability in 2 inbred strains. Frequency in 6- to 8-wk-old females was 4.8% in C57BL/6J (B6; 85 of 1,784) and 16.1% in BALB/cJ (BALB; 287 of 1,768). Affected and normal B6 (41 of each) and BALB (40 of each) were mated with males of the same strain. Septa did not prevent females from having a first litter: 95% of septate (39 of 41) and 98% of normal B6 (40 of 41) had litters within 100 d, as did 93% of each BALB group (37 of 40). First litters were born later to B6 with septa: 59% had litters within 30 d vs 93% of normal females. Similar numbers of BALB females had litters within 30 d (septa 59%, normal 54%). Females with septa had smaller first litters (average & standard deviation of pups: B6 septa 5.5 ± 2.4, B6 normal 6.5 ± 2.0; BALB septa 5.2 ± 2.9, BALB normal 6.0 ± 3.0). Females with septa remained productive (numbers with later litters spaced < 60 d apart: B6 septa 38 of 41, B6 normal 40 of 41; BALB septa 37 of 40, BALB normal 28 of 40). In both strains, later litters were similar in size (average pups in litters 2+: B6 septa 5.9 ± 1.5, B6 normal 6.2 ± 1.3; BALB septa 5.7 ± 1.6, BALB normal 5.8 ± 1.5) and frequency (average days between litters 2+: B6 septa 34 ± 10, B6 normal 35 ± 8; BALB septa 32 ± 12, BALB normal 32 ± 9). Septa did not affect the number of litters (averages: B6 septa 4.5 ± 1.4, B6 normal 4.7 ± 1.3; BALB septa 4.4 ± 1.3, BALB normal 3.9 ± 1.3). To date, the only case of dystocia occurred in a normal BALB female delivering a third litter. Septa did not affect the percentage of pups surviving to weaning (B6 septa 83 ± 15, B6 normal 86 ± 13; BALB septa 87 ± 18, BALB normal 84 ± 14). Septa were more common in female offspring of affected B6: 11.9% (44 of 369) also had septa vs 5.0% (20 of 402) born to normal females. BALB frequencies were more similar: 15.9% (57 of 358) of pups born to affected BALB had septa vs 14.5% (42 of 290) born to normal BALB. Data collected to date show that septa can affect the first litter, and that incidence does not decrease greatly when normal females are bred for one generation.

PS9 Mouse Coronavirus MHV-1 Causes Significant Respiratory Disease in NZO and NOD Mice

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Mouse hepatitis virus (MHV) is a betacoronavirus of mice. To understand the similarities of MHV pathology with COVID-19, the objective was to characterize the range of pathological lesions caused by MHV-1 in 2 collaborative cross mouse strains (NZO/HiLtJ and NOD/ShiLtJ). Groups of 8 male and 8 female NZO and NOD mice were infected intranasally with 150,000 pfu MHV-1 or media (controls; $n = 2$). Mice were weighed daily and euthanized, necropsied, and tissues collected at 8 or 12 d postinfection (DPI). NZO mice began losing weight 4 DPI progressing to an average weight loss of 8% (males) or 12% (females) on 8 DPI and 5 and 8%, respectively by 12 DPI. Pathologic changes observed in NZO mice involved the lung and central nervous system (CNS). Infected mice had widespread hepatic hydropic change but no significant hepatitis or hepatic necrosis. Severe pneumonia affected up to 75% of lung alveoli with foci of vasculitis and vascular thrombosis affecting all mice at 8 DPI and lesions had largely resolved in males by 12 DPI. CNS lesions included focal encephalitis, gliosis, vasculitis, and thrombosis. Olfactory bulb (OFB) lesions were moderate to severe in both sexes at 8 and 12 DPI. MHV was not detected in lung and liver at 8 and 12 DPI. NOD mice infected with MHV-1 began losing weight at 4 DPI. On 7 DPI, 3 mice had lost > 15% body weight and were euthanized. On 8 DPI, an additional 8 mice were euthanized for weight loss > 15%. Only 5 mice continued to 12 DPI without substantial weight loss. Pathologic changes in the NOD mice at 7 and 8 DPI involved the lung, where pneumonia affected up to 75% of the alveoli with a trend toward more severe pathology in females than males. Unlike the NZO mice, the NOD mice lacked significant pathologic changes in the liver, brain, or OFB, but there was marked necrosis of lymphocytes in the thymus, spleen, and lymph nodes and reduced bone marrow hematopoietic cells. MHV titers of 5,000 to 1,300,000 pfu/g of lung were detected in NOD mice at 7 and 8 DPI. MHV-1 reliably produced pneumonia in a strain-, and potentially sex-dependent manner in NZO and NOD mice with little hepatic impact recapitulating COVID-like human respiratory disease. MHV-1 infection in NZO mice resulted in CNS pathology similar to a subset of human COVID patients.

PS10 Weight Loss and Acute Hindlimb Paralysis in a Rhesus Macaque (*Macaca mulatta*)

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A 15-y-old female rhesus macaque (*Macaca mulatta*) from an outdoor breeding colony was hospitalized and evaluated for recent weight loss and diarrhea. Initial exam findings included palpable abdominal distension and a gallop rhythm on auscultation. Abdominal radiographs revealed significant distention of the colon and cecum with granular mineral-opacity intraluminal material, suspected to be ingested sand and gravel. Initial treatment consisted of bismuth subsalicylate, supplemental fiber, simethicone, and metronidazole for suspected cecal impaction. After 10 d of hospitalization and treatment, she was reported for having a rectal prolapse that spontaneously resolved after 30 min. Later that night, she was reported for having acute bilateral hindlimb paralysis. On physical exam, both of her hindlimbs were cold to the touch, and femoral pulses were absent bilaterally. An aortic thromboembolism was suspected. The animal was euthanized due to poor prognosis, and the presence of a 3 cm saddle thrombus was confirmed on gross necropsy. The patient also had acute renal infarcts and a severe necrohemorrhagic enterocolitis. Heavy growth of *Campylobacter jejuni subsp jejuni* was cultured. While *Campylobacter jejuni* is not typically associated with significant hemorrhage and necrosis, the severity of the enteritis in this patient was likely exacerbated by the reported rectal prolapse and sand impaction present. Histopathology revealed a severe, chronic, multifocal to coalescing lymphoplasmacytic myocarditis with interstitial fibrosis and cardiomyocyte degeneration and loss. The cause of the myocarditis could not be identified, despite numerous ancillary diagnostics for infectious etiologies. Ultimately, this patient had multiple factors contributing to a thromboembolic event, including a hypercoagulable state induced by significant inflammation and blood flow anomaly, evidenced by the gallop

rhythm. While aortic thromboembolism is a relatively common phenomenon in feline patients, few cases of saddle thrombus in nonhuman primates have been reported. This case highlights the potential risk of thromboembolic events as a sequela in patients with predisposing factors.

PS11 Facial Swelling in a Laboratory Rat

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A 6-mo-old, male, Sprague Dawley rat presented for a right-sided swelling of the mandible. The animal was on a protocol for new interventions to treat neuromas following peripheral nerve transection. Two months prior to presentation, the animal's common peroneal nerve of the right hindlimb was transected and distally sutured into the ipsilateral gastrocnemius muscle. Subsequent weekly von Frey analyses and Hargreaves tests were performed. On examination, the rat had a firm mass associated with the right mandible causing malocclusion of the teeth and subsequent right maxillary incisor overgrowth. Possible differentials included a tooth root abscess, salivary gland inflammation or neoplasia, lymph node inflammation or neoplasia, neoplasia of the mandible such as osteosarcoma, or a non-neoplastic growth. Buprenorphine sustained-release was administered, and the animal was given softened food and gel in the cage. The animal was then sedated with isoflurane for oral examination. The mass could not be visualized within the oral cavity but was palpable between the ramus of the mandible and the subcutaneous jaw tissue measuring approximately 2.5 cm in diameter. A fine-needle aspirate of the mass was attempted, but due to the boney nature of the mass, it only yielded a small amount of frank blood. Radiographs revealed a proliferative bony lesion of the right mandible. Surgical excision was not possible given the location and invasiveness of the mass. The animal was euthanized using intraperitoneal ketamine followed by carbon dioxide inhalation. Necropsy was performed. Histopathologic evaluation identified a disorganized mass of well-differentiated dental tissues expanding the mandibular ramus, with associated bone loss and remodeling. The features are consistent with a compound odontoma, which is a developmental malformation of disorganized but normal dental tissue. While odontomas can be experimentally induced, this case developed spontaneously and was unrelated to the experimental protocol. Spontaneous odontomas, although not reported commonly on laboratory rodents, should be considered as a differential diagnosis for swelling and/or mass in the oral cavity.

PS12 CRISPR Hamster Colony with Case-wide Diarrhea

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Eight 5- to 9-mo-old male and female Golden Syrian hamsters presented with intermittent soft stool between July 2021-May 2022. Four parental hamsters were CRISPR/Cas9 edited at another university to correct an OPN1SW mutation preventing the production of functional blue cones. The CRISPR lab had not reported soft stool as a colony issue. The hamsters were then transported to the current facility, resulting in diet changes and a switch from reverse osmosis water to acidified water. The researchers bred the hamsters for 2 generations with no further experimental manipulation. Three hamsters with the OPN1SW correction and 5 without were clinically affected. Soft stool and mild staining around the perianal region with no change to body condition or hydration status was found on examination. The differential diagnosis included *Clostridium piliforme*, *Campylobacter* sp., *Salmonella* sp., *Helicobacter* sp., *Lawsonia intracellularis, Spironucleus muris, Giardia* sp., CRISPR off-target effects, stress induced colitis, or nutritional associated changes. Nutritional change as the etiologic cause was less likely since onset of signs were 3 mo after the introduction to new chow. A clinical hamster with failed gene correction was submitted for necropsy and histopathology; major findings included moderate proliferative gastroenteritis, proliferative typhlocolitis, and portal hepatitis with bile duct hyperplasia and mild nodular regeneration. Protozoa were observed in the small intestines and argyrophilic bacteria were seen along the gastrointestinal tract with a Warthin-Starry silver stain. Pooled fecal contents, including samples from this hamster, were submitted for culture and PCR. The culture was negative for *Salmonella* sp. and *Campylobacter* sp. growth. PCR returned positive for *Giardia* sp. but was negative for *Lawsonia intracellularis* and *Clostridium piliforme.* Fecal PCR from another affected hamster returned positive for *Giardia* sp., *Helicobacter* sp. and *Campylobacter jejuni.* The cause of the sporadic proliferative typhlocolitis and diarrhea in this colony was likely multifactorial. This case series suggests greater association between proliferative enteropathy in adult to aged hamsters with *Helicobacter* and *Campylobacter* compared to younger hamsters.

PS13 Acute Respiratory Episode and Hoarse Bark in a Laboratory Hound

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A 9-y-old, 20 kg, intact female, pair-housed hound dog presented for acute increased respiratory effort, stridor, and a hoarse bark. Ten days previously, she received a second proprietary study-related IV infusion, serving as a normal control with no evidence of prior health concerns, and had been normal since the infusion. Prior anesthetic episodes for this dog were uneventful, with no adverse reactions to anesthesia noted. On exam, the dog was tractable but anxious, with increased respiratory effort and stridor. Butorphanol and acepromazine were administered for mild sedation. Radiographs of the thorax and neck revealed mild increased opacity in the caudodorsal lung field and mild increased soft tissue opacity in the area of the hyoid apparatus. Dexamethasone (0.25mg/kg IM) and maropitant citrate (10mg/kg SC) were administered once. The top differential diagnosis was laryngeal paralysis, and other differentials included a delayed hypersensitivity reaction to study-related infusion, and laryngeal obstruction/dysfunction. She recovered from the initial event and was placed in activity-restricted special housing but remained quiet and dysphonic with mild inspiratory stridor. This continued for the following 3 d until a sedated laryngeal exam was performed. During the sedated exam, arytenoid function was symmetrical and within normal limits; however, a mass of redundant tissue was observed on the right side of her airway, between the epiglottis and arytenoids. She was anesthetized and intubated to gain better visualization; mechanical ventilation was not required. The mass of tissue was removed and submitted for histopathology. Hemostasis was achieved and she was allowed to recover from anesthesia. Recovery was uneventful and stridor was resolved. She was returned to normal housing 2 wk later after full recovery was achieved, and there has been no recurrence of clinical signs. Histopathology revealed the mass was an everted laryngeal saccule. Everted laryngeal saccules are commonly associated with brachycephalic obstructive airway syndrome, where the saccule everts due to increased respiratory pressure. The underlying etiology for the everted saccule remains unclear.

PS14 Prolonged Menstruation in a Geriatric Rhesus Macaque (*Macaca mulatta*)

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A 26-y-old female intact singly housed rhesus macaque (*Macaca mulatta*) was presented with prolonged menstruation, lethargy, and hyporexia. She had a history of prolonged cycles and was being managed with medroxyprogesterone acetate 150mg intramuscularly every 3 mo. On physical exam she was noted to be thin with a body condition score of 1.5/5 and had a 4-5cm firm irregularly palpated uterus. Whole body radiographs, ultrasound of the uterus and blood work for complete blood cell count and blood chemistry were performed. She was treated symptomatically for pain with meloxicam (0.1mg/kg) every 24 h, buprenorphine (0.01mg/kg) every 12 h and given an iron dextran injection (10mg/kg) while awaiting results from further diagnostics. There were no significant findings on bloodwork. Ultrasound revealed the classic fried egg appearance of a uterus with chronic medroxyprogesterone acetate administration. Radiographs showed increased soft tissue opacity in the thoracic cavity with cardiac displacement to the right raising suspicion of a diaphragmatic/hiatal hernia with mild atelectasis of the left lung. Differential diagnoses included secondary infiltrative or metastatic disease (endometrial cancer), disseminated endometriosis involving diaphragm and/or lungs and trauma. Due to the poor prognosis euthanasia was elected. Gross necropsy revealed 2 bulges of liver and peritoneal fat into the diaphragm into the left and right hemithorax. The left bulge took up 1/3 of the chest and caused partial atelectasis of the lungs. The diaphragm itself was weakened and thinned and suspicious for rupture. The common bile duct was also thickened and distended containing numerous 5 mm stones. Finally, her uterus was enlarged, irregular, and nodular. Histopathology confirmed infiltration by endometrial stromal sarcoma in the diaphragm, mesentery, and liver. Her final diagnosis was regional endometrial stromal sarcoma, advanced perigonadal endometriosis with reproductive and abdominal endometriomas, diaphragmatic hernia, and cholelithiasis. Diaphragmatic invasion and hernia of endometrial stromal sarcoma is an unusual presentation of endometriosis; however, it is an important differential to consider in intact aged females with reproductive complaints.

PS15 Unidentified Finch Object (UFO): An Exercise in Laboratory Animal Forensics

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A red, muscle-like tissue, without discernable feather/hair, skin, or bone, was found on the floor of a cage housing a group of nonbreeding 6-mo-old female zebra finches (*Taeniopygia guttata*) undergoing quarantine. All birds were bright, alert, and responsive with no abnormalities on visual exam. No birds were missing or injured, and there was no change to husbandry or report of pests in the room. All eggs produced by the females appeared normal and were unfertilized. Birds were fed a fortified seed mix and offered supplemental protein mash, fresh fruit, and cuttlebone. The paper liner in the cage was changed 3 times a week. The unidentified tissue measured 3 x 1.5 x 1 cm and weighed 2.84 grams. Morphometrically, the tissue was red, wet, spongy in texture, with an indentation in the center. One side of the tissue had a small aggregate of blood clots. Differential diagnoses included autolytic body parts of a bird or pest (e.g., wild rodent), neoplasia, and fetal or neonatal remnant/abnormality after inadvertent mating. The tissue was submitted for histopathological evaluation. Microscopically, the tissue was composed of a tube-like glandular structure with numerous large folds and projections lined by a pseudostratified ciliated epithelium, supported by abundant fibrovascular stroma, embedded with numerous stromal glands containing intracytoplasmic fine eosinophilic granules (glandulae magni) along with mild multifocal mononuclear infiltration and mild congestion. A focally extensive area of hemorrhage was noted, composed of numerous nucleated extravasated erythrocytes (avian erythrocytes). The tissue was identified as normal avian oviduct (oviduct magnum). It was therefore suggested that one female zebra finch had an oviduct prolapse followed by amputation; however, the affected bird could not be identified. The underlying etiology of oviduct prolapse in avian species is commonly associated with straining caused by physiologic hyperplasia, egg-laying, or dystocia. Birds with soft-shelled eggs, nutritional imbalance, obesity, and inflammation of the reproductive tract or cloaca are susceptible to oviduct prolapse.

PS16 The Curious Case of the Mouse Colony with Broken Legs

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An investigator reported that 3 of their adult female C57Bl/6 mice had sustained "broken legs," characterized by left forelimb swelling and lameness. Upon further questioning, they reported losing >30% of previous experimental cohorts due to similar clinical signs, however the veterinary staff was not notified at that time. The mice were on IACUC-approved microbiome studies that included bacterial inoculation through oral gavage. On examination the mice were active, mildly underweight (BCS of 2–2.5/5), and they all had a weight-bearing left forelimb lameness and a focal subcutaneous left axillary mass (~0.5 cm in diameter). Given the mild, nonspecific presentation, the animals were monitored overnight. However, the following morning they were persistently hunched, grimacing, exhibiting piloerection, and the mass had increased in size for all animals (~0.8–1.0 cm in diameter). Due to the progression and severity of clinical signs, euthanasia was elected. Differential diagnoses included trauma from manual restraint and oral gavage, or an infectious etiology. Postmortem diagnostic tests performed included aspiration and cytology of the mass, computed-tomography (CT), and necropsy. Cytology revealed an extensive amount of amorphous debris with neutrophils and macrophages. CT imaging revealed normal musculoskeletal anatomy and integrity of the neck, thorax, and forelimbs. A distinct left axillary subcutaneous pocket, filled with a granular radiopaque material, travelled cranioventrally to the left lateral wall of the cervical esophagus. On gross necropsy, this communicable tract was grossly visible and the subcutaneous space of the left axilla contained a tan, paste-like material (presumably ingesta). The gastrointestinal tract contained a scant amount of fluid and ingesta. Based on the diagnostic tests performed and the experimental history, the diagnosis was esophageal perforation secondary to oral gavage. Preventative measures that were taken included investigator retraining and the use of technicians skilled in oral gavage, after which there were no further cases.

PS17 Hindlimb Lameness in a Cynomolgus Macaque (*Macaca fasciculata*)

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A 2.5-y-old, 2.64-kg, experimentally naïve, pair-housed female

cynomolgus macaque (*Macaca fasciculata*) was reported for intermittently carrying her right hindlimb in flexion or extension when ambulating. She was able to bear weight and received meloxicam (0.2 mg/kg SC once, followed by 0.1 mg/kg SC SID for 3 d). Antiinflammatory treatment appeared to provide no relief, so 5 d after presentation, she was sedated for further examination and diagnostics. There was moderate muscle atrophy, decreased range of motion of the coxofemoral joint, and crepitus of the coxofemoral joint and stifle of the affected limb. Radiographs showed a markedly irregular, sclerotic, poorly defined, and narrow right femoral head and neck with marked remodeling of the right acetabulum. Based on her physical examination and radiographs, the working diagnosis was degenerative joint disease associated with avascular necrosis of the femoral head. The patient was initiated on carprofen treatment (4.4 mg/kg PO SID) for potential discomfort while the lab decided on a plan. Femoral head ostectomy (FHO) surgery was offered; however, the lab declined in favor of continuing carprofen and transferring her to an acute-use experimental protocol. The animal was enrolled in a 2-wk study during which time acupuncture was also performed to attempt to provide additional pain relief. At the completion of the study, the animal was euthanized, and a necropsy was performed. Grossly, the right femoral head was small (~5 mm diameter), rough, and irregular with an absence of articular cartilage, and the joint capsule was thickened (~8 mm). Microscopically, there was extensive osteonecrosis of the femoral head with a lymphoplasmacytic infiltrate and bone remodeling. The bone marrow was replaced with fibrous tissue and there was multifocal lymphohistiocytic infiltrate of adjacent connective tissue. Findings were supportive of the diagnosis of avascular necrosis of the femoral head (also known as Legg-Calvé-Perthes disease). This condition has not been published in this species and has only been reported twice in other nonhuman primates, though it is a well-described condition in young small-breed dogs for which conservative (analgesia, cage rest) and surgical (FHO) options exist.

PS18 Pouch Swelling in a Fat-Tailed Dunnart *(Sminthopsis crassicaudata)*

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A 3-y-old female fat-tailed dunnart *(Sminthopsis crassicaudata)* presented with a firm mass adjacent to the right side of the marsupium. On physical exam, the mass was firm, freely moveable, and measured 9.4 mm in diameter; it did not appear to involve the teats. No other abnormalities were noted, and the Dunnart remained bright and alert with a normal appetite and body condition. Differentials included neoplasia, cyst, or abscess. The mass remained unchanged for 3 wk, with only a small increase in size to 9.9 mm, however shortly thereafter, the mass increased in size to 13.1 mm x 11.5 mm and became fluid filled and cystic, moving cyst to the top of the differential list. During palpation of the mass, clear fluid was expressed, and the mass decreased to an almost unnoticeable size. The fluid filled cystic mass reoccurred 4 d later, measuring 14.8 mm x 12.9 mm. The dunnart was induced with 5% isoflurane via induction chamber and maintained at 3% isoflurane and 1% oxygen via a nose cone. The area around the mass was clipped and cleaned with 70% isopropyl alcohol prior to aspiration using a 27-gauge needle. Approximately 1.0-1.5 mL of straw-colored fluid was aspirated and submitted for cytology. The sample was determined to be moderately cellular, consisting of predominantly degenerate and nondegenerate neutrophils with few macrophages and moderate background hemorrhage. Occasional macrophages contained phagocytosed cellular debris. Cytologic interpretation indicated pyogranulomatous inflammation. The cystic mass reoccurred 2 wk postaspiration, this time with more extensive distribution throughout the marsupium, which was also edematous. The Dunnart was euthanized at this time and submitted for necropsy. Histopathology of the marsupium and mass revealed a cystically dilated, multifocally necrotic, tubular mammary adenoma. As this is a novel species, clinical prevalence of this condition in fat-tailed dunnarts is unknown, with only 1 known report identifying mammary gland adenocarcinomas in dasyurids. This is believed to be the first published report of a benign mammary gland adenoma in this particular dasyurid species. Clinical management of this condition is similar to mice, with monitoring for tumor interference of mobility, decline in body condition, and tumor size being the major clinical removal criteria.

PS19 Inappetence and Diarrhea in Porcine Models of Hypertension

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Two female intact Yorkshire cross pigs weighing approximately 70kg on a hypertension study presented for inappetence, diarrhea, polydipsia, and acute decline. Both pigs had surgery on the same day but represented 2 different models of hypertension. The first pig was implanted with subcutaneous depots of deoxycorticosterone acetate (DOCA; 100 mg/kg) while the second pig had a constrictor ring placed around the left renal artery. Postop, both pigs were transitioned to 0.9% saline water to potentiate hypertension. Seven days after surgery, the first pig presented for inappetence, diarrhea, and fever. Blood was drawn and a broad-spectrum antibiotic (1,050 mg PO BID) was started out of concern for infection of the implantation site. Bloodwork revealed a significant hypokalemia (2.3 mEq/L); potassium gluconate (1,190 mg) was added to the saline water of both pigs. Capromorelin (240 mg PO) and famotidine (80 mg PO) were given for inappetence. Three days later, this pig rapidly decompensated and was unable to be resuscitated. Eleven days after surgery, the second pig presented for diarrhea and inappetence, and was started on the same treatment plan of capromorelin and famotidine. Two days later, the 0.9% saline water was mixed 60:40 with regular water to reduce sodium intake due to potential concerns for salt toxicity. Fourteen days after surgery, generalized body tremors and foaming at the mouth were noted in this pig and euthanasia was elected due to declining condition. Given the model and neurologic signs observed, we were suspicious of salt toxicity. Brain tissue from the second pig was submitted for histopathology and eosinophilic perivascular infiltrates were observed which is pathognomonic for salt toxicity. Previous hypertension studies in swine have added salt to the feed, however animals on this study became inappetent with this method of salt delivery. The use of saline water to potentiate hypertension is well-described in rats and this method was elected as an alternative. The model has since been refined to provide saline water during the day and regular water at night to reduce total sodium intake. This change has resulted in the desired hypertensive model without causing the clinical signs previously seen.

PS20 Pharmacokinetics of High-dose Meloxicam and Toxicity in Pilot Efficacy Studies in Female CD1 Mice

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Despite the mouse being the most used animal in research, the pharmacokinetics of analgesia drugs are not well understood. Pharmacokinetic data are needed to establish effective pain management and dosing protocols to ensure the well-being of laboratory mice. Meloxicam is a nonsteroidal anti-inflammatory drug widely used in veterinary medicine to relieve surgical pain and

inflammation. In this study, we performed a pharmacokinetic study to evaluate the plasma concentrations of 10 mg/kg and 20 mg/kg meloxicam given subcutaneously in mice. Female CD1 mice were given 10 mg/kg (2.5 mg/mL; $n = 30$) or 20 mg/kg (5 mg/mL; $n = 30$) meloxicam subcutaneously. Three mice were euthanized by carbon dioxide at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, and 48 h after treatment, and blood was collected via cardiocentesis. Plasma was analyzed via liquid chromatography–tandem mass spectrometry to measure drug concentrations. The 10 mg/kg dose half-life was 5 h, time to peak plasma concentration was 1 h, and maximum concentration was 28.5 µg/mL. The 20 mg/kg dose half-life was 4 h, time to peak plasma concentration was 0.5 h, and maximum concentration was 54.9 µg/mL. Both doses remained above the purported therapeutic level of 390 ng/mL for at least 12 h. There was no clinical evidence of toxicity. An initial study was performed to assess the efficacy of these dosages in female CD1 mice that had an ovariectomy via ventral laparotomy. Immediately after anesthetic induction, mice were given 10 mg/kg ($n = 2$) or 20 mg/kg ($n = 4$) followed by re-administration of 10 mg/kg q 12 h postop. Postoperative pain was accessed through ANY-maze and behavioral observation measuring nest building, rearing, orbital tightness, wound licking, grooming, arched posture, and activity 3, 6, 12, 24, and 48 h postop. Mice given 20 mg/kg displayed behavioral signs of pain and 2 mice experienced profound drug effects resulting in death and euthanasia. Necropsy revealed intestinal perforation and diffuse intestinal gas distension indicating adverse reactions to the high dose meloxicam. This suggests that while both 10 mg/kg and 20 mg/kg meloxicam reach therapeutic plasma concentrations for 12 h, the 20 mg/kg dose is not a suitable dose of analgesic for murine studies due to its ability to cause NSAID toxicity

PS21 Effects of Midazolam/Dexmedetomidine/Buprenorphine and Midazolam/Dexmedetomidine/Sustained-release Buprenorphine Anesthesia in C57BL/6J Mice

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Injectable anesthesia facilitates many procedures in laboratory mice and is sometimes required over inhalant protocols due to technical or experimental limitations. Ketamine/xylazine/acepromazine, one of the most commonly used injectable protocols, has been shown to have a widely variable response in mice. Because of this variability, it is important to continue to develop safe and effective injectable anesthetic protocols. We hypothesized that a combination of dexmedetomidine (dex), midazolam (midaz), and buprenorphine (Bup) or sustained-release buprenorphine (BupSR) would provide a reversible and reliable anesthetic regime with potential for postoperative analgesia. To test this hypothesis, we performed a series of 3 experiments in young, male and female C57BL/6J ($n = 28$) mice. In the first experiment, mice were anesthetized with an intraperitoneal (IP) injection of low dose (0.15 mg/kg and 4 mg/kg) or high dose (0.25 mg/kg and 6 mg/kg) dex and midaz respectively, followed by subcutaneous Bup (0.1 mg/kg). A surgical plane of anesthesia was defined by the loss of paw withdrawal reflex following a brisk application of a 300-g noxious stimulus to the hindfoot. The high dose reliably reached a surgical plane of anesthesia in all 10 mice, which lasted for 86 ± 30 min, while only 6 of 8 mice lost the righting reflex with the low dose. In the second experiment, the mice received the high-dose dex and midaz and either Bup or BupSR (1 mg/kg). All mice received 100% supplemental oxygen and the anesthesia was reversed with atipamezole (1 mg/kg) IP after 45 min. In this experiment, all 10 mice reached a surgical plane and remained at a surgical plane of anesthesia at the 45-min mark. All mice regained the righting reflex within an average of 4 ± 3 min of reversal. In the final experiment, 6 mice were anesthetized using the high dose dex, midaz, and Bup, and a terminal laparotomy was performed to confirm that the protocol was effective under surgical conditions. All mice regained the righting reflex following reversal. In summary, an injectable anesthetic protocol of dexmedetomidine, midazolam, and either buprenorphine or sustained-release buprenorphine can provide a reliable and reversible surgical plane of anesthesia in mice.

PS22 Comparison of Alfaxalone-Midazolam, Tiletamine-Zolazepam, and Ketamine-Acepromazine Anesthesia during Plethysmography in Cynomolgus Macaques (*Macaca fascicularis*) and Rhesus Macaques (*Macaca mulatta*)

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Plethysmography is used in NHPs to measure minute volume before aerosol exposure to an agent to calculate total time necessary in the exposure chamber. The consistency of respiratory parameters during the entire exposure time is paramount to ensuring dosing accuracy. Our study sought to validate an intramuscular (IM) alfaxalone-midazolam (AM) anesthetic combination administered at 5 mg/kg alfaxalone and 0.3 mg/kg midazolam for use in aerosol studies. We hypothesized that AM would provide an adequate duration of anesthesia, achieve and maintain steady-state minute volume (SSMV) for 20 min, and have anesthetic quality and side effects comparable to or better than either IM tiletamine-zolazepam (TZ) administered at 6 mg/kg and IM ketamine-acepromazine (KA) in a 10:1 mixture administered at 0.12 mL/kg. TZ and KA are currently the most common anesthetics used for aerosol studies. Two groups of NHPs, 1 consisting of 15 cynomolgus macaques and 1 of 15 rhesus macaques, received 3 IM anesthetic combinations (AM, TZ, and KA), no less than 1 wk apart. Anesthetized NHPs were placed in a plethysmograph chamber and their minute volumes were measured every 10 s to determine whether they had achieved and maintained SSMV for at least 20 consecutive min. Achieving and reliably maintaining an SSMV for at least 20 min facilitates precise aerosol dosing of a challenge agent. Quality of anesthesia, based on the NHP's ability to achieve and maintain SSMV, was higher for both species with AM compared with TZ (cynomolgus: $P = 0.0008$; rhesus: $P = 0.0142$) and KA (cynomolgus: $P < 0.0001$; rhesus: $P = 0.0008$), and AM had a longer duration of SSMV compared with TZ ($P = 0.0001$) and KA ($P < 0$ 0.0001) in cynomolgus macaques. Average SSMV was larger with AM compared with TZ in cynomolgus macaques ($P \leq 0.05$, 95% CI: 15–213 mL), but smaller with AM compared with KA in rhesus macaques ($P \leq 0.05$, −343–−7 mL). Duration of anesthesia was sufficient with all combinations but was longer in TZ for both species than both AM (cynomolgus: $P \leq 0.05$, 95% CI: 6–41 min; rhesus: $P \leq 0.05$, 95% CI: 2–23 min) and KA (cynomolgus: $P \leq 0.05$, 95% CI: 19–54 min; rhesus: $P \leq 0.05$, 95% CI: 10–31 min). These results suggest that the AM anesthetic combination would produce the most accurate dosing for an aerosol challenge.

PS23 Correlation of Noninvasive Diffuse Optical Neuromonitoring and Systemic Predictors of Return of Spontaneous Circulation During Cardiopulmonary Resuscitation

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Physiologic parameters of coronary perfusion pressure (CoPP) and end-tidal CO_2 ($EtCO_2$) are established indicators of CPR quality and are significantly associated with return of spontaneous circulation. While growing evidence supports the use of physiology-directed

CPR strategies, reliable measurement of either CoPP or $EtCO_2$ during CPR requires invasive procedures (vascular access for CoPP; endotracheal intubation for $EtCO_2$) which limit timely intervention. Here, we examine the quantitative correlation of noninvasive measurements of the change in cerebral tissue oxygen content during CPR with established systemic parameters of CoPP and $EtCO_2$. We hypothesize that increasing CoPP and increasing $EtCO_2$ will be correlated with increasing oxygen content. A retrospective study examined data collected in 1-mo-old female swine (n = 77, 9–12 kg) who underwent an asphyxia-associated cardiac arrest model followed by 20 (n = 52) or 25 (n = 25) min of CPR. During CPR, a noninvasive frequency-domain diffuse optical spectroscopy (FD-DOS) sensor continuously monitored cerebral tissue concentration of oxyhemoglobin ($[HbO_2]$). CoPP was calculated as the difference between invasively measured aortic pressure and right atrial pressure during chest compression release. $EtCO_2$ was computed as the maximum capnometry value in each respiratory cycle. The relationship between the change in $[HbO_2]$ from 1 min into CPR ($\Delta[HbO_2]_{CPR1m}$) and CoPP, and between $\Delta[HbO_2]_{CPR1m}$ and $EtCO_2$, was assessed using a linear mixed-effects model, incorporating random slope and intercept effects to account for within-subject correlations of repeated measures. Significance was determined at a level of $P < 0.05$. A positive correlation was demonstrated between CoPP and $\Delta[HbO_2]_{CPR1m}$ (slope $P < 0.001$). In contrast, a correlation with $EtCO_2$ was not observed (slope $P = 0.22$). The results indicate a commonality between mechanisms underlying generation of CoPP and oxygen delivery to the brain, but a discrepancy between that and exhalation of CO_2. Thus, the use of $EtCO_2$ alone to direct CPR may not result in effective cerebral oxygen delivery. Further study examining the use of noninvasive cerebral oxygenation monitoring for guidance during CPR is currently underway.

PS24 Morphological and Molecular Characterization of a Novel Mouse Fur Mite

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Myobia murismusculi (also known as *Myobia musculi* or MOB) and *Radfordia affinis* (RDA) of the Myobioidea superfamily and *Myocoptes musculinus* (COP) of the Sarcoptoidea superfamily are the 3 most prevalent ectoparasite species in contemporary laboratory mice. In this study, based on morphological and molecular evidence, we identified a novel fur mite from naturally infested mice obtained from research colonies in Taiwan and designated as *Myobia muris* (MOM). The nucleotide sequences of the 18S and 28S ribosomal RNA (rRNA) genes of MOM and RDA were determined and compared with previously characterized mites inhabiting animals and plants. Phylogenetic analysis results of the 18S and/or 28S rRNA gene sequences revealed that MOM belongs to the same superfamily as MOBs and RDAs, and COP is least related to MOM. Genetic distance result indicated that MOM is most closely related to MOBs and RDAs (18S rRNA: ≥ 98.7% identity [MOBs & RDAs]; 28S rRNA: 93.7% identity [RDA]) but is distinctly different from COP (18S rRNA: 86.5% identity; 28S rRNA: 74.7% identity). Morphological characters (for example, shape, size, and detailed structures on body surfaces) of MOM were observed by optical microscope examinations and compared with those of other mouse fur mites. The result showed that MOM is externally indistinguishable from MOB, with similar shape/size but different external structures to RDA, and highly distinct to COP in shape and externals. In conclusion, morphological and molecular characteristics suggest that MOM is a novel member in the superfamily Myobioidea with MOB and RDA.

PS25 Whole Genome Sequencing of Skin Microbiota in *Corynebacterium bovis* Clinical and Nonclinical Infection

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Corynebacterium bovis remains a significant problem for immunodeficient mouse colonies worldwide, yet inoculation of naïve immunodeficient mice with cultured *C. bovis* fails to consistently produce clinical disease. It is unclear why mice develop clinical signs, and the inability to recapitulate clinical *C. bovis* (CCB) experimentally remains a significant barrier to understanding its impact on cancer research. Exposure of nude mice (Hsd:Athymic Nude) and NSG (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ) mice to soiled bedding collected from NSG mice with CCB consistently transfers the clinical infection with highly predictable disease progression. Replicating CCB experimentally allowed us to investigate the skin microbiome changes throughout disease. Eighteen mice from each strain were divided into 3 experimental groups including soiled bedding exposed (CCB), cultured *C. bovis* inoculated (Cb+), and negative controls (Cb-). Skin swabs were collected throughout disease progression. Nucleic acid was extracted for whole genome sequencing (WGS). After WGS, reads were trimmed, filtered, and analyzed for community composition using KRAKEN2 or changes in genetic content through GENESHOT. Expansion of *C. bovis* in the CBB and Cb+ groups occurred for both mouse strains. For NSG mice, commensal microbes *Alistipes shahii*, *Acutalibacter muris*, and *Cutibacterium acnes* showed a significant decrease in the abundance at 3 and 5 wk postexposure. Similar trends were also observed in nude mice. Other than *C. bovis*, no bacterial species increased in abundance in either mouse strain when comparing CCB and Cb+. This suggests that CCB is a single-agent infection. However, limitations of skin microbiome studies include lower read counts, and further validation studies are in progress. Finally, analysis of the genetic content in each cohort showed enrichment of pathogenicity-associated (PA) genes, such as invasion hydrolase, drug exporters, histidine kinases/two-component sensors, lipases, and metal-binding proteins. These findings are consistent with recent literature identifying increases in PA genes in *C. bovis* isolates. Of critical importance, our study shows our ability to recapitulate CCB and identifies *C. bovis* as a likely solo agent involved in clinical disease.

PS26 From Guts to Gills: Diving into the Microbial Populations of Zebrafish through Microbiome and Targeted Infectious Agent Analysis

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Use of zebrafish as a research model is expanding. Zebrafish present advantages, but also several challenges. Centralized standards for zebrafish husbandry, production, or guidelines for excluded agents are necessary. Also, the commensal microbiome of animal models impacts research study outcomes, and it reasons that zebrafish would be no different. To improve reproducibility across institutions, there is a need for tools to perform microbiome monitoring and targeted agent identification. To improve understanding of the bacteria cohabitating with zebrafish, we evaluated the zebrafish microbiome

to identify commensal microbes and probe for the presence of known zebrafish infectious agents. 10–15 zebrafish, plus tank water, were obtained from 3 independent sources. Entire fish were processed for infectious agent PCR, while the water samples were processed through a stepwise filtration protocol. Filter membranes were tested to identify agents associated with the fish environment and determine efficacy of filtered water in identifying infectious agents present in the colony. Of the tested agents, *Pseudocapillaria tomentosa* and *Pseudoloma neurophila* were only detected in whole fish samples, while *Mycobacterium* spp. were found only in tank water. Some agents were not detected uniformly across pooled fish samples, including *Aeromonas dhakensis* and *hydrophila, Pleistophora hyphessobryconis*, and picornavirus, but were captured by tank water samples. *Plesiomonas shigelloids* and *Pseudomonas fluorescens* were identified in all fish pools and tank water. To bolster our knowledge of zebrafish commensals, we performed 16S rRNA sequencing of the bacteria associated with the intestines, scales, and gills of 3–5 fish from all 3 colonies. Sequencing analysis revealed *A. hydrophila* in gill microbiome samples, but not uniformly across samples from the same source. Scale-associated microbiome samples showed the highest levels of uniformity across individual fish and across 2 of 3 vendors. The gill-associated microbiome also showed unexpected potential problematic bacteria such as *Bdellovibrio bacteriovorus* and a bacteria associated with epitheliocystis in salmon. Gut samples showed unexpected diversity in fish from the same colony. The data from this investigation deepens our knowledge of zebrafish microbial community dynamics and optimized testing protocols for standardization of care.

PS27 Comparing Detection of Rodent Pathogens with Exhaust Air Dust and Sentinel Surveillance Testing in Facilities with Varying Census Sizes

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Interest has grown in comparing the use of PCR analysis via environmental testing methods, such as exhaust air duct (EAD) testing, for rodent health surveillance. We examined the PCR on EAD filters with sentinel mice for our normal surveillance panel (n = 15 agents), 2 additional viral agents, murine norovirus (MNV) and murine chapparovirus (MKPV), and two bacterial agents, *Corynebacterium bovis* and *Helicobacter* spp. In this study, EAD testing and sentinels were compared at day 0, 90, and 180 in 4 facilities (n = 12 rooms) with animals housed on IVC racks (n = 57 double-sided, 14 single-sided racks). All racks on study were negative for our standard agents during the study. Bacterial agents were consistently positive on EAD filter while less consistently identified on sentinel animals. EAD filters and sentinel mice tested for MNV demonstrated agreement for positive tests by PCR and by serology. For MKPV we compared pooled urine with EAD, and feces from sentinels. Six cages of sentinels were positive for MKPV by fecal PCR with 5 of 6 testing positive on urine, while only 3 of 6 EAD filters tested positive. We also examined copy numbers for all agents that tested positive which increased and then stabilized once positive. For EADs, the copy number was strongly correlated with census for both *Helicobacter* spp. and MNV suggesting that copy number may serve as a proxy for rack incidence. For sentinels, unsurprisingly, there was no correlation and more of a threshold for positivity for MNV. Overall, the results support the use of a mixed surveillance program, incorporating both EAD testing and sentinels.

PS28 Tracking Transmission of Murine Chapparvovirus to Immunocompetent Mice via Exposure to Soiled Bedding

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Murine chapparvovirus (MuCPV) is a prevalent emerging infectious agent causing kidney pathology leading to morbidity and mortality in immunodeficient mice. Detection of MuCPV is known to be delayed. In endemically infected SPF mouse colonies, MuCPV is consistently detected by PCR, but not by seroconversion, in mice older than14 mo. MuCPV can be detected by both PCR and serology in pet shop quality (PS) mice. The immune system of SPF laboratory mice may not be sufficiently developed to generate detectable levels of MuCPV antibodies. We hypothesized that PS mice, through exposure to myriad infectious agents, have a highly developed immune system that supports an earlier antibody response and could transfer this training to naïve mice. In this time course study, PS mice aged 3 to 4 wk, 6 to 10 wk, and 10 to 12 wk supplied soiled bedding to naïve mice of 2 immunocompetent strains. Baseline fecal PCR testing of PS mice confirmed the presence of MuCPV. 16 CD-1 Elite (CD-1E) and 16 VAF C57BL/6N (B6) mice received 50% pooled PS soiled bedding weekly. Alternating real-time fecal PCR testing (pooled by cage) and serological testing of individual mice by MFIA (NS-1 antigen) were performed on a biweekly basis for 40 wk. Estimated PCR copy numbers and MFIA scores were used to quantify results. Both strains exhibited a temporary elevation in PCR copies at 5 wk postexposure, suggesting that PS mice were not shedding high titers of MuCPV until this timepoint. Peak MuCPV shedding was observed at 20 wk for CD-1E versus 25 wk in B6. Average PCR copy numbers were approximately 1 $\log_{10}$ higher in the CD-1E versus the B6 mice. Seroconversion was observed at 29 wk for CD-1E versus 34 wk in B6 mice. MFIA scores at the end of the study were higher in the CD-1E (15) versus the B6 (6) mice. Exposure to the additional agents in the soiled bedding from the PS animals did not appear to expedite immune response. Due to delayed seroconversion, antibody detection cannot be reliably used to detect MuCPV in soiled bedding sentinels with less than 6 mo exposure. PCR remains the recommended method of choice for the detection of murine chapparvovirus in soiled bedding sentinels.

PS29 Husbandry Guidelines for a Novel Immunodeficient Rat Strain, the SRG rat, and Its Use for Cancer Xenograft Studies

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The SRG rat is a novel strain on the Sprague Dawley background with knockout mutations in the Rag2 and Il2rg genes. This rat strain lacks mature T, B, and NK cells, making it severely immunodeficient and a suitable host for a wide variety of commercially available human cancer cell lines, as well as patient-derived xenograft (PDX) tissue, particularly from patients with nonsmall cell lung cancer and ovarian cancer. With the larger body weight and the capability of multiple tissue collections during growth kinetics and treatment efficacy studies, the SRG rat offers capabilities not available in smaller animal models and is a valuable addition to your xenograft and oncology toolbox. The SRG rat is significantly more immunodeficient than the RNU Nude rat and requires strict adherence to husbandry guidelines to prevent infection from opportunistic organisms. Here we describe our practices for housing, breeding, husbandry, injections, and surgical manipulations using the SRG rat, as well as guidelines for dosing for treatment efficacy

studies using the SRG rat. In addition, we provide data on side-by-side comparisons of tumor formation rate and growth kinetics in the SRG rat compared to commercially available immunodeficient mouse strains to help guide your tumor xenograft studies.

PS30 Exposure to Dim Light at Night (dLAN) Disrupts Nocturnal Melatonin in Nude Rats Bearing Human Castration-Sensitive VCaP Prostate Cancer: Impact on Tumor Circadian Dynamics, Metabolism, and Proliferation

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Over 248,000 men in the U.S. alone this year will be diagnosed with prostate cancer, and over 33,300 will die from the disease. Exposure to dLAN suppresses nighttime pineal melatonin (MLT) production that influences normal and neoplastic tissue metabolism and proliferation. Previously, we showed in rodent tumors in vivo that MLT inhibits linoleic acid (LA)-uptake and conversion to 13-hydroxyoctadecadienoic acid (13-HODE), a lipoxygenase product that enhances epidermal growth factor and insulin-like growth factor-I-induced mitogenesis. Recently, we developed a tissue-isolated, castration-sensitive VCaP human prostate cancer xenograft model to test the hypothesis that suppression of the nocturnal MLT signal due to vivarium dLAN exposure accelerates tumor LA-uptake, aerobic glycolysis (Warburg Effect), and proliferative activity. In this GLAS-supported study under an IACUC-approved protocol male nude rats (Crl:NIH*Foxn1*^{rnu}; n=12/group) bearing this xenograft model were maintained on either a control 12L(300 lux):12D(0 lux) or experimental 12L(300 lux):12dLAN (0.2 lux) light/dark cycle. Results revealed (Mean ± S.D.) plasma MLT levels in controls peaked in the mid-dark phase (183.4 ± 12.8 pg/mL) and were lowest (2.2 ± 0.4 pg/mL) in the mid-light phase, and low (< 10 pg/mL) throughout the 24-h period in dLAN rats. Tumors in rats exposed to dLAN exhibited a significantly shorter latency to onset and a 2-fold faster growth rate than controls. Control group tumors revealed elevated cAMP levels, LA uptake, 13-HODE production, Warburg Effect, as well as elevated patterns of expression of signaling pathways phospho-ERK1/2, -AKT, -STAT3, and -GSK3γ during the light phase and markedly suppressed during the dark phase. In the dLAN group, these measures were elevated throughout the 24-h period. This is the first evidence in vivo, that dLAN-induced disruption of integrated circadian rhythms of signaling, metabolism, and proliferation results in accelerated growth of castration-sensitive human prostate cancer xenografts. Thus, lighting design strategies to minimize laboratory animal and human exposure to LAN that preserve the integrity of the circadian MLT signal may offer a novel approach to suppress the growth progression of human castration-sensitive prostate cancer.

PS31 Role of Oncostatin-M in Exercise-induced Breast Cancer Prevention

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As the leading cause of cancer, 1-in-4 women are diagnosed with breast cancer in their lifetime, worldwide. Epidemiologic studies and rodent models show that moderate-intensity physical activity can decrease the risk of breast cancer. Proposed mechanisms of how physical activity impacts breast cancer progression range from minimizing risk factors to reducing proliferation and increasing apoptosis of abnormal mammary cells. Myokines are muscle-derived cytokines excreted by skeletal muscle following acute exercise. Specifically, the myokine oncostatin M (OSM), has been shown to decrease breast cancer cell proliferation, in vitro. We hypothesized that OSM is involved in physical activity-induced breast cancer prevention, in vivo. Female Sprague Dawley rats were injected with 75 mg/kg n-methyl-n-nitrosourea (MNU) at 35 d of age to induce mammary adenocarcinoma. Rats were exercise trained (MNU+Ex) or remained sedentary in standard cage conditions (MNU+Sed). The study was powered with n=12 in each group to observe a significant difference in tumor free survival time. Exercise training consisted of treadmill acclimation, and progressive increases in session duration, speed, and grade, until reaching 30 min/day, 20 m/min at 15% incline. Exercise training continued 5 d/week until tumor palpation or week 18, which ever came first. Additionally, rats completed a maximal endurance test and blood was drawn in a time course (before, 30 min following, 2 h, and 24 h\ to determine OSM plasma levels. We observed no significant differences between MNU+Sed and MNU+Ex growth curves for body weight over the course of the intervention. Tumor free survival was significantly higher (P = 0.044; $P < 0.05$) in MNU+Ex animals (103.0 ± 19.0 days post-MNU) compared to MNU+Sed animals (71.3 ± 22.7 days post-MNU). Following acute exercise, OSM plasma levels were significantly higher compared to baseline OSM levels (P = 0.046; $P < 0.05$). This study provides the basis for future work aimed at observing tumor latency after OSM blockade. In conclusion, independent of differences in energy balance, exercise training increased tumor free survival in a rat model of carcinogen-induced breast cancer. The observed protection may be modulated by acute exercise-induced increases in OSM levels.

PS32 Transcription Factor SOX2 as an Indicator of Progression in Early-stage Bladder Cancer

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Bladder cancer (BC) is the fourth most common cancer type in men with approximately 70% of cases first diagnosed at an early stage. Conservative treatment for BC provides a better quality of life, but a subset of tumors will still progress and eventually lead to mortality. Currently, there is no reliable way to identify which patients are at greatest risk of progression to advanced disease. We previously reported that early-stage BC that overexpresses the transcription factor sex determining region Y-Box 2 (SOX2) have higher risk of progression. Because SOX2 functions as an oncogene in other cancers, we hypothesized that increased expression of SOX2 drives progression of early-stage bladder cancer. A lentiviral approach was used to overexpress SOX2 in MB49 murine BC cell line. To model the tumor microenvironment, empty vector and SOX2 overexpressing cell lines were recombined with fetal bladder mesenchyme dissected from embryonic day 16 Sprague Dawley rats. Recombinants were subsequently surgically implanted under the renal capsules of male and female C.B-17/IcrHsd-*Prkdc*^{scid} mice (n=10 MB49 SOX2, n=10 MB49 empty vector). Mice were euthanized and necropsied at 2-3 wk postimplantation, and tissues were collected for histologic analysis. A scoring system was developed to encompass clinical morbidity, ascites production, local tumor invasiveness, and tumor extension to nearby organs. Each variable was individually semiquantified, then added to give a combined score. The Wilcoxon rank sum test was used for all comparisons. Gross pathology results showed no

significant difference between the total scores of the SOX2 MB49 allograft and empty vector allograft mice. However, when compared to empty vector controls, SOX2 MB49 bearing male mice had significantly more ascites ($P = 0.004$) and splenic tumor involvement ($P = 0.04$). SOX2 MB49 bearing female mice had significantly higher mortality ($P = 0.02$) compared to female controls. Furthermore, histological analysis demonstrated SOX2 MB49 male mice had significantly greater renal tumor invasiveness ($P = 0.04$) and tumor size ($P = 0.02$) compared to control males. In conclusion, SOX2 upregulation may drive aggressive tumor development in early-stage BC making it a candidate biomarker for tumor progression.

PS33 Atraumatic Sampling Interface into GBM Xenograft Rat Model via Cerebral Open Flow Microperfusion (cOFM) Probe and Culex Automated Blood Sampling System

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In the US, glioblastoma multiforme (GBM) accounts for 14.6% of all primary brain tumors and 48.3% of malignant brain tumors. The median 2-y survival rate for patients with GBM is between about 10%-26%, dependent on treatment and the patient's response. Developing new drugs is challenging due to the heterogeneity of GBM and low exposure to therapeutic agents due to the blood-brain barrier (BBB). Cerebral open flow microperfusion (cOFM) is a minimally invasive sampling technology that allows sampling of interstitial fluid (ISF) inside the brain tissue with intact BBB. The macroscopic openings and the material properties of the probe allow sampling of ISF components and drugs regardless of size or lipophilicity. This allows monitoring of pharmacokinetics (PK) across the BBB. A study showed that cOFM can also be used to apply glioma cells into the brain and allow a glioma to form around the cOFM probe. This provides atraumatic access into the center of glioma and moreover the direct application of potential drug candidates into the GMB bypassing the BBB.We propose a study design where the cOFM probe is used to establish direct access to an orthotypic human GBM xenograft from U87 cells in immune-deficient rats. The cOFM probe is implanted 14 d prior to dosing, to assure surgical trauma recovery and BBB re-establishment. Catheters for blood sampling and dosing are implanted 3 days before dosing. An antibody anticancer drug is dosed via intravenous (IV) catheter as a single bolus injection. The PK profile of the therapeutic antibody in the tumor ISF and blood is continuously and automatically assessed via cOFM combined with the Culex sampling system over 7 d. This design enables collection of PK data from macromolecules. Mean elimination half-lives of up to 20 d, like for antibodies, need a setup that allows long-term studies in awake animals. As a result of the automated sampling of both ISF and blood, the stress level of animals is as low as possible. Combining cOFM with Culex allows continuous sampling of ISF and blood in awake animals and thus assessing the full panel of PK parameters in one single animal over several days. This reduces the number of animals needed compared to conventional biopsy PK studies.

PS34 Implementation of Minimally Invasive Brain Tumor Resection in Rodents for High Viability Tissue Collection

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The present protocol describes a standardized paradigm for rodent brain tumor resection and an automated tissue preservation. It uses a multifunctional, nonablative resection device and an integrated tissue preservation system adapted from the clinical version of the device. This protocol requires minimal surgical skills to perform the resection process in less than 2 min/animal via the same burr hole used for the initial tumor implantation with minimal-to-no blood loss. To validate this protocol, 20 GL261-bearing mice (C57BL/6 female, 6-8 wk of age) were divided into 2 groups: a control untreated group (n=10) and a treatment group undergoing surgical resection using the MIRS (n=10). In addition, each group was stratified into 2 subgroups based on the baseline tumor burden as quantified by the IVIS imaging system: a group with smaller tumor burden (mean bioluminescent signal = 5.5e+006 ± 0.1e+006 photons/s) and a group with larger tumor burden (mean bioluminescent signal = 1.69e+007 ± 0.2e+007 photons/s). After resection, mice were followed for survival and tumor burden. Mice undergoing resection by MIRS had prolonged median survival rate compared to the control mice (22 versus 16 d in the animals with small tumor burden and 19 versus 12 days in the animals with large tumor burden, $P < 0.05$). The resection process resulted in a significant decrease of the tumor burden and the resected tissue demonstrated high in vitro and in vivo viability. Based on the post-resection MRI, the resection volume was consistent across animals and can be adjusted based on the number of rotations performed using the cutting aperture. In summary, this paradigm can accurately mimic the clinical version of the surgical resection process in patients and represents a robust preclinical model in brain tumor research which will expand opportunities to explore unanswered questions about perioperative management and therapeutic discovery for brain tumor patients.

PS35 X-Ray-assisted, Ultrasound-guided Injections (USGI) to Initiate and Treat Focal Lung Tumors in Mice

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Current models of lung tumors in mice cause many diffuse tumors, only capturing the late stages of the disease in patients. The lack of reproducible ways to induce a singular tumor in the lung makes the development and evaluation of therapies for early lung tumors challenging. Attempts at a manual percutaneous or transpleural surgical orthotopic approach proved unsuccessful and so a USGI approach was developed. Although ultrasound cannot visualize the lung interior, several simple anatomical landmarks are employed to arrive at the location of interest. According to our approach images are first taken using an x-ray micro-tomography system (uCT) with a mouse in the same orientation and placement as it would be for ultrasound. The uCT is used to determine 3 coordinates; first of is the cranial distance into the left lung from the liver/lung interface (z-axis). The second coordinate is the depth from the pleural line (y-axis). And the third is medial distance from the skin line to the injection point (x-axis). We then translate these coordinates as we are visualizing in real time with ultrasound. The ultrasound system is equipped with precision manipulators for the injection system, positioning stage, and transducer mount all of which allow for accurate coordinate adjustments. The location for injection was selected using the following criteria: ease of visualization with uCT longitudinal monitoring, allowance for non-detrimental margin of error, and focus on the single lobe of the left lung. Based on more than 400 injections, about 85% of mice grow a tumor at the site of interest. The same procedure is then used the deliver therapeutics intratumorally. This approach constitutes a reproducible methodology for inducing and evaluating lung cancer treatments using minimally invasive ultrasound guidance.

PS36 Comparing User Measurement Variability of Subcutaneous Tumors with 3D Thermal Imaging and Calipers

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Repeatable tumor measurements are key to accurately assessing tumor growth and treatment efficacy. Our previous work showed that a novel 3D and thermal imaging system (3D-TI) for measuring subcutaneous rodent tumors significantly reduced interoperator variability across 3 in vivo efficacy studies. Here we investigated this reduction in interoperator variability across a much larger dataset. The dataset of 6,532 paired 3D-TI and caliper interoperator repeats was obtained from tumor scans and measurements in 27 laboratories across 289 studies, 153 operators, over 20 animal strains, and 100 cell lines. Interoperator variability of the measurement methods was analyzed using coefficient of variation (CV), intra-class correlation (ICC) analysis, and significance testing. Median 3D-TI CV of 0.127 was significantly lower than the median caliper CV of 0.175 ($P <$ 0.00001). The effects of large interoperator variability at critical points in the study were also investigated. At randomization, changing the operator performing caliper measurements resulted in 59.4% probability that a rodent would be reassigned to a different group. The probability that this would occur when using 3D-TI was significantly lower at 29.2%. In studies where tumor was expected to regress, substituting an operator mid-study resulted in tumor volume increase of approximately 500mm3 when using calipers. The same effect was not reproduced when using 3D-TI. We conclude that 3D-TI offers a significant reduction in interoperator variability in comparison to calipers and can improve reproducibility of in vivo studies across a wide range of animal strains and cell lines.

PS37 Development of an Outcome-based Acclimation Program for Nonhuman Primates

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Acclimation is the process by which animals adjust to a new environment or experience and is typically focused on duration rather than outcomes. The development of an innovative outcome-based approach changes this focus by setting standards that NHP should achieve to demonstrate adequate acclimation prior to study transfer. With senior leadership support, a global team of NHP behavior experts was established and tasked with defining clinical and behavioral expectations of the well-acclimated NHP. In addition to clinical health parameters, behavioral measures were identified, including social compatibility, positive socialization with humans, expression of normal species-specific behavior, and a demonstrated ability to respond to positive reinforcement training (PRT) for handling. To facilitate behavior assessment, a scoring system was developed. For each behavioral measure, a scoring scale from 1-5 was defined, with 5 being an ideal score. Enhanced human-animal socialization activities were included in the plan to promote socialization. A proof-of-concept study was designed and conducted to validate if the new acclimation program and associated measures could achieve the desired welfare outcomes. This study included 26, 2-to-4-y-old cynomolgus macaques. All macaques were examined upon arrival and throughout the acclimation study by site veterinarians and maintained good clinical health. Within 28 d, all animals achieved or exceeded the target behavioral scores for social compatibility, normal behavior and socialization. All macaques also demonstrated an ability to be trained through PRT for handling.

PS38 Use of a Primate Welfare Assessment Tool to Encourage Continuous Institutional Improvement

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Primates play an important role in biomedical research and there is an ethical obligation to provide the best welfare throughout their life. Regular animal welfare assessments based on primate-specific, evidence-based inputs and behavioral outputs are important for promoting effective animal care and use programs by ensuring an animal's affective state, behavior, and physiology are suitable for scientific study; deviations from approved protocols or negative welfare states are identified and corrected quickly; and that animal use, housing, and husbandry standards are optimal, continue to meet animal needs, and provide positive experiences. Welfare assessments should also consider cumulative use and humane endpoints. Recognizing the need for more formalized welfare assessment, a Primate Welfare Assessment Tool (PWAT) was developed to establish global guidelines and best practices, benchmark current practices, and monitor progress of improving welfare across sites. The PWAT was developed, beta tested, and a pilot was conducted in fall 2021. Two sites located in different continents used the tool, including room, site, and culture of care assessments, and a user survey was completed following use of the tool. Based on the user survey, the assessment was easy to complete, and personnel were satisfied or very satisfied with the look, feel, and accessibility of the forms in SmartSheets. "Easy to use," "site-level assessment," and "animal behavior" were named as the most important features, and the PWAT was considered valuable or very valuable for assessing and improving primate welfare. Personnel gave the PWAT 8.7/10 for their overall experience with using the tool. The PWAT took ~7h to complete over 6 rooms for the room-level assessment, 30 min for the site-level assessment, and <1h for the culture of care assessment. Subsequently, full site implementation occurred in March 2022 to benchmark practices cross 13 sites. The PWAT tool and dashboard were successful in helping sites to identify areas for improvement and recommendations for each site, assisting IACUCs to make decisions as to what aspects of their program to prioritize to improve primate welfare.

PS39 Developing Engaging Annual Animal Welfare Reinforcement Training

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Annual animal welfare refresher training is fundamental within any institution to help reinforce key concepts, principles, and standards to ensure that employees are aware of expectations when working with animals. However, developing these types of trainings can be challenging due to several factors. Not only does the training need to be interesting and relevant for all learners, but we must also consider the variety of species worked with, type of research conducted, cultural and language differences, learning modalities, level of education, length of time in the industry, and job role. To avoid presenting the same training year after year, we have implemented a multistep process that includes selecting a trending topic while maintaining a focus on our key values for working with animals, organizing a multicultural and multidiscipline development team, conducting robust beta testing prior to implementation, allowing for completion by instructor-led sessions or e-learning, providing the training in multiple languages, and gathering feedback to help improve future trainings. This process allows us to develop and

implement training that reinforces key concepts related to animal welfare and a culture of care, while introducing new information for our target audience, which includes anyone who works with animals (for example, animal caretakers, research technicians, or post-life technicians), anyone who supervises individuals who work with animals, and anyone who oversees animal work (scientists, study directors, or principal investigators). Data collected shows leaners find these refresher trainings relevant, topics presented help introduce, bring awareness to, and help identify opportunities to improve animal welfare and a culture of care. Trainings are archived and can be referenced in the future and/or used for training new employees. We have found using this process to be an effective way to develop and implement annual animal welfare refresher training and are beneficial in other training initiatives.

PS40 Efficacy of a Novel Battery-operated Tumbler Device Compared to Live Animal Sentinels for Mouse Pathogen Detection

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The search for alternatives to live animal sentinels in rodent health monitoring programs is fundamental to the 3Rs. We evaluated the efficacy of a novel battery-operated tumbler device that rotates soiled bedding in contact with sample collection media against the use of live animal soiled bedding sentinel mice. Four rodent racks were used, each with 3 test cages; a cage with a tumbler device that ran 10 min twice weekly, a cage with a tumbler device that ran 60 min twice weekly, and a cage housing 2 female Crl:CD1(ICR) mice. Every 2 wk, each cage received soiled bedding collected from all cages on each respective rack. In addition to soiled bedding, the tumbler devices contained various sample collection media (a Reemay filter that remained in the tumbler for the entire duration of the study, a Reemay filter that was replaced each month and pooled together as one sample, and a sticky swab added at every bi-weekly cage changeout and later pooled together as one sample) and a Reemay filter located at the exhaust outlet of the cage. Direct PCR using a fecal pellet, body swab, and oral swab was performed on the sentinel mice for comparison to the animal-free methods. Out of 16 total pathogens detected, the monthly Reemay filters from both the 10-min tumblers and 60-min tumblers detected 81.61% and 75.77% of pathogens, respectively while live animal sentinels detected only 44.23% of pathogens. Sticky swabs in both the 60-min (23.81%) and the 10-min tumblers (15.48%) detected the least number of pathogens. These results indicate that the novel tumbler device is an effective and reliable tool for use in rodent health monitoring programs and a suitable replacement to live animal sentinel mice. Reemay filters rotated for 10 min in contact with soiled bedding and replaced monthly performed the best for pathogen detection.

PS41 Impact of Automated Genotyping and Increased Breeding Oversight on Efficiency of Overall Mouse Breeding Colony Management

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Mice have become increasingly popular as genetic tools, facilitated by the production of advanced genetically engineered mouse models (GEMMs). GEMMs often require in-house breeding and production by research groups, which can be quite complex depending on the design of the GEMM. Identification of methods to increase the efficiency of breeding practices offer opportunities to optimize and reduce the number of animals bred for research while maintaining similar research output. We investigated the use of commercial automated genotyping and centralized breeding management on overall breeding colony productivity in a colony of multiple GEMM lines. This study involved a 3-group study design, where the first group continued their standard breeding practices (group A), the second used standard breeding practices but outsourced genotyping in place of inhouse genotyping (group B), and a third group outsourced genotyping and had assistance with routine breeding practices from the laboratory animal care team (group C). Compared to standard practice (group A), groups B and C produced more cages and mice over time, which appeared to be driven primarily by an increase in the number of breeding cages in each colony. Higher numbers of breeders correlated with an increased number of litters and generation of new cages. The increases in colony productivity measures were further enhanced in group C compared to group B. The overall cost associated with producing new animals was lowest in group B, followed by group A and C. Although, by the end of the study, cost to produce new mice was comparable between all 3 groups. These data suggest that by optimizing breeding practices and management, fewer animals could be used to produce the same amount of progeny and reduce overall animal usage and production.

PS42 The Effect of Noise, Vibration, and Light Disturbances from Daily Health Checks on Breeding Performance and Nest Building in Mice

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Animals must be assessed for health each day in order to ensure animal wellbeing and compliance with regulations. At our institution, there is variability in health check techniques for mouse cages housed on individually ventilated caging (IVC) racks, where some technicians tend to partially undock the cage or use an LED flashlight to improve visualization of the cage environment. These variations have a clear impact on the cage microenvironment, particularly on intracage noise, vibration, and light exposure. Changes in noise, vibration, and light exposure have previously been shown to affect multiple welfare and research-related parameters, including breeding performance, fecal corticosterone levels, cognition, and even immune function. We sought to assess the effect of partial cage undocking or LED flashlight use during daily health checks on fecundity and nest building scores in C57BL/6J mice in order to assess the least disturbing method of performing health checks for mice on IVC racks. Additionally, an accelerometer, microphone, and light meter were used to measure intracage noise, vibration, and light exposure in each group. Breeding pairs (n=100 pairs) were randomly assigned to 1 of 3 health check groups: partial undocking, LED flashlight, or control. We hypothesized that the mice exposed to a flashlight or cage undocking during daily health checks would have decreased fecundity and poorer nest building scores compared to the control mice. However, there was no statistically significant difference in fecundity or nest building scores between either experimental group when compared to the control group. These results indicate that a short duration, once daily exposure to partial cage undocking or an LED flashlight during daily healthy checks does not impact breeding performance or animal well-being, as measured by nest scores, in C57BL/6J mice.

PS43 Emergency Respiratory Device for Use in Rodents

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Respiratory distress and subsequent hypoxia is a side effect commonly seen in rodent anesthesia. In severe cases, this can lead to apnea and death. While both mechanical and manual positive pressure mask ventilators are commonly used in veterinary medicine for larger species, these devices are much less common in rodent species. Rodents are commonly used in research as well as being a prevalent companion animal, thus the need for an effective mobile ventilation device is warranted. Using inexpensive equipment commonly found in most veterinary practices, we created a device modeled after commercial bag ventilators. It can be attached to the end of a catheter tip being used with intubation or to a readymade mask to provide positive pressure ventilation and can be modified to work in both rats and mice based on the volume of air titrated. While this device was initially intended to be used during instances of respiratory distress during anesthesia, it can also be used during nonanesthetic related emergencies as well. This device was used during a study where rats were anesthetized and underwent intratracheal instillation of a test compound. Using this emergency device, we were able to successfully resuscitate 5 out of 6 rats that had succumb to respiratory arrest during this study. Postmortem necropsy and histology revealed no pathology of the lungs related to resuscitation. This device was also trialed on postmortem rats and mice to verify adequacy of forced air volume using set volume syringes. Adequate volume to inflate the lungs but did not cause barotrauma was noted. Overall, we were able to show that the addition of this user-friendly device for emergency resuscitation of rodents provides an easily made, quickly assembled, and cost-effective way to decrease animal deaths and improve clinical and research outcomes.

PS44 Evaluation of a Novel Scoring System to Assess Postoperative Pain in Rats

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Rat pain scoring presents a challenge in veterinary medicine as, being prey animals, rats tend to mask signs of pain and distress. The Rat Grimace Scale is a commonly used tool for evaluation of pain in rats. However, it is primarily based on still images and can be difficult to apply in real-time. Using a novel scoring system originally developed by the University of Finland, we evaluated 2 surgical models for evidence of postoperative pain, comparing it to our facility standard using the of dichotomous variables of "painful" versus "non-painful." The novel rat pain scoring system evaluated several variables, including posture, fur quality, movement/activity, orbital tightening, and fecal quality on a scale of 0-3, while ensuring minimal stimulation of the animals during evaluation. A total of 125 rats were evaluated, with 80 animals receiving an intrathecal or epidural dose of a test article, and 45 animals receiving a spinal contusion at the level of C5 or T7. All animals received buprenorphine SR perioperatively, therefore scoring was primarily used to identify insufficient analgesic or break-through pain. Scoring was performed twice daily starting the day prior to surgery and continued for 3 to 5 d postsurgery. All animals were noted to have a pre-operative score of 0. The highest score achieved during the intrathecal/epidural dosing was a total score of 1 and all animals were noted to be nonpainful. Total scores for the spinal contusion model reached a maximum value of 7. However, all of these animals were noted to be nonpainful using the traditional scoring system. These variations between the scoring systems support the need for a more refined scoring system. Additionally, it was noted that model type and strain of rat can impact scoring, and therefore these should be taken into consideration when using this novel pain scoring system.

PS45 Jacketed Telemetry in Rats: Noninvasive Method for Refinement of Cardiorespiratory Monitoring During Exercise

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Protocols involving physical exercise are used to study physiology and pathophysiology of major functions both in humans and animals. Refining the way exercise-induced variations in cardiorespiratory function are monitored is of one of the keys to achieve enhanced animal welfare and results' quality. The aim of this work was to evaluate the feasibility of cardiorespiratory monitoring using the novel DECRO telemetric jacket for rats during continuous treadmill exercise. For this purpose, the effects of an incremental exercise protocol on a training treadmill were monitored with a non-invasive telemetric jacket on an experimental group of sedentary rats (n = 9). This protocol consisted of a progressive increase of speed level (from 10 to 35 cm/sec max) every 2 min. Heart rate (HR), respiratory rate (RespR), and activity level (AL) were measured during a control state (Baseline) and during the last 30 s of each speed levels. All animals were successfully dressed with the jacket and were placed in their cage. After 25 min of baseline, HR was 392±36 bpm, RespR was 173±53 brpm, and AL was 15±12 mg. All animals managed to run with the jacket until 35 cm/s speed. The monitoring tool measured a significant overall physiological increase in HR (+26%, 104±21 bpm, $P < 0,01$) and RespR (+73%, 126±22 bpm, $P < 0,01$) induced by exercise at 35 cm/s. In conclusion, this work provides evidence that this novel telemetry jacket can be used to monitor cardiorespiratory parameters adaptation during a standard forced exercise protocol in a noninvasive manner. Such an alternative to the current tools (implanted telemetry, metabolic chamber) could be used to refine exercise protocols requiring monitoring and offers new perspectives to study cardiac and respiratory changes in various pathological models.

PS46 Survival Cerebrospinal Fluid (CSF) Collection: A Novel Method for Serial Collection of Cerebrospinal Fluid from Rats

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The blood brain barrier (BBB) is a specialized cellular barrier critical to controlling the passage of substances into the cerebrospinal fluid (CSF), which protects the brain against circulating toxins and pathogens. Many central nervous system drug discovery programs require the successful collection of clean CSF samples to assess exposure levels because of penetration and distribution of new chemical entities through the BBB. Rodents are the most frequently used animal model for these studies. However, collection of clean CSF samples (void of blood) has historically required terminal surgery (sampling) under anesthesia, sacrificing animals to collect a single sample. Furthermore, since only 1 sample could be collected per animal, the robustness of the data was rate-limiting and warranted a 3Rs alternative approach. Our goal was to refine rat CSF collection by reducing the number of animals used and improving the quality of the samples collected in a conscious animal. We evaluated a novel indwelling Cisterna Magna (CM) catheter for ease of use, duration of use, impact on animal health, and quality of samples. This novel CM catheter reliably enabled repeated collections of CSF from a single animal for the duration of our study. Three

animals were used in this validation. We were able to collect clean samples from each animal for up to 2 wk post implantation. In addition, we were able to pair the catheter with a specialized collection device to tightly control the exact volume of CSF collected, protecting the animal's health while eliminating any samples containing blood.

PS47 Prevalence and Global Distribution of the Murine Bacterial Pathogen *Chlamydia muridarum*

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Following the recent and unexpected detection of *Chlamydia muridarum* (*Cm*) in mice from 2 distinct genetically engineered mouse (GEM) colonies, a multifaceted investigation was undertaken to investigate the institutional and global prevalence and distribution of the organism. A *Cm*-specific PCR assay was developed and testing subsequently implemented for resident colonies in 8 vivaria from 3 academic institutions, 58 imported mouse shipments from 39 academic institutions, and mice received from 55 breeding colonies from 4 commercial vendors. Additionally, to estimate *Cm*'s global prevalence in laboratory mouse colonies, a database containing 11,387 metagenomic fecal microbiota samples from 120 institutions and a cohort of 900 diagnostic samples from 96 institutions were examined. Results indicate significant prevalence amongst academic institutions with *Cm* detected in soiled bedding sentinels from 62.9% of 97 animal holding rooms from the 3 institutions; 32.7% of the incoming mouse shipments; 14.2% of the institutions submitting microbiota samples; and 16.2% of the diagnostic sample cohort. All samples from commercial breeding colonies were negative. The considerable prevalence of *Cm* is likely attributed to widespread global interinstitutional distribution of unique mouse strains and failure to recognize that some of these mice were from enzootically infected colonies.

PS48 Pneumonia Associated with *Chlamydia muridarum* Infection in NSG Mice

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Chlamydia muridarum (*Cm*), the only natural chlamydial pathogen of mice identified to date, is used to model human *C. trachomatis* infection. Despite experimental use, *Cm* has not been isolated from laboratory mice since its initial discovery. We recently determined that *Cm* is moderately prevalent in academic mouse colonies. Naïve NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG) mice were cohoused with *Cm* shedding mice (n = 2γ) and/or their soiled bedding (n = 5γ; 4γ) to investigate *Cm* transmission and pathology. All mice developed severe clinical disease (lethargy, hunched posture, unkempt haircoats, dyspnea, and weight loss) 21-35 d after initial exposure. These mice were subject to complete necropsies, complete blood counts, and aerobic/anaerobic lung cultures. Histiocytic and neutrophilic bronchointerstitial pneumonia was detected in all mice. Chlamydia inclusions were associated with regions of bronchiolar and alveolar inflammation, detected by immunohistochemistry (IHC) targeting of Chlamydia's major outer membrane (MOMP), and hybridized with a *Cm* reference sequence probe. IHC was conducted in a larger tissue set in 2 mice; MOMP was detected in duodenal, jejunal, ileal, cecal, colonic, tracheal, and nasopharyngeal epithelium in both mice and nasal epithelium of 1 mouse. Positive ISH staining was observed in small intestinal, cecal, colonic, and tracheal epithelium of both mice. Formalin fixed paraffin embedded lung scrolls were *Cm* PCR positive in both mice. Additional microscopic findings associated with *Cm* included colonization of small/large intestinal surface epithelium (11 of 11 mice), neutrophilic rhinitis (2 of 11 mice), neutrophilic tracheitis with intraepithelial inclusions (2 of 11 mice), and vaginitis/endometritis/salpingitis with intraepithelial inclusions (1 of 7 female mice). All mice had moderate neutrophilia with mild monocytosis (8 of 11) and polycythemia (5 of 11) observed in select mice. *Cm* was isolated from lungs, cecum, and feces from an NSG used as a contact sentinel. It is well documented in the literature that experimental *Cm* infection results in a robust immune response (myeloid and Th1-type T-cell responses in immunocompetent mice) and clinical disease/pathology (select immunocompromised mice). *Cm* infection can confound experimental results and should be excluded/eradicated from enzootically infected laboratory mouse colonies.

PS49 Effectiveness of Various Antibiotics for Treating *Chlamydia muridarum*-infected Mice

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Chlamydia muridarum (*Cm*), a gram-negative intracellular bacterium, causes subclinical respiratory disease in immunocompetent mice and moderate to severe pneumonia in immunocompromised mice. We recently reported that *Cm* is moderately prevalent (~15 %) in contemporary academic mouse colonies. As *Cm* effects both innate and adaptive immune responses, it can confound research, and therefore may need to be eradicated from infected colonies. Treatment could be challenging as the bacterium can form persistent state aberrant bodies in response to environmental stressors, including antibiotics. Tetracyclines are the first-line drugs for treating chlamydial infections in many species. Macrolides, fluoroquinolones, penicillin, and sulfonamides are also used. Treatment of *Cm* in mice has been limited. It is necessary to identify a *Cm* treatment protocol to treat large mouse colonies that is easily administered, effective, and inexpensive, while minimizing confounding effects on the experimental model. We treated *Cm* infected wild-type and genetically engineered mice for 7 or 14 d with 0.008% doxycycline in water; 0.025% enrofloxacin in water; 0.08% sulfamethoxazole and 0.016% trimethoprim in water or feed impregnated with either 625 PPM doxycycline, or 0.124% sulfamethoxazole and 0.025% trimethoprim, or 0.12 % amoxicillin. NSG mice were used as contact sentinels with *Cm* negative posttreatment mice to evaluate efficacy as they are exquisitely sensitive to infection. Mice treated with all antibiotic protocols, except enrofloxacin, were negative by PCR for up to 58 d posttreatment. All NSG cohoused mice remained *Cm* negative and disease free. Only enrofloxacin failed to eradicate *Cm*. These results provide insight into effective treatment strategies for *Cm* and a framework for a future more extensive study to identify the most effective, least confounding treatment protocol for eradication of *Cm*.

PS50 Mouse Papillomavirus 1 Outbreak in a Rodent Facility: What We Know, What We Are Learning, and Questions Left Unanswered

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Papillomaviruses are environmentally stable, species-specific, nonenveloped, DNA oncoviruses. Mouse papillomavirus 1 (MmuPV1), initially described in athymic nude mice in an Indian laboratory animal facility, is a valuable model for human papillomaviruses. However, the natural course of MmuPV1 infection, strain susceptibility genetics, transmissibility, and infection kinetics remain unclear. Here, we describe a MmuPV1 outbreak in a 10,000-cage, IVC rack system facility (room capacities:~200-1000 cages) with historic MmuPV1 research restricted to an ABSL-2 room. Papillomatous lesions developed on muzzles and tails of nude, but not other, mice housed distant from the ABSL-2 room. Histopathologic lesions were consistent with viral papillomas, demonstrated malignant progression, expressed MmuPV1 E6, E7, and L1 RNA, and stained positive for capsid protein. Whole genome PCR and sequencing showed an identical viral genome to the originally used MmuPV1. Additional symptomatic nude mice 2 mo later supported within room transmission. Whole-facility screening revealed another room housing MmuPV1-infected asymptomatic animals. Containment/mitigation measures included room quarantine, testing and culling, depopulation, and disinfection (combined vaporized hydrogen peroxide, 180 °F washing, and autoclaving). Disinfection was insufficient to eliminate DNA from racks. Adding 1-3 cycles of 10% bleach (30 min contact) and rinsing removed residual DNA. Infectivity of the residual material remains unclear. Current efforts include repopulation of the originally identified rooms following disinfection, rederivation and/or culling of positive animals from other rooms, and active surveillance for new outbreaks. We recently identified, early during disease course, an additional room outbreak through the use of in-house developed MmuPV1 PCR as part of our active surveillance. The outbreak has so far spanned 13 mo with up to 20% of mice PCR+ in each identified room with additional animals euthanized to control virus spread. Caution is recommended for institutions considering proposed live MmuPV1 research as clinical signs are limited to nude mice, commercial testing is limited, and escape under ABSL-2 conditions is possible.

PS51 Investigation of a Diarrhea Outbreak in Nsg and Related Immunodeficient Strains

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We investigated an outbreak of diarrhea in immunodeficient mouse strains housed in a barrier facility. Clinical signs included intermittent watery diarrhea or soft stool, resulting in bedding-coated feces or fecal smearing on the cage walls. Lactating dams were prone to acute deaths during mid-lactation. Affected strains were characterized by the presence of either SCID (Prkdcscid) or RAG null (Ragnull) mutations and the common gamma chain null (IL2rgnull) mutation, e.g., NSG, NRG, FRG and B6 or BALB double knockouts. Serology of dirty bedding (n=14) and contact (n=5) sentinels, fecal PCR (n=9) and aerobic/anaerobic cultures (n=8) of diarrheic mice failed to identify a pathogen. ELISAs for *C. perfringens* (γ, γ, γ) and *C. difficile* (A, B) toxins were negative. Histologically there was extensive superficial epithelial apoptosis and luminal sloughing of the cecum and colon with mild to severe regenerative hyperplasia. Ulcerative enteritis was unique to moribund lactating females. Naïve NSG mice (n=4) co-housed with diarrheic NSG mice developed diarrhea demonstrating that the diarrhea was infectious. However, CD-1 mice (n=5) co-housed with diarrheic NSG did not develop diarrhea. Naïve NSG mice co-housed with exposed CD-1 mice did not develop diarrhea suggesting that CD1 mice are unlikely to serve as a reservoir. NSG mice (n=3) gavaged with intestinal homogenates from diarrheic mice developed diarrhea; NSG mice gavaged with intestinal homogenates from normal mice did not. If the intestinal homogenate was filtered through a 0.2 um filter, the NSG mice (n=3) did not develop diarrhea suggesting that the pathogen was filterable. NOD SCID (NOD.Cg-Prkdcscid/J, n=3) and RAG KO (B6.Cg-Rag2tm1.1Cgn/J, n=3) mice gavaged with intestinal homogenates from diarrheic mice did not develop diarrhea. Gavage of B6 DKO (C57BL/6NTac.Cg-Rag2tm1Fwa Il2rgtm1Wjl, n=3) developed slight diarrhea. Naïve NSG mice (n=2) co-housed with the gavaged NOD. SCID and RAG KO mice did not develop diarrhea. In contrast, NSG (n=2) co-housed with B6 DKO mice developed severe diarrhea. Microbiome analysis revealed an increase in a Bacteroidales bacterium in diarrheic mice but not in nondiarrheic control mice in both the cohousing and gavage experiments. However, the species differed between the 2 experiments. Microbiome analysis of the gavaged mice 25d after the initial analysis showed a decrease in the Bacteroidales bacterium. The results suggest that the increase in the Bacteroidales bacterium is a consequence of the diarrhea and not the cause. We conclude that this unidentified, non-viral, diarrhea pathogen is infectious and that only SCID (Prkdcscid) or Ragnull mice with the common gamma chain knockout (IL2rgnull) are susceptible. Other immunocompetent or immunodeficient strains are probably not susceptible and are unlikely to serve as reservoirs for the diarrhea.

PS52 Generalized Septicemia Caused by *Elizabethkingia miricola* in a Laboratory Colony of African Dwarf Frogs (*Hymenochirus boettgeri*)

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An outbreak of morbidity and mortality in African Dwarf frogs (*Hymenochirus boettgeri*) was reported following arrival at our animal facility. Animals were found dead on arrival or became moribund shortly thereafter and over the following 3 wk. Grossly, some affected animals showed multifocal hyperemia in the inguinal and axillary areas and on the limbs, and a majority presented with mottled tan discoloration along the ventral abdomen. Histologically, lesions were consistent with generalized septicemia, characterized by a granulomatous meningoencephalitis, otitis media, peritonitis, myocarditis and pericarditis, nephritis, pneumonia, and arthritis. Gram staining identified gram-negative rod-shaped bacteria free within tissues and within macrophages. Results of coelomic culture identified moderate to numerous *Elizabethkingia miricola*, and water testing of various affected tanks showed elevated levels of nitrites and ammonia with *Citrobacter*, *Aeromonas*, *Pseudomonas*, and *Staphylococcus* spp. cultured from several tank biofilters. *Elizabethkingia miricola* is a newly recognized and rapidly emerging opportunistic pathogen in anurans, and has been reported as a cause of septicemia in humans. This report documents the first occurrence of *E. miricola* septicemia in African Dwarf frogs and illustrates the importance of recognition of this potential pathogen in the laboratory setting for amphibian research colonies, as well as those individuals directly working with them.

PS53 Novel Hepatitis: A Variant in Cynomolgus Macaques (*Macaca fascicularis*)

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A group of 43 Mauritian cynomolgus macaques *(Macaca fascicularis*) arrived at the facility for institutional quarantine in apparent good health. On quarantine clinical pathology, 15 animals had moderate-to-severe elevations in liver enzymes without clinical signs (77-687 IU/L ALT, 51-233 IU/L AST). Clinical pathology was repeated in the cohort 2 wk later, which indicated resolution of liver enzymes in all 15 original animals, but novel elevations in 9 animals (78-1007 IU/L ALT, 71-120 IU/L AST). Macaque serology panel (Herpes B, SIV, SRV, STLV, measles), TB skin tests, and fecal parasitology were all negative except for seropositivity in some animals vaccinated for measles. Some fecal samples
cultured *Yersinia* sp. and *Campylobacter* sp. without clinical signs. Given the transient elevations in liver enzymes, additional diagnostics were pursued to rule out differentials associated with self-limiting hepatitis in macaques. All macaques were seropositive for cytomegalovirus and were negative for *Mycobacterium tuberculosis* on fecal PCR. All 43 animals were seropositive for Hepatitis A IgG antibodies and 7 for IgM, indicating recent and/or ongoing infection. Pooled fecal samples were submitted to a commercial lab for Hepatitis A PCR. The results were not positive enough by the lab's assay standards to make a definitive identification of the viral pathogen. However, several samples generated an amplicon which was sequenced and suggested a novel Hepatitis A variant was being shed in the feces. A shipment of 27 animals from the same vendor arrived 5 wk later. Only 2 animals presented with liver enzyme elevations on clinical pathology. All animals were positive for Hepatitis IgG, 2 of which were also positive for IgM. Considering the serology results and fecal PCR amplicon sequencing, the transient liver enzyme elevations observed during quarantine were most likely the result of an active Hepatitis A infection. We give an overview of Hepatitis A in nonhuman primates, biocontainment best practices, and considerations for research study assignment following transient hepatitis.

PS54 Vasculitis Presentation for a Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Infection

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A 32-d-old male Yorkshire-cross swine presented with erythematous ear margins 4 d after arrival. No procedures had been performed and the animal was otherwise clinically within normal limits. Rule outs for ear margin erythema include trauma, contact dermatitis, bacterial (*Pasteurella multocida*, *Streptococcus suis*, *Haemophilus parasuis*, *Erysipelothrix rhusiopathiae*), or viral infection (PCV2 and PRRSV). The animal was segregated to prevent nose to nose contact. A presumptive diagnosis of vasculitis was assigned and a broad spectrum antibiotic was started (ceftiofur CFA). Twenty-four hours later, approximately 95% of the ear pinnae had become purple. At 48 h, the distal aspect of the tail also developed erythema, and the ear pinnae became pruritic with cutaneous scaling. The pig remained active and appetent. However, given the presumptive vasculitis diagnosis, with clinical disease progression, the animal was considered unsuitable for research and was euthanized. Gross necropsy abnormalities consisted of generalized lymphadenopathy (+/- hemorrhage), and mottled red/dark red lungs that failed to fully collapse, with expanded, prominent interlobular septae. Hematologic abnormalities consisted of mild anemia (RBC=4.47 x10^6/µL; 4.87-7.88) and lymphopenia (3.47 x10^6/µL; 4.02-12.5). Serum chemistry abnormalities were limited to hypoproteinemia (5.4 g/dL; 7.0-8.9). Viral PCR was negative for porcine circovirus 2 (PCV2) and positive for PRRSV-2. Microscopic findings confirmed necrotizing vasculitis in ears, tail, and brain, with lymphoid depletion and necrosis in the tonsils and multiple lymph nodes and also inflammation in the heart, liver, and lungs. PRRSV virulence can be highly variable. While PRRSV is well known for its effects on lymphoid, reproductive, and respiratory systems, it can also present as generalized inflammation involving multiple organ systems and thereby affecting a broad variety of research models. Observation of cutaneous lesions consistent with vasculitis should raise concern for organ involvement. Euthanasia and diagnosis are warranted for cases of presumptive vasculitis.

PS55 Porcine Circovirus 2 Infection with Secondary Hypersensitivity and Suspected Bacterial Septicemia in a Laboratory Swine

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An experimentally naive 4-mo-old, 52 kg, intact female Yorkshire-Landrace cross was reported for loose stool. Empirical treatment with bismuth (10 mg/kg PO x 5d) was initiated, with a corresponding improvement in clinical signs. Four days after the initial presentation, the animal developed a rash on the caudal aspect of the hind legs. On physical examination, the rash consisted of papules on the caudal aspect of the rumps and hocks; the animal was also slightly lethargic and pyrexic (104.4 F). Differential diagnoses at this time included porcine dermatitis and nephropathy syndrome (PDNS), swinepox, and contact dermatitis. The rash spread to the lateral thighs and worsened over the course of 3 d, with a resolution of fever and lethargy following meloxicam administration (7.5 mg PO once). The rash gradually receded, but was still present as mild scabs at the time of planned research euthanasia 11 d post-rash appearance. A diagnostic necropsy was performed. Gross examination revealed multifocal scabs on the caudal rump, lateral, and medial hocks; pale kidneys, with the right having pinpoint mottling of the cortex; adhesions of the parietal pleura; thickened ileum; enlarged lymph nodes. Histopathology revealed dermatitis, enteritis, tubulointerstitial nephritis, perivascular inflammation, pleuritis, and epicarditis. Porcine circovirus 2 (PCV2) was also detected at significant levels in the spleen (ct 19.3). This gilt was negative for both African swine fever (ASF) and classical swine fever (CSF), with no clinically significant organisms cultured on bacterial assessment. Heart and lung lesions were determined to be chronic and consistent with a previously resolved pneumonia and pericarditis. Multiple inflammatory processes suggest an allergic hypersensitivity reaction to the bedding (skin and enteric lesions) and bacterial septicemia (spleen and kidney lesions) as the main differentials, although no specific pathogens were isolated. This gilt was vaccinated against PCV2 and while histologic lesions were not consistent with PDNS, PCV2 is suspected to be a possible contributing factor for the development of these clinical signs.

PS56 Pharmacokinetics of Sustained-release, Intravenous, and Oral Meloxicam in Yucatan Minipigs (*Sus scrofa domestica*)

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Swine are a popular animal model in biomedical research due to their

physiologic similarity to humans and are used for surgical training and as models for dermatology, xenotransplantation, and cardiovascular research. Such studies often include painful procedures, and the NSAID meloxicam is frequently used as part of peri- and postprocedural analgesia protocols, typically via repeated subcutaneous (SC) or oral (PO) dosing. The sustained release formulation of meloxicam (MSR) represents a potential refinement of a single subcutaneous injection providing analgesia for multiple days, minimizing additional distress and discomfort to swine from repeated handling and dosing. However, to our knowledge, there are no published pharmacokinetic (PK) data validating the duration of MSR plasma concentrations in swine. The purpose of this study is to compare PK data for IV, PO, and sustained release meloxicam formulations in Yucatan minipigs. Six Yucatan minipigs, 3 male and 3 female, are used in a crossover study wherein each animal receives all 3 meloxicam formulations in series, IV, MSR, and PO, separated by 5-d minimum washout periods. Serial plasma samples collected from awake pigs following each meloxicam administration are analyzed via HPLC. Plasma collection timepoints for each formulation are: IV– 0m, 2m, 5m, 10m, 20m, 30m, 1h, 2h, 4h, 6h, 8h, 12h, 24h, 32h, 48h; MSR– 0m, 30m, 1h, 2h, 4h, 6h, 8h, 12h, 24h, 32h, 48h, 54h, 72h, 80h, 96h, 120h, 144h, 168h; PO is given daily for 5 d to reach steady state with sampling at 0m, 15m, 30m, 1h, 1h, 30m, 2h, 4h, 6h, 8h, 12h, 24h, 32h, 48h, 54h, 72h, 75h, 80h, 96h, the last PO dose is given and the PK curve begins at 120h, 120h 15m, 120h 30m, 121h, 121h 30m, 122h, 124h, 126h, 128h, 132h, 144h, 152h, 168h. Preliminary data indicate that the manufacturer recommended dose of MSR (1.2 mg/kg) yields a higher 1-hr plasma concentration than either IV or PO formulations dosed at 0.4 mg/kg (MSR 3,536 ng/ml, IV 2,897 ng/ml, PO 851 ng/ml), and that MSR plasma concentrations decline more slowly over 8 hr (MSR 1,803 ng/ml, IV 844 ng/ml, PO 188 ng/ml). Moreover, the 8-hr MSR plasma concentration was more than 4-fold greater than presumed therapeutic levels (> 400 ng/ml), implying analgesia is provided over an extended period of time.

PS57 Metabolic Cage Analysis of Surgically Catheterized C57Bl/6J Mice (*Mus musculus*) Treated with Carprofen and Sustained-release Buprenorphine

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Regulations require that appropriate analgesia be provided for animals for proper pain control. Common analgesics used in mice are carprofen (C) and sustained-release buprenorphine (SRB). However, given the potential gastrointestinal side effects these analgesics have in various species, the impact of these analgesics on mice used in metabolic studies is concerning. To investigate how C and SRB can affect food consumption and locomotor activity, we anesthetized and surgically catheterized the jugular vein and carotid artery in C57Bl/6J mice (n = 6 male/group, n = 10–11 females/group) and assigned them to the following groups: 1. Sham surgery and C only (Sham+C), 2. Catheterization and C and SRB(Sx+C+SRB) and 3. Catheterization and C only (Sx+C). The mice were placed in instrumented metabolic cages where continuous measurements of food and water consumption and locomotor activity were automatically collected before and after the surgery. Postoperative signs of pain based on coat quality, eye appearance, coordination and posture, and overall condition (0 = normal, 13 = significant pain) were assessed at 6 h and daily for 3 d. We hypothesized that, whereas Sx+C mice would have similar food consumption and locomotor activity as Sham+C mice, Sx+C+SRB mice would have decreased food consumption and increased locomotor activity compared to Sham+C mice. Our results demonstrate that although food consumption did not differ between the groups, during the initial 14-h postoperative period Sx+C+SRB mice traveled a significantly greater distance than Sham+C and Sx+C mice, and the Sx+C mice exhibited significantly lower movement over the entire 3-d postoperative period ($P < 0.05$). Pain scores were highest 6 h postoperatively in the Sx+C group, followed by Sx+C+SRB and Sham+C groups ($P < 0.05$). Interestingly, by postoperative day 2, pain scores were highest in the Sx+C+SRB group. Taken together, our results suggest that treatment with SRB causes a transient hyperactivity without compromising food consumption, whereas carprofen alone most likely does not alleviate immediate postoperative pain. In conclusion, researchers should carefully consider the effects that analgesic drugs can have on mice when designing metabolic or behavioral experiments.

PS58 Opioid-induced Hyperactivity in Surgically Catheterized C57Bl/6J Mice

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For rodent postoperative pain management, long-lasting buprenorphine is favorable compared to the standard buprenorphine formulations because a single subcutaneous injection can provide up to 48 h of analgesia. The 2 long-lasting buprenorphine formulations that are currently available for mice are sustained- and extended-release buprenorphine. Although sustained-release buprenorphine (SRB) use in mice is considered extra-label, extended-release buprenorphine (XRB) has recently been FDA-indexed. However, our previous study comparing the pharmacokinetic parameters of surgically catheterized SRB- or XRB-treated mice revealed XRB-treated mice had a 3- to 4-fold increase in buprenorphine plasma concentrations (SRB C_{max} = 3.76 ng/mL vs. XRB C_{max} = 13.52) and anecdotal reports of hyperactivity. Several studies have reported that opioid-treated mice can have decreased food consumption and increased hyperactivity. Thus, to investigate differences in food consumption and hyperactivity between SRB- and XRB-treated mice, we placed surgically catheterized 13- to 15-wk-old male (n = 15) and female (n = 20) C57Bl/6J mice treated with either SRB (1 mg/kg, SC, once) or XRB (3.25 mg/kg, SC, once) in instrumented metabolic cages before and after surgery and assessed cage-side clinical signs of pain. We hypothesized that mice treated with XRB would have increased activity levels and decreased food consumption postoperatively, compared to SRB-treated mice. Although cage-side observed clinical signs of pain and food consumption were similar between groups, XRB-treated mice had a significant increase in activity levels during the postoperative period (SRB 133.25 ± 4.9 m/h vs. XRB 210.09 ± 35.84 m/h, $P < 0.05$). Hyperactivity was most prominent during the initial 12-h light-phase postoperative period in XRB-treated mice suggesting that higher plasma buprenorphine concentrations result in an opioid-induced hyperactivity. Together these results demonstrate that despite XRB-treated mice having higher buprenorphine plasma levels and transient postoperative hyperactivity, XRB provides adequate analgesia that does not inhibit normal feeding behavior or induce clinical signs of pain. Thus, we recommend the use of either SRB or XRB to alleviate postoperative pain.

PS59 Assessment of Rodent Activity as a Measurement of Efficacy of Postoperative Analgesics and Animal Well-being Using a Novel Device, the Rodent Fitbit

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Accurate postoperative pain assessment and management is crucial for good animal welfare and science. We sought to evaluate the efficacy of postoperative pain management in laboratory mice objectively by using a novel method to continuously monitor activity in the home-cage environment. To determine if activity levels are impacted by analgesics, we evaluated the activity levels in mice provided Ethiqa XR (3.25 mg/kg), Meloxicam SR (6 mg/kg), combined Ethiqa XR/Meloxicam SR and topical Bupivacaine block compared to mice provided sterile saline (5 ml/kg) subcutaneously. C57BL/6 male mice (n = 6) were implanted with Rodent Fitbit devices near the nape supracutaneously with 3-0 PDS suture, and activity levels were measured for 3 d prior (baseline) and 4 d after analgesic administration. Activity levels increased with mice provided Ethiqa XR/Meloxicam SR compared to saline controls on N0. To determine the effective analgesic period based on stimulus-evoked nociception with electric Von Frey filaments and nonstimulus evoked nociception with Rodent Fitbits, we performed right hind paw incision model under isoflurane, to assess the effectiveness of the analgesics of interest. C57BL/6 male mice (n = 6) were implanted with the Rodent Fitbit device and activity levels, in addition to Von Frey and Grimace scales, were measured for 3 d prior to preoperative analgesic administration and the right hind paw incision of the flexor digitorum brevis muscle (baseline). Activity levels, Von Frey, and Grimace scales were also observed over 5 d postoperatively to assess postoperative pain within surgical mice and the effectiveness of the analgesic treatment. Preoperative analgesic usage of Ethiqa XR/Meloxicam SR and Ethiqa XR was shown to reduce pain induced by a paw incision pain model and demonstrated less activity alteration compared to previously mentioned treatment groups. In summary, measuring activity levels appears to be an effective indicator for detecting pain and verifying the efficacy of analgesics in mice.

PS60 Comparison of Thermal and Mechanical Nociceptive Testing in Naïve Sprague–Dawley and Fischer 344 Rats

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Rodents have been used extensively for studying pain. Multiple validated methods of pain testing are utilized in animals. Surprisingly, there is no direct comparison of thermal and mechanical pain testing methods in experimentally naïve rats of different genetic backgrounds. Understanding this variability can help better select animal models and pain testing methods for studying analgesics. We hypothesize that Sprague–Dawley (SD) rats will have greater variability in nociceptive responses compared to Fischer 344 (F344) rats. Published data suggest that SD rats have greater variability; however, no direct statistical comparisons have been made. In the present study, nociceptive testing was performed in experimentally naïve SD and F344 rats. Both male and female, 10- to 14-wk-old F344 and SD rats were used to measure nociceptive responses in 3 different paradigms: the Hargreaves test, Tail Flick test, and Randall-Selitto test. Hargreaves and Tail Flick tests utilized an electronically controlled light source to generate a noxious thermal stimulus to the left hind paw or tail tip; the latency to withdrawal of the limb or tail, in seconds, was recorded. The Randall-Selitto test was performed by using an operator-controlled, handheld instrument to generate a noxious pressure stimulus to the left hind paw; force to withdrawal of the limb, in grams, was recorded. Five rats of each sex and genetic background underwent one type of test, which was performed on day 0 and day 7. The Randall-Selitto test had a statistically lower coefficient variation compared to the Hargreaves Test, with no statistical differences observed within each test due to sex, genetic background, or testing day for all 3 tests. Nociceptive responses for the 3 tests were only statistically different at day 0 for the Randall-Selitto test between male and female F344 rats. The current findings show that the Randall-Selitto test has the least variation, and sex and genetic background do not alter variation for nociception via the Randall-Selitto, Hargreaves, and Tail Flick tests in rats. Additionally, a 1-wk washout period was sufficient to generate repeatable nociception results.

PS61 Physiologic and Behavioral Effects in Laboratory Mice Anesthetized with Isoflurane Using a Red-tinted Chamber Compared to a Traditional Translucent Chamber

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Isoflurane is characterized as a distressful agent for rodents, with reported behavioral and physiologic effects. Utilizing a 'darkened home cage' has been recommended during CO_2 administration for rodent euthanasia, which is arguably a similar animal experience to anesthetic induction with isoflurane. Based on the premise that rodents perceive red light as darkness, we compared physiologic and behavioral markers of stress in 2 inbred strains of mice (C57BL/6J and BALB/cJ; 24 males and 24 females per strain) anesthetized with isoflurane in either a red-tinted (dark) induction chamber or a traditional (clear) induction chamber. Physiologic stress was assessed using plasma levels of norepinephrine, epinephrine, and corticosterone. Stress-related behaviors (that is, rearing, face wiping, and jumping) were recorded on video and scored from initiation of induction to loss of consciousness. Compared to the traditional translucent chamber, we hypothesized that mice anesthetized in the red-tinted chamber would exhibit decreased markers of physiologic stress and fewer stress-related behaviors. Due to differences in retinal pigmentation and visual capacity, we further hypothesized this effect would be more pronounced in C57BL/6J mice compared to BALB/cJ mice. Ultimately, no significant correlations were found between chamber type and physiologic stress hormones in either strain. Further contradicting our hypothesis, stress-related behaviors were observed most frequently in mice anesthetized in the red-tinted chamber, including 1) significantly higher rearing frequencies in BALB/cJ mice; 2) higher behavioral stress scores in BALB/cJ and male C57BL/6J mice; and 3) increased face wiping behavior when considering all mice combined. These findings suggest that laboratory mice do not experience significant reductions in physiologic measures of stress and, particularly albino strains, may actually display increased expression of stress-related behaviors when anesthetized in a red-tinted induction chamber. Based on our findings and a growing body of literature on the unintended effects of red light, we do not recommend red-tinted chambers for anesthetic induction in laboratory mice.

PS62 Acute Effects of Anesthesia on Ultrasonic Vocalizations and Neuroendocrine Responses in Neonatal Rats

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Minimization of potential pain and distress of rodents being anesthetized is a touchstone of veterinary clinical medicine, codified in the AVMA Veterinarian's Oath. While significant resources have been allocated to the evaluation of this in post-weanling rodents, minimal information is available regarding the humaneness of anesthesia and anesthetic overdose euthanasia in neonatal rodents. Hypothermia is the most reported anesthetic protocol for neonatal rodents less than 10 d old, however, there are risks associated with hypothermia including pain on warming. Traditional behavioral and physiological outcome measures used for adult rodents undergoing anesthesia are not suitable for evaluating neonates. We investigated the effects that different anesthetic methods have on the well-being of neonatal rats measured by ultrasonic vocalizations (USVs) and norepinephrine (NE) responses. We hypothesized that USVs and NE responses would be strong indicators of distress in neonatal rats undergoing anesthesia and that neonatal rats undergoing hypothermia anesthesia would experience more distress than those undergoing inhalational anesthesia. Male and female Sprague–Dawley rats (n = 540) at postnatal (PN) days 2, 5, 8, 11, and 14 were randomly assigned to 1 of 7 experimental groups: hypothermia (PN 2, 5, & 8 only), isoflurane vaporizer (induction: 5%, maintenance: 1–2.5%), isoflurane drop method, sevoflurane vaporizer (induction: 8%, maintenance: 3–5%), sevoflurane drop method, baseline control, and no anesthesia, each with at least n = 14. Animals underwent USV collection prior to anesthesia and at 10- and 120-min postanesthetic recovery. At 120-min postrecovery, animals were decapitated, and blood was collected for NE analysis. Overall, time to surgical plane of anesthesia and time to recovery from anesthesia were significantly increased in hypothermia groups compared to other groups (P < 0.0001). For USVs, there were significant differences in the total number (PN 2 & 8) and duration (PN 8) of calls at both postrecovery timepoints between hypothermia groups and other groups. These findings suggest that inhalational anesthetic methods are preferred for anesthesia of neonates when compared to hypothermia.

PS63 Behavioral evaluation of Laboratory-housed Ferrets (*Mustela putorius furo*) in Different Cage Sizes

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The domestic ferret is a common animal research model for infectious disease and behavioral studies. Ferrets are social animals that are commonly pair housed. Regulatory standards for minimum housing space exist for common laboratory animal species but not for ferrets; minimum or optimum cage dimensions have also not been investigated in ferrets. Additionally, cage sizes reported in the literature are highly variable. Optimum space is an important animal welfare consideration, given that smaller cage sizes have been linked to increased incidence of stress-related or boredom-related behaviors in some laboratory animal species. Here, we performed a crossover study to evaluate 2 different cage sizes (single: 18 × 31 × 31 in. [45.7 × 78.7 × 78.7 cm] and double: 18 × 31 × 62 in. [45.7 × 78.7 × 157.5 cm]) for pair-housed ferrets (n = 12). In phase 1, 3 ferret pairs were housed in single cages and 3 pairs were housed in double cages, with all other variables kept constant. After 1-wk acclimation, continuous video recordings during 5 time periods per day were captured, 5 d per wk, to allow for later assessment of the ferrets' behavior. In phase 2, the pairs of ferrets were housed in the opposite cage size from phase 1 and, following 1-wk acclimation, were recorded for a 2-wk period. Recordings were assessed using a scoring system for positive, neutral, and negative behaviors (including play, aggression, boredom, and rest) to examine activity budgets and behavioral indicators of welfare by cage size. Cages were also divided into quadrants to score space utilization. Automated motion capture events over a 20-hr period per day, 5 d per wk, were also captured as a proxy for activity level. This study can provide valuable information needed to guide animal care and use programs regarding appropriate ferret housing to ensure animal welfare.***Data will be added to the abstract following analysis, per conversation with John Farrar.*

PS64 A Novel Scoring System for Humane Endpoints in Mouse Cecal Ligation and Puncture Sepsis

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Animals used in biomedical research to model sepsis often experience high mortality due to difficulty with the prediction of death based on premortem analysis and translating findings to human medicine. The use of death as an endpoint does not align with the practice of minimizing animal pain and distress. Visual scoring schemes would allow researchers to determine humane endpoints but require a high interobserver agreement for accurate results. The objective of this study was to establish a scoring system for septic mice following cecal ligation and puncture (CLP), based on 3 visual parameters with scores ranging from 0 to 3. In the first of this study's 2 components, we evaluated the interobserver agreement of the scoring system for CLP-septic mice. Observations of post-CLP mice conducted between users comprised 283 data points and demonstrated near-perfect agreement (greater than 0.81) by weighted Cohen's kappa statistic (0 to 1.0); respiratory parameter γ-score = 0.82, activity-stimulus γ-score = 0.90, and eyes γ-score = 0.81. The second component assessed the ability of the scoring system to predict mortality. C57BL/6J mice (n = 80, male and female) were monitored until death or up to 7-d post-CLP with the scoring system and subcutaneous temperature transponders. Results showed that the scoring system discriminates between surviving and nonsurviving CLP-septic mice, with significant differences between scores. The scoring system demonstrated accuracy in predicting mortality, with an AUC of 0.907 and high sensitivity and specificity for the activity-stimulus (sensitivity: 94.55%, specificity: 92.0%) and eyes (sensitivity: 94.44%, specificity: 73.08%) parameters. Subcutaneous temperature measurements represented a noninvasive quantitative predictor of mortality (sensitivity: 92.60%, specificity: 92.31%). Retrospective analysis of the observational score data indicated that death could accurately be predicted by cumulated activity and eye scores of 5 or greater. Our scoring system demonstrates generalizability to diverse animal users through the high interobserver agreement. Furthermore, this scoring system represents a refinement and can be implemented to replace death as an endpoint and determine humane endpoints for CLP-septic mice.

PS65 Efficacy of Mechanical Cage Washing to Remove Viral, Bacterial, and Protozoal Murine Pathogens

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Infectious agents have varying susceptibilities to thermal inactivation or mechanical removal from cages via heated, pressurized water. We

evaluated the ability of select agents (*Candidatus savagella* [SFB], mouse norovirus [MNV], *Tritrichomonas* sp., and *Entamoeba muris*) to survive the cage wash process and infect naïve animals. Agents were chosen due to their prevalence in rodent colonies, environmental stability, and/or their potential to influence experimental outcomes. Cages that had housed mice infected with all 4 organisms were assigned to 1 of 3 treatment groups: sanitization in a tunnel washer (82.2 °C [180 °F] final rinse for 20 s; $n = 40$); sanitization in a tunnel washer followed by autoclaving (121 °C for 20 min; $n = 40$); or control (bedding change only; $n = 40$). The presence of these agents in the cage was assessed by PCR performed on swabs collected from the empty soiled cage interior before and after treatment. Additionally, to determine if residual nucleic acid could produce infection, 2 Swiss outbred (J:ARC(S)) female mice were housed for 7 d in each cage after treatment. The procedures above were repeated so that each pair of J:ARC(S) mice ($n = 10$ pairs of mice/treatment group) were housed in a cage from the same treatment group for 4 consecutive, 1-wk-long periods. Swabs collected from soiled cages were PCR positive for SFB, MNV, *Tritrichomonas*, and *Entamoeba* in 99%, 39%, 63%, and 73% of the cages tested, respectively. Cages in the tunnel wash group that were positive for SFB, MNV, *Tritrichomonas*, and *Entamoeba* pretreatment remained positive after washing 8%, 0%, 43%, and 10% of the time, respectively. No cages from the autoclave group tested positive for any of the agents after treatment. No mice housed in cages in the autoclave or tunnel wash groups became infected with any of the agents, however, 70%, 50%, 0%, and 90% of the pairs of mice exposed to untreated cages were positive for SFB, MNV, *Tritrichomonas,* and *Entamoeba*, respectively. Our results suggest that nucleic acids from SFB and protozoal organisms may remain in cages after mechanical cage washing, however, the nucleic acids were not infectious or were below the minimal infectious dose.

PS66 Drivers and Benefits of European-style Pen Enclosures

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The housing environment for NHPs used in research plays a key role in determining their welfare. Use of European-style pen enclosures is increasing globally in response to changes in regulations, guidelines, and the expectations of research funders/sponsors, as well as growing awareness of their benefits for animal wellbeing and study outcomes. We introduce and illustrate the pen enclosures concept, present unpublished survey data on the prevalence of European-style pens, and summarize published studies that have found features of these enclosures to improve behavioral markers of welfare, including additional enriched space, group housing, functional vertical space, and use of floor substrate. It will also address common misconceptions and provide practical advice on this housing approach. Pen enclosures provide a greater quantity and quality of space than conventional cage units, allowing performance of a wider range of natural behaviors, greater opportunities for environmental enrichment, and socialization in species-appropriate groupings, all of which improve NHP welfare. In contrast, small, relatively unenriched cage environments can lead to behavioral and other abnormalities, which are possible confounds in experimental studies. Pen enclosures typically span the full height of the room, with a solid floor covered with substrate for hygiene and extended bouts of foraging behavior. The larger 3D space, with vertical barriers and multiple high-level perches, allows the animals to manage their social interactions more effectively and maintain group stability. Enclosure fronts constructed of horizontal, widely spaced bars, and the addition of verandas, increase useable space for the occupants, improve visibility into and out of the pen, and facilitate positive human-animal interactions. Experience with this housing system in academic and industry laboratories demonstrates that perceived problems such as ease of capture and cleaning can be solved with good enclosure design and staff training. Stocking density need not be reduced, and cleaning time can be less. Additional benefits include calmer, less aggressive, more cooperative animals, improved staff satisfaction, and greater confidence for openness with the public.

PS67 Space Use Considerations in Larger Housing Enclosures for Cynomolgus Macaques (*Macaca fascicularis*)

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Over the past several decades, many advancements have been made in the captive care of nonhuman primates to improve their quality of life. Housing considerations are a critically important area of welfare to evaluate, as this is where the primate spends its entire life. Taking into consideration the behavioral and physical needs of the animal should be key when making decisions about improvements to primate housing. As housing enclosures continue to evolve and look to incorporate more opportunities for the animal to express natural behavior, it is important to understand how the animal uses the space, especially when given more space. We focus on the use of space by cynomolgus macaques (*Macaca fascicularis)* in 2 types of European Union (EU) standard housing (Directive 2010/63/EU). We have worked to improve our EU housing unit to incorporate more structures to elicit natural behavior and improve efficiency with animal training for procedures. Our legacy unit (unit A) is equipped with a balcony covered with small mesh, holding cages, and 2 thermal neutral perching options. Our new housing unit (unit B) is equipped with a horizontal bar balcony, a swing, an elevated tunnel, holding cages, and multilevel thermal neutral perching options. Data collected on space use suggest the balcony in units A and B and tunnel in unit B are preferred locations within the enclosure, but the entire volume of the unit is not heavily used. Animals housed in these units have several opportunities for perching/resting, but our observations suggest a high rate of use of the balcony and tunnel over the other options. This demonstrates the importance of animal choice and may suggest that inclusion of balconies and/or perching tunnels are preferred enhancements over simply adding more space or volume.

PS68 Evaluation of New Outdoor Run Spaces for Young Rhesus Macaques: Animal Management and Animal Welfare

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Improving animal welfare through the use of innovative animal housing in research settings is a high priority, and quantitative evaluation is essential for making empirically based decisions. A novel type of housing was developed, giving rhesus macaques living in indoor caging access to outdoor runs. We evaluated this novel housing with 24, 2-3-y-old male rhesus macaques living in pairs. Outdoor access with additional dynamic enrichment was provided 24/7 during the assessment period. The novel housing required adjustments for both animals and humans to facilitate daily husbandry, including positive reinforcement training to shift animals between indoor and outdoor spaces, and new area management protocols, census labeling, and cage identifiers. Treatment of clinical health issues was managed by using the indoor cages and restricting access outdoors as needed. We found subjects spent 69% of their time outdoors when the runs were available, demonstrating a preference. Statistical analysis of behavioral data revealed more locomotion and social play while they had run space access, and a decrease in solitary play during the posttest condition. Other behaviors (abnormal, inactivity, anxiety/fear, contact aggression, and prosocial) remained unchanged. Fecal cortisol levels declined when subjects had access to the outdoor spaces; alopecia scoring and body condition scoring did

not change across study phases. Overall, the subjects showed a strong preference for using the outdoor runs, expressed more physical activity and social play, and showed evidence of diminished psychosocial stress when the outdoor spaces were available. This housing configuration posed many new challenges for staff and required consistent collaboration among husbandry, veterinary, and behavioral staff to succeed.

PS69 Automated Cognitive Testing Systems Facilitate Data Collection while Enhancing Behavioral Management

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For many scientific investigations, the collection of behavioral data from nonhuman primates is of great importance. Data collection procedures have evolved over the decades, beginning with paper and pencil methods and are continuously moving toward more sophisticated techniques. While the systematic collection of behavioral responses provides invaluable data for scientific studies, it can also provide considerable enrichment opportunities for the subjects and should be an important component of well-integrated research and behavioral management programs. In addition, behavioral data collection procedures can provide insights into changes in the psychological and physical health of nonhuman primate subjects. We have been using automated cognitive testing systems that involve touch screens in our chimpanzee, rhesus monkey, and squirrel monkey colonies to explore a variety of cognitive processes in these nonhuman primates, including cognitive changes as a function of aging and chemotherapy. We have also been using an eye-tracking system with our chimpanzees to better understand the ways that these animals attend to and process visually presented stimuli. Many of our subjects freely participate in hundreds of trials per day with these devices, working for small pellet or fluid rewards, strongly suggesting that performance of the cognitive tasks is enriching for the animals. Importantly, in our projects, no food or fluid control is required. Furthermore, once patterns of responding are established, deviations from a subject's typical pattern may be indicative of welfare/health issues and can be rapidly recognized and then addressed. The setup and use of the touch screen and eye tracker systems will be described, and preliminary response data will be presented. Additionally, several cases will be described in which diminished responding by subjects in testing situations preceded health-related changes.

PS70 Social Housing Male Macaques (*Macaca mulatta* and *Macaca fascicularis*) with Females Following Castration or Vasectomy

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Social housing is critical for improving the psychological well-being of NHPs housed in research settings. Isosexual pair housing is a common practice used to accomplish this goal. However, due to dominance-related behaviors, adult male macaques can be difficult to social house in this manner. This is especially true for smaller colonies with limited number of primates to form compatible isosexual pairs. Creating male/female pairs following castration or vasectomy is an alternative to the traditional same-sex dyad. The males in this report were vasectomized or castrated when requested by research personnel. In general, vasectomies are the default method for reproductive control at our facility as they are minimally invasive, do not alter the hormonal profile of male macaques, and require minimal recovery time. Twenty-six male/female pairs (cynomolgus=21; rhesus=4; male cynomolgus with female rhesus=1) were established following castration or vasectomy. Success rate of pairing was 96% with partners remaining compatible from 40 to 1122 d. Most separations were due to experimental reasons (76%) followed by less-than-ideal compatibility (23%). Only 1 pair of adult rhesus were separated due to aggressive interaction with injuries. Social housing castrated/vasectomized males with females is an effective method to create long-term compatible pairs with little to no aggression during introduction. This is especially true for adult male macaques who would otherwise remain single housed due to a lack of compatible same-sex companions.

PS71 Video Conference Technology as a Tool for Pair Introduction in Rhesus Macaques (*Macaca mulatta*)

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Pair housing is known to promote welfare for macaques (*Macaca mulatta*) in captivity. However, finding compatible partners can be challenging, particularly when the animals are not located near one another. Because macaques show interest in videos of conspecifics, we examined the use of video conference technology as a potential tool to assess compatibility in 84 rhesus macaques (2-22 y old) prior to pair introduction. We set up a video conference session on an tablet in front of the cage between potential partners. Pairs were either isosexual (12 female-female, 21 male-male) or heterosexual (n=9), but in all cases, the partners were unfamiliar to one another and housed in different rooms at the time of the video session. Video sessions were 10 min in duration and were captured using the screen record function on the tablets. We scored attention to screen, anxiety (yawn, scratch, body shake) and prosocial behaviors (lipsmack, present), and examined whether these behaviors predicted future pair success (co-housed in full contact for at least 28 d). We predicted that monkeys who spent time paying attention to the tablet, particularly if they demonstrated prosocial behavior, would be more likely to be successfully paired than those that did not. In general, monkeys spent relatively little time attending to the tablet (average 17.6 +/- 1.67% of time). The amount of time monkeys paid attention to the tablet did not predict pair success (beta estimate = -0.06, NS); however, pairs in which attention was primarily shown by 1 animal (skewed) had a higher chance of success than those in which both individuals showed similar levels of attention (beta estimate = -4.66. $P = 0.03$). Neither prosocial (beta estimate = 0.89, NS) nor anxiety (beta estimate = -1.95, $P = 0.07$) behavior correlated with pair success. It is possible that one video session was not enough to see an effect. While preliminary, our data suggest that video conferencing technology may be useful as a tool for introducing unfamiliar partners prior to a socialization attempt.

PS72 Advancing Welfare through Behavioral Management Program Assessment and Evaluation

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We assessed our behavioral management program to determine the effectiveness of several recent alterations to our program. We used our established quantitative behavioral data collection method and objective scoring systems which are invaluable tools that behavioral managers can use to measure the impact of program changes on the behavioral welfare of primate populations. Qualitative reports of behavior problems, issues with enrichment, or concerns about monkey social interactions from animal care, veterinary and research staff members, in concert with quantitative measures, expand our

understanding of the day-to-day behavioral repertoire and needs of the animals in our care. Such analyses provide insight as to where best to make effective and practical changes to management and care, to make the best use of available resources (personnel time, money) toward advancing animal welfare. Three evaluations will be discussed. First, the impact of providing more frequent fresh produce and destructible enrichment in a target population of 133 rhesus macaques over 6 mo (74% of them showing decreased stereotypical locomotion). Second, the impact of providing smaller pieces of produce to monkeys housed in pairs and protected contact resulted in a reduction in reports of social problems by animal care staff who fed them (from 20% to 14% of pairs reported for aggression). Third, the impact of reducing the frequency of quantitative behavioral observations on the detection of behaviors of concern (a 33% reduction in observation frequency maintained a high detection accuracy for some behaviors (e.g., stereotyped locomotion 92.5% still detected)). All of these data-based program evaluations are used to determine if the changes achieve the intended goals (such as reducing stereotyped behavior and aggression) without jeopardizing our knowledge of behavioral problems exhibited in our population of nonhuman primates. This type of approach guides meaningful changes to behavioral management program implementation.

PS73 Current status and Challenges of Behavioral Management Programs for Nonhuman Primates in Southeast Asia

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Behavior management contains a variety of components which can be found in the regulations and guidelines related to laboratory animal use and care. It includes social housing, enrichment, and animal interactions and training. Despite a couple of training workshops held regarding behavioral management programs over the last decade in Southeast Asia, there is no up-to-date information regarding the level of understanding and challenges of conducting behavioral management on NHPs in the region, so a survey was conducted to understand the current situation. A survey was sent to eight facilities and all eight responded. It included questions about what components of behavioral management have been implemented, identifying what is needed to carry out the work, and resources needed to improve behavioral management implementation. The results indicated that all participating facilities considered all or some behavioral management components have been implemented in their facility, and enrichment is the main implemented element. However, the results also indicated that all the facilities consider themselves to have insufficient knowledge to implement behavioral management fully, and also find it difficult to obtain relevant educational resources. No regular training or continuing education related to social housing, enrichment, and animal training are readily available. Most of the facilities also showed a strong desire to learn about animal training or methods for interacting with NHPs in their facilities. When asked about challenges to implementing behavioral management programs, facilities considered time and manpower as major problems in conducting behavioral management tasks daily. Although personnel in each institution spent more than 30 min daily to perform the tasks related to behavioral management, institutions wished to spend less time and perform these tasks more efficiently. It is hoped that the survey results provide better insight into the status of behavioral management programs in Southeast Asian countries. It would be beneficial to all institutions in Southeast Asia if knowledge-based course work or regular training that they could access were established.

PS74 Case Study of Guanfacine to Mitigate Social Wounding by a Male Pigtail Macaque (*Macaca nemestrina*)

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Managing a breeding colony of nonhuman primates requires the maintenance of compatible social groups while promoting genetic diversity. In our breeding colony of pigtail macaques (*Macaca nemestrina*), we form single male, multi female groups, rotating males between groups every few years. One male had a history of wounding the females he was housed with, even after he was maintained on fluoxetine. Considering this social incompatibility, he was introduced to a new group of females and housed in a larger indoor/outdoor cage, but he continued to wound all the adult and juvenile females frequently, often necessitating veterinary treatment. Here we report the evaluation of a new therapeutic intervention: guanfacine. Guanfacine, an γ_2A adrenergic agonist, has been successfully applied in reducing self-injurious behavior in rhesus macaques (*Macaca mulatta*), with the proposed mechanism being a correction to the dopaminergic system. Given the pathways that guanfacine mediates, and the resultant reductions to impulsivity shown in animal models as well as in human patients, we theorized that it may represent a viable treatment for this socially reactive pigtail macaque. At the time of study, the 8-y-old male lived with 9 adult females and 10 offspring. Baseline data were collected during a 4-wk period when the male was on fluoxetine (20mg). Over a 6-wk period, he was then transitioned to guanfacine (10mg), while being weaned off fluoxetine. He was then observed for a further 4 wk while maintained on guanfacine. During all study periods, 10-min focal follow observations were used to record the male's behavior (45 total h of observations) and ad lib wounding records were kept throughout. Over the course of the study (from baseline to maintenance on guanfacine) wounding rates and severity fell. The male showed a significant decrease in agonistic behaviors ($t=-2.04$, $P = 0.045$), with average daily agonistic behavior rates dropping by 13% at the end of the study period from baseline. Although the male showed reduced agonism, there was no change in his rates of affiliative interactions with females or breeding events. This case study suggests guanfacine may aid the care of socially reactive male macaques in combination with behavioral management strategies.

PS75 Biobehavior Management

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A strong veterinary-behavioralist relationship is crucial to the successful housing of laboratory NHPs. Our approach to biobehavioral management is to incorporate biological, behavioral, and environmental factors to influence health-related outcomes to ultimately improve the overall research program. This allows us to take an individualized approach to psychological wellbeing of our nonhuman primate patients. We present various ways that the veterinarian and behavior staff work together with the research team to optimize housing and wellbeing for NHPs s in our research program. This includes minimizing weight loss in postoperative animals with the combination of supplemental feeding, social housing, human-animal socialization, and appetite stimulants. Enrichment and feeding strategies are used to exercise NHPs to combat obesity or for physical therapy to promote circulation, ambulation, and reduce edema. During routine exams, body condition scoring is used, and social housing considerations are

made before dietary adjustments are solidified. Destructible enrichments are used to deter wound picking or to deter agitation for animals acclimating to jacket and tether systems. Additionally, we have worked to use pharmaceuticals and supplemental agents to deter self-injurious behavior and promote social housing. These strategies are used daily within our program and are modified to meet the individual nonhuman primate and study needs. When combined with our strong operational acclimation program that targets minimizing fear and aggression, we find our NHP patients are better prepared for our housing and research environment, and we are better able to respond to their individual needs.

PS76 Feasibility of a Gel-based Diet in Dosing Oral Albendazole and Fumagillin to Treat *Pseudoloma neurophilia* in Adult Zebrafish (*Danio rerio*)

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Pseudoloma neurophilia is a microsporidian parasite that disrupts research by inducing behavioral and physiological changes in zebrafish. While there is no treatment for *P. neurophilia*, albendazole and fumagillin have been used to treat microsporidian infections of other species. We investigated the efficacy of oral albendazole and fumagillin in the treatment of *P. neurophilia.* Prior to completing this aim, we performed a pilot study to validate a commercially available gel-based zebrafish diet to dose zebrafish with fumagillin or albendazole. We hypothesized that the medicated diets would be palatable, contain appropriate drug concentrations, and administration would not produce adverse clinical events. High-performance liquid chromatography analysis of the diets demonstrated that the fumagillin diet contained three times more fumagillin than anticipated, while the albendazole diet contained 66% of its anticipated concentration. Consequently, the albendazole dose was increased by 33%. Next, 30 adult AB zebrafish were assigned to one of 3 groups (10 fish per group, 4 wk of feeding): fumagillin at 15 mg/kg, albendazole at 2 mg/kg, or no medication (control). We observed fish daily for adverse clinical events and recorded the time for each group to finish their daily ration. Fish in the albendazole and control groups consistently finished their diets within 15 min. Fish in the fumagillin group finished their diet within 60 min. Only one adverse clinical event was recorded in the study for one fish in the albendazole group, which became progressively emaciated during the study and was later found dead. Histopathology of this fish did not reveal study-related pathology. These results confirm that the gel-based medicated diets are safe and palatable when administered to adult AB zebrafish. We plan to use these diets to investigate treatment of *P. neurophilia,* which presents an exciting opportunity to reduce the impact of this pathogen on animal welfare while improving the quality of zebrafish research.

PS77 Short-term Administration of a Diet Containing Rapamycin, Acarbose, and Phenylbutyrate Induces Resilience to Osteoarthritic Changes in *Aged Mice*

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Osteoarthritis (OA) is a common cause of decreased quality of life for aging individuals. Over 60 million people in the United States—a fifth of the population—have been diagnosed with some form of OA. Treatments may be limited to pain management, including drugs such as opioids with a high risk of side effects and potential for abuse. Other treatment options include expensive and invasive surgical procedures such as joint replacements, which have the potential for breakdown and failure. We are suggesting the use of a novel drug combination of rapamycin (14 ppm), acarbose (1,000 ppm), and phenylbutyrate (1,000 ppm), given in the diet to increase resilience to OA in mice. This drug combination has been shown to delay age-related cognitive and physical decline in prior studies, and studies have considered these drugs separately for management of OA. In this preliminary study, a total of 80 20-mo-old male and female C57BL/6 mice were tested for physical fitness using several assays including a box maze, a rotarod trial, and a horizontal beam assay. Following this, half of each cohort had surgery performed to induce OA, and the others had a sham surgery performed. Half of each surgical group (n = 10) was then maintained on a standard mouse diet and half was maintained instead on a formulated mouse diet containing rapamycin, acarbose, and phenylbutyrate. All mice were monitored 3 times per week for 2 mo, including a multimodal pain assessment. At the end of the study, the box maze, rotarod, and horizontal beam assays were performed again to compare all groups to their presurgery performance. In general, mice fed the drug cocktail diet performed significantly better on these assays per analysis via T test. At the end of the study, treated mice with surgically induced OA performed better ($P < 0.05$) on the rotarod and horizontal beam assay versus their untreated counterparts. Treated male mice also performed more successful maze trials, although females did not. These observations provide support for the potential efficacy of this drug cocktail even when given at an advanced age and after the inciting insult. In summary, this prototype cocktail may be part of a therapeutic strategy for those suffering from or at risk for osteoarthritis.

PS78 Impact of Supplier-derived Gut Microbiomes on Feed Intake and Fecal Energy Loss

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Excessive food intake and obesity are prevalent in the general population. Excessive food intake can lead to obesity and other comorbidities and ultimately become a burden on the healthcare system. The gut microbiome (GM) influences metabolic regulation and intake and satiety. The GM of specific pathogen-free (SPF) mice varies in composition and richness between suppliers, and the different SPF microbiomes significantly influence body weight (BW). We sought to determine whether the difference in BW conferred by these GMs is associated with differences in food intake or fecal energy loss. Two colonies of CD1 mice were rederived in surrogate dams harboring either a high microbial diversity GM (GM4) or low microbial density GM (GM1). Pairs were bred and litters were culled to a uniform size upon birth. There were approximately 14 breeding animals and 40 offspring used for this study. At 3 wk of age, offspring were weaned into same-sex pairs and weighed. The food hopper was also weighed on that day and on 3 consecutive days. This was repeated at 6 wk of age and at 9 wk of age. At 6 wk of age, fecal samples were also collected for bomb calorimetry analysis to measure fecal energy loss. At 3 wk of age, there was a significant difference in body weight, with GM1 weighing more than GM4 (P = 0.001), with no effect of sex. Regarding food intake, there was a significant difference between GM1 and GM4 in the total feed consumed per cage at 3 wk of age (P = 0.004), but this difference was abrogated when intake was normalized to BW. At 6 wk of age, there was a significant difference in intake between GM1 and GM4 with GM1 consuming more feed in total ($P < 0.001$) as well as per gram of mouse. These results show that supplier-origin GMs influence voluntary food intake which may, at least partially, explain the observed differences in BW. The data from this study demonstrates the utility of this platform to study how GM influences food intake,

use of energy, and body weight.

PS79 Modulating Maternal Microbiota to Generate a Novel Mouse Model for Fragile X Syndrome

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Maternal microbial dysbiosis has been implicated in adverse postnatal health conditions in offspring. We made a novel observation that progeny of CD-1 Swiss outbred stock mice fed a westernized diet (WD) with low fiber and extra fat during pregnancy exhibited higher frequencies of stereotypy (times nasal area crossed the horizontal axis), hyperactivity (times mouse crossed a quadrant), cranial features (ear pinnae diameter and craniometric analysis), and lower brain expression of Fragile X mental retardation protein (FMRP), similar to what is typically observed in Fragile X syndrome (FXS) in humans. We hypothesized that gut dysbiosis and inflammation during pregnancy influenced the prenatal uterine environment, leading to abnormal phenotypes in offspring. Pregnant CD-1 mice descending from mice fed WD were randomly subdivided with half receiving 3.5 × 105 organisms a day of an anti-inflammatory probiotic *Lactobacillus reuteri* in the drinking water. We found that oral in utero supplementation with *L. reuteri* sufficiently inhibited the FXS-like phenotypes in offspring mice from mothers fed a WD. Whole blood collected by terminal cardiac puncture from pregnant females on embryologic day 14 was used to assess cytokine profiles, which found increased circulating levels of pro-inflammatory cytokine interleukin-17 in WD-fed mice relative to mice fed standard chow as well as those fed WD with *L. reuteri*. To test our hypothesis of prenatal diet contributions to FXS, we performed Caesarian births using dissimilar CD-1 foster mothers to eliminate effects of maternal microbiota transferred during vaginal delivery or nursing after birth. We found that foster-reared offspring still displayed a high frequency of FXS-like features, indicating significant in utero contributions. By contrast, matched foster-reared progeny of *L reuteri*-treated mothers did not exhibit the FXS-like typical features, supporting a key role for microbiota during pregnancy. Our findings suggest that diet-induced dysbiosis in the prenatal uterine environment is strongly associated with the incidence of the FXS-like phenotype in progeny but can be alleviated by addressing gut dysbiosis through probiotic supplementation.

PS80 Facilitating Mouse Studies of Post-acute Sequelae of COVID-19 (PASC)

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COVID-19, caused by SARS-CoV-2, and its chronic form, Post-Acute Sequelae of COVID-19 (PASC), remain significant public health concerns. Transgenic mice are an effective model for acute COVID-19 research, but PASC studies are currently lacking due to the prohibitive costs of performing such studies in an Animal Biosafety Level 3 (ABSL-3) containment setting. The goal of this study was to determine the natural timing of clearance of the SARS-CoV-2 virus from mice and establish a protocol for transfer of animals from ABSL-3 to ABSL-2 for PASC studies. We hypothesized that infected mice would clear viral infection by approximately 3 to 4 wk postinfection (WPI). Six- to 18-wk-old, B6.Cg-Tg(K18-ACE2)2Prlmn/J (hACE2) mice (N = 48/sex) were intranasally inoculated with a pre-alpha strain of SARS-CoV-2 in an ABSL-3 containment setting. Environmental samples, oral swabs, and fecal samples were collected weekly up to 8 wk postinoculation and cohorts of surviving mice were necropsied at 4, 7, and 8 WPI when lung and brain were collected. Viral loads in all samples were quantified via RT-qPCR. Survival was significantly affected by sex, with males more susceptible (P = 0.002), but not age (P = 0.005). SARS-CoV-2 viral RNA copies were present in the lungs of mice at 4, 7, and 8 WPI, indicating that the mice had not yet cleared infection by the culmination of the study and raising the possibility of persistent infection.

PS81 Rhesus Macaques Demonstrate a Differential Early Immune Response in the Respiratory Tract after Inoculation with Wild-type Compared with Live-attenuated Measles Virus

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Measles virus (MeV) is a highly transmissible virus that spreads through infection of the respiratory tract. Both wild-type and live-attenuated strains of MeV (LAMV) are known to replicate readily in respiratory epithelium, but the local immune response in the respiratory tract has not been well-studied. To investigate the early immune response within the lungs, 2 male and 6 female rhesus macaques were infected intratracheally with either wild-type MeV (n = 4) or LAMV (n = 4). Bronchoalveolar lavage (BAL) was performed at 0, 3, 11, and 14 d post inoculation (DPI). MeV RNA in the BAL cells was quantified by quantitative reverse transcriptase-polymerase chain reaction. BAL fluid was evaluated for cytokine and chemokine production by electrochemiluminescence immunoassay and for MeV-specific IgG production by enzyme-linked immunosorbent assay. Viral RNA load was similar in both groups at DPI 3, but viral RNA persisted until at least DPI 14 in animals inoculated with WT MeV compared with LAMV, who had no detectable RNA by DPI 11. Higher titers of IgG were present in the BAL fluid at DPI 11 and 14 in WT-inoculated animals compared with LAMV-inoculated animals WT-inoculated animals had a larger increase in IP-10/CXCL10 at DPI 11, while LAMV-inoculated animals had larger increases in IL-12 at DPI 11 and INF-y at DPI 14. WT-inoculated animals had lower IL-1γ levels at DPI 3 compared to LAMV-inoculated animals, but higher levels at DPI 14. Neither group showed significant changes in either IL-1γ or TNF-γ levels through DPI 14. Further study is warranted to clarify the role of the local respiratory tract immune response in controlling MeV infection and promoting protective immunity after infection or vaccination.

PS82 Effect of Altered Postnatal Joint Loading on the Developing Mouse Meniscus

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The meniscus is a fibrocartilaginous structure within the knee joint that serves a critical role in the transfer of mechanical forces. Meniscal injuries are common and disrupt the specialized function of the meniscus, compromising overall joint health. Due to the limited healing capacity of meniscal tissue, solutions to improve meniscus repair are needed. Knowledge of the factors regulating postnatal meniscus maturation may be helpful in guiding novel regenerative strategies. To determine the effect of altered mechanical loading on meniscus development in the postnatal period, unilateral sciatic nerve resection (SNR) was performed in male and female CD1 Col-1/Col-2 fluorescent reporter mice on postnatal day (D) 1 or 14, with subsequent gait and joint tissue analysis on D14, 28, or 42. We hypothesized that SNR would impair ambulation of the injured limb, resulting in altered loading across the knee, and reduced growth and maturation of the meniscus. Custom gait analysis performed on D14 and 28 revealed significant gait alternations in all mice receiving SNR, consistent with sciatic denervation (n = 3–8 per treatment group). Further classification of gait alterations at D42 were evaluated via a gait analysis system, which confirmed the persistence of sciatic denervation (n = 7–9 per treatment group). On D42, mice receiving SNR exhibited 27% reduction in maximum intensity of the operated hindlimb (SNR P1, P = .01; SNR P14, P = .02), as well as over 65% reduction in paw print width (P < .001). Histological analysis evaluating tissue morphometry, endogenous fluorescent signals, and enzymatic activity (tartrate-resistant acid phosphatase and alkaline phosphatase) surprisingly revealed minimal differences in the knees between mice receiving SNR and control mice (n=5–8 per treatment group). Furthermore, mechanical properties of whole meniscus (probed via atomic force microscopy nano-indentation testing) revealed no significant difference (P =.97) in elastic modulus between mice receiving SNR (n = 5) and control (n = 4) mice. In contrast, preliminary tensile testing of the Achilles tendon showed reduced tendon stiffness (51%) and maximum force (40%) in mice receiving SNR. Knee joint and meniscal tissue formation developed normally in the face of marked and persistent gait deficits from sciatic denervation. These results do not support early postnatal mechanical loading as a primary driver of knee joint and meniscus maturation.

PS83 Gastric Coinfection with Thiopeptide-positive *Cutibacterium acnes* Alters *Helicobacter pylori*-induced Pathogenesis in Murine Model of Gastric Cancer

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Helicobacter pylori (*H. pylori*) is the leading cause of gastritis and gastric neoplasia in humans. During the cascade from inflammation to cancer, the transcription factor FOXM1 becomes increasingly expressed in both humans and mice. Other gastric bacteria may also play a role in *H. pylori* pathogenesis. *Cutibacterium acnes* (*C. acnes*), a commensal in multiple organs, is isolated from stomachs of *H. pylori*-colonized and uncolonized humans. Given some strains of *C. acnes* produce thiopeptides that inhibit FOXM1 expression, we hypothesized that coinfection with thiopeptide-positive *C. acnes* would alter *H. pylori*-induced pathogenesis. 69 germ-free INS-GAS mice (40 males, 29 females) were dosed with *H. pylori* alone, *H. pylori* followed by *C. acnes* 1 wk later, *C. acnes* followed by *H. pylori* 2 wk later, *C. acnes* alone, or remained uninfected. Animals were orally gavaged with 2 × 107 bacteria. At 17 wk postinfection, histopathology, bacterial colonization, and gastric cytokine mRNA expression analysis for Il1γ, Ifn-γ, Tnfγ, Il17a, Il22, iNOS, Foxp3, and Foxm1 were performed. Serum antibodies for *H. pylori* and *C. acnes* were measured by ELISA. In males only, 32-plex gastric tissue cytokine array was performed, and RORγT expression in gastric lymph node CD4+ T cells was evaluated by flow cytometry. Histopathology inflammation scores were increased in both males and females dosed with *C. acnes* prior to *H. pylori* compared to *H. pylori alone*, however, this group exhibited reduced gastric pro-inflammatory markers by cytokine array. This coinfection group also had decreased *H. pylor*i gastric colonization in males, but not females. Males dosed with *H. pylori* followed by *C. acnes* exhibited reduced gastric pro-inflammatory cytokines, regulatory cytokines, and Foxm1 expression, while coinfected females exhibited reduced Il1γ only, compared to *H. pylori* alone. Serum pro-inflammatory IgG2a antibodies and gastric lymph node RORγT expression were lower in coinfected male mice compared to *H. pylori* alone. Meanwhile, coinfected females showed no difference in pro-inflammatory IgG2a antibodies compared to *H. pylori* alone. This study demonstrates that coinfection of thiopeptide-positive *C. acnes* with *H. pylori* perturbs biomarkers of importance in *H. pylori*-induced pathogenesis.

PS84 Shedding of Wild-type Adeno-associated Virus in Naïve Cynomolgus Macaques (*Macaca fascicularis*)

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Nonhuman primates are an important animal model in the development of adeno-associated virus (AAV) gene therapies. Previous studies have shown that exposure to AAV elicits immune responses against the viral capsid which leads to the production of neutralizing antibodies. Neutralizing antibodies compromise the subsequent use of AAV as a systemically delivered gene therapy vector. The presence of neutralizing antibodies therefore presents a challenge in studies of AAV vector performance in nonhuman primates. Despite the importance of maintaining nonhuman primates that are neutralizing antibody-negative for AAV gene therapy studies, little is known about the transmission of wild-type AAV in nonhuman primate research colonies. To evaluate for shedding of wild-type AAV in a research colony, we followed a cohort of 19 naïve Cynomolgus macaques (*Macaca fascicularis*) upon arrival to our institution over the course of 6 wk, periodically collecting feces, urine, oral, and nasal swabs. DNA was extracted from these samples and analyzed for the presence of AAV viral DNA using a quantitative polymerase chain reaction assay with primers designed to detect a highly conserved region of the viral genome. Over the course of the 6 wk, AAV viral DNA was identified in samples from 13 of 19 animals. AAV viral DNA was identified in all sample types, although more frequently and at higher levels in urine and fecal samples than in oral or nasal swabs. This work emphasizes the importance of considering AAV viral status in the determination of housing arrangements and husbandry practices when maintaining neutralizing antibody-negative animals for studies evaluating AAV gene therapies. Further work is necessary to correlate the shedding of viral DNA with new infections in research animals and with the development of neutralizing antibodies. This presentation will include a discussion of neutralizing antibody screening and surveillance, as well as husbandry considerations when maintaining a colony of nonhuman primates for AAV gene therapy studies.

PS85 Severe Acute Myocardial and Hepatic Disease in 2 Göttingen Minipigs

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Two 5 to 6-mo-old female Göttingen minipigs from a single cohort of 40 pigs were found 1 mo apart laterally recumbent, but responsive, with elevated respiratory rate and effort, elevated heart rate, and shivering despite normothermia. Pig 1 had a darkly hyperemic snout and pinnae. No abnormal respiratory sounds were auscultated on either pig; lung radiographs were within normal limits. Given a herd history of porcine circovirus (PCV), both pigs were treated empirically with meloxicam, ceftiofur to prevent secondary bacterial infection, and supportive care. Serum chemistries showed elevated ALT, AST, BUN, creatinine, and CK in both pigs. Both pigs declined over 3-4 d, becoming hypoxic and hypothermic; Pig 2 was markedly icteric by euthanasia. Serum submitted from Pig 1 was negative for both PCV2 and PCV3 by rPCR. Sterilely collected lung, mesenteric lymph nodes, and liver from Pig 1 did not indicate bacterial infection as a primary etiology. Microscopic exam yielded diagnoses of necrotizing and hemorrhagic cardiomyopathy with systemic vasculopathy consistent with mulberry heart disease (MHD) and hepatosis dietetica in both pigs with signs of duration up to 1 week. MHD is a vitamin E/selenium-responsive syndrome, typically affects pigs <7-8 wk of age, and affects the entire cohort fed the same diet. This presentation was unusual given the pigs' age, duration of the lesions, systemic distribution of the vasculopathy, and low number of pigs in the cohort affected. Trace mineral analysis demonstrated copper deficiency with normal selenium levels. Feed analysis confirmed appropriate vitamin and mineral levels. Thorough history revealed a recent housing change to runs with aged, galvanized steel that had not previously housed pigs, leading to a presumptive diagnosis of zinc toxicosis from chewing on the metal. High zinc concentrations competitively inhibit copper absorption, in this case leading to copper deficiency and a coagulopathy mimicking classic MHD and hepatosis dietetica. The cohort was immediately moved to different housing; no further pigs were clinically affected. Tissues were collected from littermates and from pigs that had been co-housed with Pig 1 and Pig 2 upon study completion 2 mo after the second case for comparative analysis.

PS86 Abdominal Mass in a Rhesus Macaque (*Macaca mulatta*)

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A 17-y-old, single-housed, male rhesus macaque (*Macaca mulatta*) was sedated in 2017 for routine semiannual vet exam where on abdominal palpation, an approximately 1.0 cm diameter round, firm mass was palpated in his upper mid-abdomen. His body condition score was 3.5/5, he had minimal alopecia on bilateral forelimbs and he weighed 11.27 kg. The remainder of the physical exam, including complete blood count and chemistry, was unremarkable. On abdominal ultrasound, no distinct masses were appreciated. He remained on veterinary observation with sedated exams performed semiannually and during protocol related PET scans where the mass remained unchanged. During his August 2021 exam, a significant loss in body weight (now 8.4 kg) and decrease in body condition (2/5) was observed despite a history of normal appetite. He had significant alopecia, primarily at the ventral head and neck, bilateral forelimbs, and hindlimbs. The previously noted midabdominal mass had subjectively increased in size to 3.0 cm diameter with a secondary, round, firm mass (1.0 cm) palpated in the right cranial abdomen. On ultrasound, the 2 masses had a heterogeneous center with hyperechoic encapsulation and were associated with bowel loops. PET/CT scan showed a focal area of abnormal perfusion associated with an intraluminal mass within the colon. The primary differentials were trichobezoars, diverticulosis, lymphadenopathy, IBD, and neoplasia. Supplemental feed and extra enrichment were initiated, and an exploratory laparotomy was planned. During the laparotomy, the ascending and transverse colon had a plicated appearance with 4 distinct firm-to-hard, irregularly round, intraluminal masses. The descending colon was moderately fluid distended. An enterotomy was performed and the 4 larger masses were retropulsed to the site for removal. No other abnormalities were found within the GI tract and abdomen. On cut section, the masses appeared to be chronic trichobezoars that consisted of a very firm mat of hair encapsulating a core of hair and ingesta. Post laparotomy, he continues to have normal appetite and eliminations while being maintained on supplemental feed and extra enrichment, with regular exams during protocol related imaging.

PS87 Skin Lesions in an Athymic Nude Rat

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A 9-mo-old, group-housed, intact male athymic nude rat was examined for pinpoint erythemic lesions and several 2-5 mm raised masses on the skin of the proximal tail, scrotum, and dorsal surface of the hind paws. This rat was housed on a wood chip-based bedding, was the only rat affected, and had no prior experimental history. There were no changes in mentation and the lesion was intermittently pruritic. The differential diagnosis list included external parasitism, bacterial infection, immune-mediated inflammatory response, contact dermatitis, phenotypic presentation, or trauma. A skin scrape was performed to rule out parasite burden. The rat was separated from its cage mate and placed on enrofloxacin water (25 mg/kg/day); however, over the course of 1 wk, the lesions previously described remained unchanged and the disease progressed as new lesions appeared on the muzzle and pinnae. A swab culture sample was taken of expressed material from a mass for anaerobic and aerobic bacterial culture and sensitivity. Hairs were plucked for dermatophyte culture. The rat was placed on alpha dry bedding to assist in ruling out environmental-associated hypersensitivity. There was no response to any of the treatments, so the rat was given one dose of dexamethasone (0.6 mg/kg) intramuscularly. There was mild improvement to the pinnae, but lesions on the rest of the body were unchanged. From initial exam, body weight decreased by 3.5%. The rat was submitted for comprehensive necropsy including histology and sample submission for bacterial culture, hematology, and clinical chemistry. Folliculitis and dermatitis were observed on histology with minor findings in the lungs, liver, and adrenal glands. No intralesional bacteria were observed on hematoxylin and eosin stain but gram stain is pending. CBC and chemistry revealed a mild mature neutrophilia. Culture results from a limb confirmed previous results with significant growth of methicillin/penicillin/cephalosporin resistant *Staphylococcus cohnii*, while culture of the spleen yielded no growth. A presumed source was not identified in this case. There is limited literature coverage of *Staphylococcus cohnii* as a causative agent of disease in rats; thus, the presentation for this rat is unique.

PS88 Wasting in Cane Toads (*Bufo marinus)*

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A colony of wild-caught cane toads, *Bufo marinus,* presented for exam after spontaneous deaths and loss of body condition over the last month. They were caught in Florida, where they are invasive, 3 mo

prior for behavioral testing and neurological mapping. At that time, ticks were removed from animals. One emaciated toad was submitted to necropsy and before euthanasia, found to be unable to move or eat, with no skin lesions but nostrils flaring, increased respiratory rate and abdominal effort. On necropsy, all abdominal organs were gelatinous, with no coelomic fat, lungs are thin with sigmoid shaped brown lesions, tongue tip is dark brown and short, and though skeleton appears intact, has minimal muscle. Differential diagnoses included chytridiomycosis, mycobacterium, Rickettsial disease, post shipping septicemia, parasites, ranavirus, hypovitaminosis A, mucor amphibiorum, and chemical contamination. Amphibian PCR panels ruled out chytrid, ranavirus, and mycobacterium. Blood smear ruled out Rickettsial disease. Other animals appeared to have squamous metaplasia of the eyes and lips. Affected animals were quarantined, empirically dewormed with ivermectin subcutaneously for 2 mo, provided higher calorie food items (waxworms), changed to a formulated diet, given a week of azithromycin, syringe fed, and bathed with saline more frequently. On histology review of necropsy slides, lungworms and their ova were found in the lungs, as well as cutaneous larval migrans in toe webs. Suspected to be Rhabdias species, where L1s are free living in the environment and L3s undergo migration. The remainder of the colony recovered body condition over the next 2-3 mo with no further larvae found after last deworming. Wild-caught amphibians present unique challenges as a laboratory species and when clinical disease appears, require aggressive intervention and thorough diagnostics.

PS89 Chronic Intermittent Inappetence and Recurrent Emesis in a Duchenne Muscular Dystrophy Laboratory Dog

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A 12-y-old, 21 kg, male intact golden retriever with Duchenne muscular dystrophy presented for evaluation of chronic intermittent anorexia and emesis. the dog had a 2-y history of intermittent anorexia and cervical pain managed with chiropractic care, a joint health supplementation, and diet change. On initial exam the dog was clinically normal, had small bilious emesis spots noted in the kennel, and was treated with a single dose of omeprazole. An abdominal ultrasound was performed and no abnormalities were noted. Over the following 2-wk period the inappetence became more frequent with intermittent watery emesis. During this timeframe the dog lost approximately 1.5 kg of body weight. A follow-up clinical exam was performed and routine blood work was submitted. On physical exam the dog exhibited pain in the upper central abdominal quadrant and appeared quiet. Complete blood cell count and serum biochemistry yielded no significant abnormalities. Treatment for presumptive gastritis and esophagitis with omeprazole and sucralfate was initiated. During the 12 h following the exam the dog's condition declined and it succumbed to what was later determined to be a cardiac event. Necropsy was performed and marked left ventricular hypertrophy, a 3 cm mass in the caudal esophagus obstructing the lower gastroesophageal sphincter, two 4-5 cm masses in the liver, thinning of the stomach and intestines, and significant thinning/fibrosis of the skeletal muscles were noted. Histopathology of the esophageal mass revealed a leiomyoma and the liver masses were nodular hyperplasia. Further review of literature revealed that humans with Duchenne muscular dystrophy have an increased likelihood of myogenic tumors that progress to high-grade lethal sarcomas. Further investigation of other affected and carrier muscular dystrophy laboratory dogs is warranted to document if they share this phenotype.

PS90 The Illusive Intermittently Inappetent New Zealand White Rabbit

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A naïve adult female New Zealand White (NZW) rabbit was obtained from Western Oregon Rabbit Company on 17 Nov 2021. She was first reported for hyporexia on 1 Jan 2021, which resolved the same day with supportive care. On 25 Jan 2021 fecal production was decreased, and she was treated as a standard GI stasis case with supportive care: SQ LRS (60mL) and maropitant citrate SQ (1mg/kg). The following day there was no improvement, an excessive amount of shed hair was noted in the home pen, and she had a guarded abdomen and increased borborygmi. The animal was tachypneic, heart rate was WNL, temperature was 103.8° F, and blood glucose was 81 mg/dL. More aggressive supportive care was initiated: IV fluids(10mL/kg/hr), maropitant citrate IV (1mg/kg), meloxicam SQ (0.3 mg/kg), and lactulose PO (2mL). She was brighter during the evening, but became stressed when assisted feeding was attempted. IV fluids were stopped overnight but an additional 60 mL of SQ fluids were given for overnight support. The following morning, she had not produced any feces and was now dysphoric with splayed legs. Her abdomen felt gas filled with increased borborygmi and a few discrete firm structures were palpated. Due to her declining condition and being an inappropriate surgical candidate for her protocol, euthanasia was elected. Necropsy findings included small intestinal, multiple white, soft raised lesions in the stomach, pale streaks in the heart, and markedly enlarged ovaries with areas of hemorrhage. Histologically, there was lymphoma in the stomach, small and large intestine, mesenteric lymph nodes, ovaries, uterus, heart, liver, and spleen. The intestinal intussusceptions coincided with mural expansion by the neoplastic lymphocytes. Lymphoma is the second most common neoplasia of rabbits and typically involves the GALT, mesenteric lymph nodes, liver, spleen, and bone marrow. Renal involvement, particularly of the renal cortices, is pathognomonic. This case was unusual given its lack of renal involvement. Rabbits are exquisitely adept at hiding their illness and GI stasis is often a side effect of a deeper problem. Without an instigating event, there should be concern of an underlying condition. This case underscores that lymphoma should always be a differential diagnosis.

PS91 Sudden Death in PLP-Cre Female Breeder Mice

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A pregnant female B6.Cg-Tg(Plp1-cre/ERT)3Pop/J mouse with a 13-d-old litter was found dead with blood around her urogenital opening. The pups were fostered to another female breeder within the colony. Forty-eight hours later the foster female became moribund with vaginal bleeding and was subsequently euthanized. Two weeks later, a third female breeder mouse of similar genetic background was found dead without any observed antemortem clinical signs. In each of these cases, the pups were injected with 25 mg/kg tamoxifen at 7 to 9 days of age, intraperitoneally. No experimental manipulations were performed with the adult breeders. All pups and adult male breeders in the affected cages did not develop any illness. To investigate, all 3 female mice were necropsied. Diagnostics included histopathology, fecal PCR and serology. Top differentials included *Pasteurella pneumotropica*, genotype-related abortion, and tamoxifen toxicity. Serology samples were negative for all screened pathogens. Fecal PCR revealed *Pasteurella pneumotropica* subtype Jawetz. Gross necropsy findings for all 3 mice

included a distended uterus filled with hemorrhagic fluid. The livers were diffusely pale. Histologically, the uterine tissues were hemorrhagic and majority of the hepatocytes had accumulation of large fat droplets. No etiologic agent was identified on histology. Because *Pasteurella pneumotropica* does not typically cause hemorrhagic disease, and no indications of infection were identified on histology or gross necropsy, attention was redirected towards the use of tamoxifen in the pups. Tamoxifen is a chemical commonly used to modify gene function in rodents via tamoxifen-dependent Cre recombination. It is hypothesized that residual tamoxifen or harmful metabolites were being consumed by the pregnant females, causing steatohepatitis, uterine hemorrhage, and death. In utero injections of tamoxifen in mice has been reported to cause adverse effects on perinatal pups and the pregnant dam. This is the first report of tamoxifen-related toxicity in pregnant females who were passively exposed by direct contact with pups who received tamoxifen injection. When using this model, subsequent breeding should be delayed to prevent unintended death of female breeders.

PS92 Subcutaneous Cervical Swelling in a Research Hound

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An 11-mo-old, 25 kg, pair-housed intact female hound presented with ventral cervical swelling. The dog had not undergone any experimental procedures prior to this presentation. Physical examination revealed a 9 cm diameter pendulous, fluid-filled subcutaneous mass. Multifocal scabbed lesions were also present along the lateral aspect of the neck. Differential diagnoses included thymic branchial cyst, hygroma, hematoma, abscess, or neoplasia. Aspiration removed 60 mLs of serosanguineous fluid from the mass and cytology demonstrated large numbers of erythrocytes. Radiographs noted a soft tissue opacity that appeared to be nonadherent to deeper tissues. Over 24 h the mass grew to 14 cm in diameter; a compression jacket was placed, and warm compresses (TID for 15 min) were initiated to reduce swelling. However, after continued expansion of the mass over the next day, a Penrose drain and pressure wrap were placed under sedation. Antibiotics (Clavamox 15 mg/kg BID PO) and one dose of carprofen (Rimadyl 4.4 mg/kg SQ) were administered. Daily bandage changes were performed and serosanguineous fluid continued to drain from the mass. On the fourth day of bandage changes, the drain came out and the mass had increased in size and firmness. As a result of unsuccessful conservative management, en-bloc surgical resection was performed. The animal was placed in dorsal recumbency and the mass, along with an approximately 1 cm margin of normal tissue, was resected via an elliptical incision. Grossly the mass was composed of a central cavity containing serosanguineous fluid and lined by a thickened, fibrous wall. The surgical site was flushed with sterile saline, the subcutaneous and skin tissues closed, and 2 Penrose drains were placed paramedian to the incision. Carprofen (Rimadyl 4.4 mg/kg SQ SID) and Clavamox (15 mg/kg PO BID) were continued postoperatively. Histological analysis of the mass revealed large quantities of spindle cells (fibroblasts) in interlacing streams associated with prominent small blood vessels, hemorrhage, multiple fibrin deposits, small quantities of mucinous matrix, and scattered hemosiderin-laden macrophages (chronic hemorrhage). Based on gross findings and histopathologic examination, the mass was most consistent with an expanding hematoma encapsulated by prominent granulation tissue, presumed to have been caused by cagemate trauma. Postoperatively the dog made a full recovery with no recurrence of the swelling.

PS93 Tarsal Swelling in a Long Evans Rat

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A 3-mo-old, female, Long Evans rat presented with an acute, mild, left hind limb lameness, along with two ~1 x 2 mm (medial aspect) and ~2 x 3 mm (lateral aspect) dark red, cutaneous lesions at the level of the left tibiotarsal joint, and a mild, diffuse, erythemic, soft tissue swelling of the same joint. The rat was bright, alert, and responsive, with a body condition score of 3/5, adequately groomed, active, and exhibited a normal posture. Despite the mild lameness, the rat was able to ambulate around the cage well and also able to rear up normally. About an hour prior to the physical examination, the rat underwent a training session with an elevated plus maze (EPM) where its left hind limb was caught between a mechanized arm and a platform for about 20 s. The rat was food restricted for the duration of the behavioral testing training period. A 1 mg/kg dose of meloxicam and a 1 mg/kg dose of buprenorphine-SR were administered subcutaneously following the traumatic injury for analgesia. Meloxicam was added to the drinking water the next day and the rat was monitored by the laboratory group daily. On recheck 3 dlater, the rat had a large ~0.5 x 1 cm, hard, bony swelling at the medial aspect of the left tibiotarsal joint. The rat was very active and clinically sound with normal ambulation and rearing. Differential diagnoses included traumatic fracture or dislocation, bone contusion, osteomyelitis of infectious origin, or a neoplastic process. Radiographs of the affected limb revealed a complete transverse fracture of the tibia above the tibiotarsal joint, as well as a complete oblique fracture of the fibula proximal to the tibial fracture. Euthanasia was elected and the affected limb was submitted for necropsy, which confirmed the radiographic findings on gross pathologic exam. Histopathologic findings revealed a bone fracture with early callus formation, consistent with the traumatic injury 1 wk prior to necropsy. This case emphasizes the difficulty in evaluating clinical signs in rats as few species would have mild to no lameness with complete tibia/fibula fractures, even when analgesics are provided.

PS94 Nonweight Bearing Left Leg Lameness in a Cynomolgus Macaque (*Macaca fasicularis*)

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A 6-y-old, 3.4 kg intact female cynomolgus macaque (*Macaca fasicularis*) pair housed indoors underwent a heterotopic heart xenograft transplant, abdominal telemetry implantation, and central venous line placement, upon which the animal recovered unremarkably. Two mo postoperation, the animal's allograft heart was explanted. Another month later, the animal became nonweight bearing on her left leg. The animal was started on meloxicam daily. Three days later, under sedated physical examination, bilateral leg radiographs were acquired, bloodwork was collected, and a dose of buprenorphine sustained-released was administered. On physical exam, she had a body condition score of 2/5. Her left hip had crepitus and mild resistance on the left coxofemoral joint. There was moderate left calf muscle atrophy. Both complete blood count and serum chemistry were unremarkable. Leg radiographs showed severe narrowing and degradation of the left femoral head. The right femoral head appeared normal. Differentials include avascular necrosis of the femoral head, degenerative joint disease, hip dysplasia, or trauma. The animal improved on meloxicam, but her lameness never fully resolved, despite chronic meloxicam treatment.

Euthanasia was elected. On necropsy, the left femoral head was severely narrowed and deformed, the articular surface was rough and irregular. There was an absence of articular cartilage when compared to the right femoral head. On histologic examination of the left femoral head, the articular cartilage was completely lost, and there was moderate erosion and loss of subchondral bone and marrow fibrosis. Due to the lack of venous thrombi and lack of necrosis of the subchondral bone in the histologic sections, a diagnosis of avascular osteonecrosis of the femoral head could not be confirmed. However, the evidence of viable subchondral bone and the absence of articular cartilage supported the diagnosis of idiopathic chondrolysis, a disease that has only been described in 2 cynomolgus macaques. In the 1 published report, the authors only discuss the radiographic and histopathological findings in detail. The reported case here describes the clinical signs and treatment options.

PS95 Understanding the Impact of Culture on Job Performance

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"She's always late to meetings." "He never says what he really means." "They never speak to us when they are in the animal facility." "I haven't seen my director in 2 years!" Do you ever feel sometimes that your greatest points of contention and stress have nothing to do with the technical part of your job? Although not easy to define or measure, culture plays a significant role in our daily activities and job performance. Call-outs, poor communication, perceived disrespect, and disengagement are all reflections of culture and can play an essential role in our attention to the necessary details of our job, even to the point of compromising animal welfare. In the session, we will define culture and consider how it relates directly to job performance. We will also discover easy ways to assess culture and identify steps for improvement.

PS96 Results from the 2020 ACLAM-ASLAP Workforce Demographics and Salary Survey of Laboratory Animal Veterinarians

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Previous economic and workforce surveys of the American College of Laboratory Animal Medicine (ACLAM) and the American Society of Laboratory Animal Practitioners (ASLAP) have been conducted approximately every 3-4 y. The goal is to summarize the demographic and workforce characteristics of the laboratory animal medicine sector of the veterinary profession in the United States. With ACLAM and ASLAP's interest in broadening the survey to include race and ethnicity information, expanded employment information, and inclusion of veterinarians employed in this sector who are not members of ACLAM nor ASLAP, a new survey was conducted in 2021 to aggregate 2020 data from laboratory animal veterinarians. The Qualtrics web-based survey was distributed to ACLAM, ASLAP, and American College of Animal Welfare members and posted on relevant websites and social media in 2021 and was available for 74 d. 826 responses were received, accounting for 50.5% and 60.2% of ACLAM and ASLAP membership, respectively, 11.7% of the respondents were not ACLAM or ASLAP members, and 677 responses were analyzed after exclusion criteria were applied. Income and experience results from this survey (reported as mean, median, first, and third quartiles) include the total annual gross professional income (shown in 1000 US Dollar units) of full-time laboratory animal veterinarians for ACLAM (188, 172, 137, 220; n=493) and non-ACLAM respondents (114, 117, 80, 141; n=119), and the years of laboratory animal medicine experience post-DVM for male respondents (21, 20, 11, 31; n=260) and female respondents (14, 12, 7, 20; n=417), among other findings. Additional results include race and ethnicity, geographic details, training program completion, employer and role details, and cross-response analysis.

PS98 Into the Crawl Ball: Adventures in Spiny Mouse Care and Management.

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African spiny mice are uncommon research animals with sparse information published regarding their care and keeping in the research setting. Our team had to import, house, and care for this novel species to our program in 2019. We had questions like: How do you safely change cages for animals that slough their skin as a defense mechanism? What enrichment do they use? What are they like? We have learned a good bit about how to care for these animals over the last few years and will share the ups and downs of managing and caring for a breeding colony of African spiny mice. Our spiny mouse population has expanded from 17 animals to our current census of 185, and our enrichment strategies have evolved from that first group of 11 males and six females. In addition, we have had to adapt to research needs for this colony, including working through how to do cage changes for complex divided caging. We have a thriving colony of spiny mice and want to share our experience with the AALAS community.

PS99 How to Establish an in Vivo Research Services Core in Academic Institutions

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A successful in vivo research services core (IVRS) was established in 2016. Multiple departments participated in the day-to-day activities of this core, including legal, finance, regulatory, and animal care. The establishment of this core required specialized legal documents for research and husbandry contracts, a detailed cost analysis for determining per diem charges, quote preparation, and hiring highly qualified staff to perform in vivo research. Marketing is essential for client acquisition. This includes creating a website and preparing brochures and posters. IVRS advertises its services on the main website, in local research incubators, and on national platforms such as the Science Exchange Marketplace. The IVRS core provides research support to faculty and industry and husbandry services for industry clients using their technical staff. IVRS offers research services, including in vivo model development, animal model selection, study design, training in complex procedures, and diagnostic tests. The IVRS Core is self-sufficient and provides revenue for the university.

PS100 Developing an Electronic Veterinary Case Management System in a Good Laboratory Practice Environment

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An internally developed and validated electronic veterinary medical case management system linked to the study data capture software has the potential to improve animal welfare by increasing compliance, minimizing data entry errors, and increasing information accessibility. A document control software, an electronic data collection system, and a custom report builder were used to create a system for electronic veterinary case management. The document control software allows for case workflow: new case submission, veterinary assessment and plan, study director approval, case outcome, and veterinary final approval steps. The electronic data collection software allows for activity scheduling, treatment administration documentation, animal observation records, and other objective data. The data from document control and electronic data software are compiled into custom reports using the report builder. Custom reports allow data access across departments that facilitate veterinary workflow, treatment administration, clinical case monitoring, and clinical history summary. All software used is compliant with Good Laboratory Practice regulations. All software in use has increased the efficiency of data review for completeness and accuracy. The system has enhanced regulatory and treatment plan compliance. While the system is still undergoing ongoing refinements to improve process efficiency and comprehensiveness, the electronic veterinary case management system has provided significant improvements in the consistency of treatment administration, increased regulatory compliance, and accessibility of veterinary case information across departments.

PS101 Implementing Change, Experience fom the Vivarium: Improving in Vivo Study Management by Adopting A Cloud-based Software Platform

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Implementing necessary digital change in a vivarium is not always an easy task, especially when it comes to replacing software. At the same time, the ability to reproduce animal experiments is a growing issue for in vivo research. Studies have shown that as many as 90% of oncology studies are not reproducible. The lack of repeatability and the need for change are linked. The legacy software and spreadsheets currently used can be difficult to set up, keep updated, and navigate by multiple team members. Moving away from these outdated methods is often difficult due to the perception of complexity in setting up new systems and the fear of retraining staff on new systems. Modern cloud-based study management software was adopted and implemented in a vivarium which had significant concerns over the length of time it would take to set up, the difficulty in getting it to connect with other internal systems, and how the research IT and vivarium staff would react to new software. The implementation of the cloud solution was conducted over a 2-d period working with the research IT department. As all elements of the software platform were designed in collaboration with scientists, all of the vivarium end-users were trained and onboarded within this 2-d period. The intuitive study setup and data collection improved workflow efficiency and reduced human error mistakes during measurements. This supported approach to change management involving all the relevant team members. Allowed for a positive and well-received in-vivo study management platform to be implemented quickly and effectively.

PS102 Evaluation of a Strategy to Increase Census Capacity by the Introduction of Higher Density Caging in the Same Footprint

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Faced with a space crunch and no immediate prospects of a new vivarium, our institution instituted a strategy to replace static mouse caging (49 cage single racks and 98 cage double racks) with 70-cage IVC racks that fit in the same footprint, increasing capacity by 43% (70/49) and increasing change interval from weekly to every 2 wk. Autowater had to be implemented later, requiring a long period when internal water bottles were used. Feasibility evaluations and upgrades to enable the project included capacity of HVAC systems, electrical and emergency power capacity, rack, tunnel washer, and autoclaves capacity, staffing levels, cage storage, and staging capacity, and "hood time" (hours of use for the animal transfer stations). This 43% increase in capacity was applied in 2 of our facilities in a multistage floor-by-floor installation project that replaced 370 single-sided racks and 114 double-sided racks with IVCs over the course of approximately 1.5 y, increasing capacity from 29,694 to 42,420 cages. Because these facilities had been constructed during an era when twice weekly cage and bottle changes were the norm, there was enough spare rack washer and tunnel washer capacity, and there was enough locker/breakroom space for the larger staff needed. Because the IVC cage parts stacked more closely than static, the 43% increase did not have a proportionate increase in autoclave capacity, which was sufficient to take the increased census. The most challenging situation was with hood time, as daily checks and cages changes took more time, and the hoods were significantly less available for investigators. This situation was partially ameliorated by adding shared procedure rooms and autowater implementation in one facility, which eliminated weekly water bottle changes. Detailed calculations and metrics will be shared, including cage wash throughput, staffing levels, and three years of customer satisfaction ratings and hood availability ratings.

PS103 Quality versus Quantity of Feed for Mice: Redefining Ad Libitum Diet

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The amount of feed provided to laboratory mice in a standard mouse cage is much more than they consume in a week, causing a substantial amount of waste and consumption of stale feed. This limits their access to fresh feed and is wasteful as a significant amount of feed is discarded during routine cage changing. This study first determined the daily feed usage of adult mice as a baseline. The mice were placed in a standard clean cage setup with a feed filled to the top of the feed hopper and monitored for two weeks. We weighed the initial amount of feed and weekly thereafter. The rate of feed consumption for the first week was compared to the second week. This trial was repeated three times for a total of 6 wk. The daily average feed consumption, as expected, was 4.18 g/mouse/d. The data on the feed consumption rate showed a significant drop in the second week of every period (average: 28.36%). After the study, a new standard was determined as the maximum amount of feed necessary per feeder. This new standard feed level is close to the amount the mice use in a week. Therefore, providing more fresh feed during regular cage changes decreases the amount of feed discarded. This study allowed us to redefine our ad libitum level, provide better quality feed to the mice, and reduce feed waste to 60%.

PS104 Aging Under Pressure: Conditions that Affect Water Valve Failure

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Every rodent facility utilizing an automated watering system will encounter cage flooding due to water valve failure. The impact can be significant, as resulting animal fatalities can impact both animal welfare and research outcomes. To date, the specific mechanism of water valve failure is understood; however, information regarding other factors that may contribute to the failure process, such as duration of valve use and water pressure, has not been examined. A study artificially simulating water valve aging combined with an analysis of drinking valve failure indicated there may be a correlation between age of the valve and failure rates. This led to the implementation of a valve replacement program, which has significantly reduced cage flooding. While testing a new valve, we found differences in flow between different facilities. Upon further investigation we realized the location of the pressure reducing station affected pressure at the rack level. We learned that lower pressures may contribute to higher valve failures.

PS105 Polycystic Kidney Disease in an Adult Common Marmoset (*Callithrix jacchus*)

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A 7-y-old, socially housed, female common marmoset (*Callithrix jacchus*) presented for a routine, semiannual physical examination. Relevant clinical history included insulin-resistant diabetes mellitus and moderate-severe hepatomegaly. Examination revealed severe, bilateral renomegaly. On abdominal ultrasound, multiple, anechoic foci of varying sizes were found in the symmetrically enlarged kidneys (3.0 x 2.0 cm per kidney). Blood chemistry revealed moderate azotemia (urea nitrogen: 73 mg/dL; creatinine: 0.50 mg/dL) and severely elevated gamma-glutamyl transferase (43 IU/L). A complete blood count showed moderate, nonregenerative anemia [hematocrit (HCT): 25.9%]. The common marmoset remained clinically stable and was assigned to a terminal study. One year later, a firm mass in the caudal abdomen was identified on routine examination. Ultrasonographic imaging revealed a large, anechoic, thin-walled, well-circumscribed mass (1.0 x 2.0 cm) that occupied her pelvic region. Blood work showed worsening azotemia (urea nitrogen: 156 mg/dL; creatinine: 0.90 mg/dL) and anemia (HCT: 22%). The differential list for her renomegaly included spontaneous progressive glomerulonephropathy, polycystic kidney disease (PKD), hydronephrosis, amyloidosis, and neoplasia. Differential diagnoses for the caudoventral mass were ovarian cyst and neoplasia. The common marmoset was euthanized due to progressive azotemia and poor body condition. On necropsy, a firm cyst arose from the right ovary. The kidneys were enlarged and firm with irregular, pitted cortices and numerous, small, clear, cortical cysts. Based on histomorphological features, the common marmoset was diagnosed with PKD. Whole genome sequencing (WGS) was performed to identify mutations in genes associated with kidney diseases in people. WGS of the affected common marmoset revealed a homozygous dominant mutation in *PKHD1*, a gene linked to autosomal dominant PKD in human patients. Comparison of genome sequences from over 130 marmosets in our colony revealed homozygous dominant *PKHD1* gene mutations in approximately half of these animals. Histological and comprehensive WGS findings that may contribute further understanding of kidney disease presentation in marmosets will be discussed.

PS106 Lysosomal Storage Disease in a Sentinel Mouse

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Twelve female Crl:CD-1(ICR) mice presented for routine sentinel health monitoring. After CO_2 asphyxiation followed by cardiac exsanguination, 11 mice appeared grossly normal, however significant abdominal distention was noted in 1 mouse. On abdominal palpation, a structure, presumed to be the liver, felt smooth with no masses, but was enlarged and extended beyond the ribs. Differential diagnoses were chronic liver disorders including: lipidosis, amyloidosis, lymphoma, and infectious hepatitis. A complete blood cell count was performed, revealing an inflammatory leukogram, and a mild nonregenerative anemia and thrombocytopenia. On necropsy, the liver was pale and enlarged, but sank in formalin, moving hepatic lipidosis down the differential list. Diffuse pallor throughout the abdomen was noted, including the gastrointestinal tract, pancreas, and reproductive tract. A soft tissue structure located near the root of the mesentery had a multilobulated, foamy appearance, and was pale. This structure was presumed to be a mesenteric lymph node. A preliminary diagnosis of lymphoma was made, and the mouse was submitted for histopathology. On histology, there were no indications of lymphoma or any neoplastic processes. All grossly affected abdominal organs had foamy macrophage accumulation, and Wright-Giemsa staining displayed the characteristic "sea-blue" cytoplasm confirming a diagnosis of lysosomal storage disease (LSD). LSD is a metabolic disorder that results from defects in the enzymes used to breakdown various substances. Accumulation of the unprocessed material causes tissue damage, necrosis, and organ system failure. There was a 2017 published case report describing spontaneous LSD in a Crl:CD-1(ICR) mouse, where cholesterol ester storage disease was the most likely diagnosis where the viscera was affected while sparing the central nervous system. Unfortunately, the brain was not examined in this case, which would have provided a more complete diagnosis. LSD is a disease of autosomal recessive inheritance and is not a concern in terms of mouse colony health.

PS107 Chronic Elevation of Circulating Gamma-glutamyl Transferase in a Rhesus Macaque (*Macaca mulatta*)

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A 17-y-old, male rhesus macaque (*Macaca mulatta*) enrolled in a chronic addiction study was monitored for persistent elevation of circulating gamma-glutamyl transferase (GGT) since 2018. The monkey remained asymptomatic and was maintained on S-adenosyl methionine. The animal had longstanding vascular access ports and indwelling catheters for 14 y, which had been repeatedly removed and replaced due to dysfunction or infection over that time. From May 2018 to October 2021, GGT and alkaline phosphatase (ALP) levels gradually increased, peaked in November 2020, then slightly decreased and stabilized thereafter. GGT was consistently elevated well above normal range while ALP remained near the upper normal limit. During that period, alanine transaminase (ALT), ALP and aspartate aminotransferase (AST) levels waxed and waned, remaining just above normal range. The animal had marginal hypoproteinemia and hypoalbuminemia. Total bilirubin and cholesterol remained normal. Considering the experimental drugs given, these changes were initially attributed to possible drug induction, biliary disease and/or cholestasis. In August 2021, the animal had palpable hepatomegaly. In April 2022, the animal was asymptomatic but had markedly elevated ALP (999U/L) and GGT (342U/L). Radiographs and ultrasound confirmed hepatomegaly. Histopathology on liver biopsies were interpreted as moderate diffuse amyloidosis. Euthanasia was elected due to poor long term

prognosis. At necropsy, the liver was markedly enlarged, occupied a third of the abdomen, and was mottled and streaked with off-white material, interpreted as amyloid, which was subsequently diagnosed histologically as marked hepatic amyloidosis. About 40% of the median liver lobe was replaced by a large subcapsular hematoma which compressed the gallbladder. Secondary systemic amyloidosis is a progressive disease in macaques that has been associated with chronic inflammation. Despite the high prevalence of this condition in macaques, it remains a challenge to diagnose, and may develop well before animals become symptomatic. The presence of severe lesions in this asymptomatic animal suggests that closer monitoring of GGT, liver imaging and biopsies might be warranted for chronically implanted animals.

PS108 Pyometra Secondary to Medroxyprogesterone-Acetate Treatment in an Adult Cynomolgus Macaque (*Macaca fascicularis)*

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A 12-yr-old, 4.2kg female cynomolgus macaque (*Macaca fascicularis)* developed menorrhagia with regular cycling. Over several months, her menses became progressively longer and heavier, and on physical exam, an enlarged (5.0 x 2.0cm) and firm uterus was palpated. An abdominal ultrasound revealed a thickened, possibly nodular endometrium and small hypoechoic extra-uterine spots that were suggestive of cysts. Differential diagnoses included endometriosis, an endometrial polyp or fibroid, and uterine neoplasia. Based on the high clinical suspicion of endometriosis, she was started on 2mg/kg medroxyprogesterone-acetate (MPA). Her menses began 14 d after the initial MPA dose and continued for 70 consecutive d. Three additional MPA doses were administered through this period, increasing to 6mg/kg. She also developed a mild anemia and was started on iron supplementation. Consistent with endometriosis, the uterus decreased in size (2.5 x 3.0 cm) about 1 mo after initiation of MPA. However, due to the failure to control menses after 3 mo on MPA, she was transitioned to a short-term study with an endpoint of euthanasia 2 mo later. One month after transitioning projects, she had her first regular length menses. During a study-related surgery 2 wk before scheduled euthanasia, foul-smelling vaginal discharge was noted in the absence of other clinical signs. Notable findings on gross necropsy included a markedly enlarged uterus (9.0 x 4.0cm) with myometrial and endometrial thickening, necrotic tissue, and purulent material between the myometrium and endometrium. *Bacteroides fragilis* was cultured from the uterus. Histology revealed marked endometrial hyperplasia of the uterus with regions of necrosis and neutrophilic infiltrates. Both ovaries had multiple cystic follicles. Endometrial hyperplasia has been reported in macaques secondary to estrogen-producing ovarian pathology and exogenous estrogen administration. Pyometra is rare in both humans and macaques with only one reported incident in a rhesus secondary to prolonged MPA treatment. Prolonged progestin exposure likely created a favorable environment for bacterial growth which culminated in a pyometra. This case highlights an important risk for endometriosis treatment with progestin in macaques.

PS109 Efficacy of a Commercial Primate Diet Containing Fenbendazole in Treating Gastrointestinal Parasites in a Captive Baboon Colony (*Papio* sp.)

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An institution houses approximately 1,000 baboons (*Papio* spp.), nearly all in group housing. In 2019, group fecal samples showed a 78% positivity rate for *Trichuris* sp. and 23% for *Strongyloides* sp. using a formalin-ethyl acetate concentration technique. A pilot study was performed to assess the ability of a commercial diet containing fenbendazole to treat animals infected with *Trichuris* sp. Animals were held in group outdoor sheltered housing on a concrete substrate. The diet was given to 25 baboons in 2 groups, aged 2.5 to 14.5 y, for 5 d, followed by another 5-d treatment 2 mo later. Animals were assessed by individual fecal egg count (FEC) using a Modified McMaster technique prior to initiation of treatment, and again at weeks 4 and 14. Baseline FEC results showed a mean of 281 eggs per gram (EPG) in group 1 (range 50-1025 EPG, n=12) and 288 EPG (range 25-1675 EPG, n=13) in group 2. Two weeks after completion of the first round of treatment, this was reduced to 0 EPG in all animals, for a 100% reduction. Animals remained negative by FEC through week 14, 1 month following completion of the second round of treatment. This regimen was next assessed for the ability to mitigate *Trichuris* sp. and *Strongyloides* sp. infection throughout the colony in various sheltered outdoor groups on concrete substrate, with some experiencing periodic indoor housing. The fenbendazole diet was given concurrently to all baboons daily for 5 d, repeated at 2 mo, and again every 4 mo thereafter, beginning in January 2020. Animals were assessed by group fecal examination at 6-mo intervals, coinciding with semiannual health examinations. The prevalence of *Trichuris* sp. declined from 78% in 2019 to 4% in 2020 and 0% in 2021, and the prevalence of *Strongyloides* sp. declined from 23% in 2019 to 0% in 2020 and 2021. These results using the medicated diet were achieved in a large socially housed research baboon colony and without changes in routine husbandry practices.

PS110 Parasitic Enteritis (*Aggregata* spp.) in a Wild-caught California Two-spot Octopus (*Octopus bimaculoides*)

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An experimentally naïve, singly housed wild-caught male California two-spot octopus (*Octopus bimaculoides*) of unknown age was presented for acute onset of decreased food intake, decreased activity, sloughing skin lesions, and generalized poor-doing. Uneaten live prey (fiddler crabs of the family Ocypodidae) were present in the tank at the time of evaluation. Differential diagnoses included senescence, primary or secondary infection (bacterial, viral, and parasitic), and water quality issues. The animal was euthanized and swabs from cutaneous lesions were collected for aerobic and anaerobic culture prior to necropsy. Gross lesions were limited to multifocal to coalescing regions of depigmentation and ulceration of the mantle and more severe ulceration with loss of underlying tissue on several arms. Though not apparent grossly, severe, chronic, ulcerative typhlitis and enteritis with intralesional coccidian macro- and microgametes were identified histologically. The Apicomplexan morphology was consistent with an *Aggregata* spp. infection, which was credited with the animal's rapid clinical decline. Cutaneous lesions were characterized by loss of normal epithelial cell populations, edema, karyorrhectic debris, and abundant bacterial rods. Culture results revealed light growth of coagulase-negative *Staphylococcus* and mixed *Vibrio* spp. which were considered secondary opportunistic pathogens and commensals. *Aggregata* infection has been documented as a common cause of disease in various *Octopus* spp., particularly in senescent animals. As an Apicomplexan parasite, *Aggregata* requires a 2-host life cycle with asexual and sexual stages in crustaceans and cephalopods, respectively. Because sourcing of this animal precluded a complete history, it is possible that infection was precipitated by the onset of senescence—a period of gradual biologic deterioration associated with anorexia that ultimately culminates in death following reproductive activity in *Octopus* spp. The use of wild-caught octopuses and crustacean prey species in research settings

inherently increases the risk of naturally acquired infections and should be taken into consideration for the captive management of these species.

PS111 Silly Rabbits, Autoimmune Disorders Are for Immunocompetent Animals: A Case Report of a SCID-associated Autoimmune Disorder in Nude Rabbits

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A related cohort of 3 juvenile naïve FOXN1 mutant nude rabbits (NuRabbit) was euthanized due to nonspecific clinical signs that decreased quality of life. All NuRabbits were maintained on prophylactic antibiotics and housed under enhanced biosecurity. Prior to weaning, the NuRabbits showed mild-moderate recurrent conjunctivitis responsive to topical broad-spectrum antibiotics. Around 2-mo-old, the NuRabbits developed lethargy, slow progressive weight loss, and a correlated loss in body condition score despite an adequate appetite. Bloodwork was performed for 2 of the 3 NuRabbits. A complete blood count showed an expected lymphopenia for both animals with a differential cell count of primarily heterophils (91%) for one animal and eosinophils (95%) for the other. Chemical analysis showed mild hypoproteinemia, mild azotemia, and mild elevated liver enzymes. The NuRabbits continued to decline in attitude and body condition despite additional antibiotics, caloric support, appetite stimulants, and supportive care. All 3 developed a progressive dry cough with scant serous nasal discharge and tachypnea. Cardiothoracic auscultation remained unremarkable. Bacterial pneumonia was suspected; however, repeated cultures performed on multiple various samples including fresh post-mortem lung, liver, and nasal wash grew only normal commensal organisms. The NuRabbits were euthanized between 2.5-4 mo of age. Histopathologic evaluation showed severe lymphocytic, heterophilic, plasmacytic, and eosinophilic infiltration in skeletal and heart muscle, liver and gallbladder, reproductive organs, eyes, and joints. Interstitial pneumonia was characterized by eosinophilic infiltration stemming from unexpected bronchiolar associated lymphoid tissue. An autoimmune disorder is suspected, either primary to genetic or local dysregulation or secondary from a microbial insult. Case reports of SCID rodent models and patients that develop autoimmune disorders theorize the pathogenesis through decreased central and peripheral tolerance, increased environmental insults, or persistent and chronic infections. This is the first case report of NuRabbits demonstrating a SCID-associated autoimmune phenomena.

PS112 Best Study Conduct and Data Management Practices for Improving Animal Study Reproducibility

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The Reproducibility Project: Cancer Biology attempted to replicate experiments from high-impact cancer biology publications. Researchers found that the original positive results "were only half (40%) as likely to replicate successfully than original null results (80%)" and concluded that there are "opportunities to improve the transparency, sharing, and rigor of preclinical research to advance the pace of discovery." The field of in vivo research presents unique challenges to generate results with a high-level of integrity, detail, and reproducibility on a consistent basis. The findings of the Reproducibility Project underscore the need to critically examine, improve, and standardize processes for animal study conduct. Researchers must understand the factors contributing to poor data quality and irreproducible study results and implement effective best practices for data integrity, study conduct, and scientific rigor. Researchers will gain an understanding of some success factors and practical approaches and solutions to make animal studies more reproducible.