

# Abstracts of Scientific Presentations

## 2018 AALAS National Meeting

### Baltimore, Maryland

#### Poster Sessions

##### **P1 Food for Thought: The Development of Drug-loaded Diets to Improve Both Science and Welfare**

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As an in vivo cancer unit, we grow human tumors in immunodeficient mice such as CD-1 nude, which may require the addition of hormones to promote the growth of prostate and breast tumors. Traditionally, the supplement was delivered by subcutaneous slow-release pellets. However, due to supply issues with the 5- $\alpha$ -DHT pellets, we had to develop another way to provide this hormone. We decided the best method for both staff and animals would be to supply it in a fortified diet, which would be less invasive and stressful to the animals while being simpler for the staff to deliver. We worked with a commercial diet company to formulate and produce the diet to ensure correct dosage delivery. Then we tested it using the LNCaP prostate tumor cell line and found it to be as effective in stimulating growth as the pellets. After this success, we decided to adopt this same method for 17- $\beta$ -Estradiol delivery, because these pellets can also cause additional side effects such as bladder calculi and urine scald, which we hoped would be alleviated by changing the delivery method. A test using the MCF-7 breast tumor cell line demonstrated that growth, while slower than in the pellet bearing mice, was sufficient for our studies, with no side effects apart from slight urinary retention, which was eliminated by removing the fortified diet for a few days. This led us to consider whether oral therapeutic drugs could also be delivered via diet, since ensuring the required dose is achieved via drinking water is difficult, due to spillage or reluctance to drink water containing unpleasant tasting compounds. Also, oral dosing can be stressful with welfare implications for the animal, requiring frequent restraint and invasive procedures. To this end, we have recently been involved in a therapy trial whereby a novel test compound for oral delivery was formulated as a diet. Administration via diet provides a simple solution for oral drug delivery and is easily transferable to a broad range of other animal model systems requiring regular oral dosing of substances such as stimulation of transgenic models. It is, therefore, a major refinement in welfare terms by reducing the need for invasive implants or injection regimes and by reducing side effects, while still generating consistent scientific outcomes.

##### **P2 The Postapproval Survival Surgery Review: A Postapproval Monitoring Tool to Evaluate Compliance and Improve Animal Welfare**

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In order to ensure compliance with regulatory requirements and optimal animal welfare, our Institutional Animal Care and Use Program has developed a unique and innovative aspect of the postapproval monitoring program, the postapproval survival surgery review (PASSR). The IACUC instructs all researchers who conduct survival surgical procedures or who assist with survival surgery (such as monitoring anesthesia and monitoring post-op) to have a PASSR

once approximately every 3 y. The purpose of this in-person training is to review protocol requirements and campus-specific policies and guidelines that involve survival surgery to assure that quality standards are being met regarding the administration of anesthetics as well as pre-, intra-, and postoperative analgesics. These meetings, held by the training and compliance staff members, also provide laboratory personnel with an opportunity to obtain guidance on best practices or review any aspects of their protocols that require clarification. Since its inception in 2009, this important component of postapproval monitoring has identified multiple instances of significant incongruencies between approved protocols and surgical records reviewed, as well as internal protocol contradictions. The most commonly identified issues are postoperative analgesia not being administered per protocol (for example, 1 of the multimodal analgesics is missing, dose intervals and quantities not followed), missing documentation, and inadequate surgical preparation. At our campus, 210 laboratories are currently approved to conduct survival surgery and 21 of them (10%) had noncompliance issues identified between 01/01/2016 and 06/30/2018. Only 3 of the 21 labs had never had a PASSR. For the 18 labs that did have one, the average number of days elapsed from the time of training to the time at which we identified a noncompliance issue was 705 with a very wide range (4 days to nearly 5 years). In order to evaluate the effectiveness of this model, correlations (or lack of) between noncompliance instances found during semiannual inspections and time elapsed since last PASSR, the presence of PI during the meeting, and other potentially influential factors will be presented, as well as lessons learned and potential limits of this model.

##### **P3 Operant Conditioning of Laboratory Beagles**

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We have a large (350+) colony of chronically housed beagles. In early 2016, several of our animal care technicians came up with the idea of training the beagles with some basic commands in order to help the techs better perform their husbandry duties. Examples of behaviors for training included dogs that would refuse to move into their home cage for feeding/separating, dogs that would leap out of the cage when the door was opened, dogs that would keep their feet in their food bowls when the techs would attempt to remove the bowl. We began with a small pool of 10 dogs, the worst offenders of these behaviors, and created a plan using a clicker and target training to teach the dogs to move on command and to hold still when the door to their cage was opened. In 2018, we have now successfully trained over 150 dogs using operant conditioning strategies. Our dog training program has also grown to include the training of dogs displaying stereotypic and other concerning behaviors. We describe the processes we have used in our training, our success rate, maintenance of training, and some obstacles that we have faced along the way.

##### **P4 Accurate Animal Tracking: From Weaning to Necropsy**

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Using radio frequency identification for electronic study tracking, including animals, surgery, dosing, necropsy, data acquisition, and management can improve accuracy, quality, and efficiency during lab

animal research. Monitoring test subjects can start before the study begins. Using programmable RFID microchip technology and software to capture, process, and analyze data, researchers can create a complete and accurate record for each individual. The tracking can start before any dosing begins, as far back as weaning. Using this technology we were able to track groups of research animals starting with the implantation of a medical device and vascular catheters until necropsy. Monitoring outcomes is an important part of a surgery lab. Documenting and working on improvement, surgery model, procedures, or surgeon is key to maintaining the 3Rs. We complete over 60,000 surgeries per year so monitoring quality and efficiency in a paper-based system leaves us with a lot of extra data entry and delays recognition of issues. We looked for an easy-to-use program that could monitor individual animal, surgical procedures, surgeon success/failure, and surgeon efficiency. Using this new surgery application we have been able to increase efficiency and monitor and identify surgeon success and retraining needs. We have been able to provide a complete history of what the animal received for analgesics, anesthetics, and when they received them. We can also monitor animal checks, when they occurred, and any recurring issues that appear post-surgery. With all of this data, we have improved training, efficiency, identification of surgery issues, and procedural problems. We can also identify highly successful surgeons and use them to train our new staff as well as staff that may be having issues with certain surgeries.

#### **P5 A Focus on Human Interaction and Increased Welfare in a Large Beagle Colony**

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We have a sizeable population (350+) of long-term beagles that are used mainly as pharmacokinetic, toxicology, safety, and surgical models. All of our dogs are purpose-bred and enter our colony around ~6-9 months of age and remain until study termination or a pre-determined retirement date (~4.25 years of age). While we do have a well-established dog play program in place, which incorporates volunteers from other areas of our campus taking dogs to a designated play area on a rotating schedule, over the past ~2 y we have placed heavy emphasis on increased quality human interactions and training with our dogs. The emphasis has mainly involved tasking our behavioral, husbandry, and veterinary staff (in addition to the dog play volunteers) with providing additional human interaction activities whenever possible. Additional human interaction activities begin at dog receipt and can include, but are not limited to, encouraging staff to treat all of our dogs as individuals, training of dogs, naming of animals, taking dogs for walks around the vivarium, petting/interacting with as many dogs as possible when working in a specific room, removing dogs in compatible groups for open floor play time (home room/ designated playroom), brushing sessions, outdoor play in compatible groups, increased enrichment strategies, and play with novel and challenging toys. While we have found this program to be extremely rewarding on so many levels, for both staff and our dogs, as well as bringing about a significant increase in animal welfare, there have also been lessons learned throughout the process.

#### **P6 Using of Microsampling Technique for Rat Toxicology Study: Advantage and Evaluation of Impact**

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Serial blood sampling is regularly conducted on rodents to evaluate toxicokinetic profile and clinical pathology in a toxicology study. However, due to the limit of blood volume, serial blood sampling is difficult to perform on a single rat. Large animal numbers are required to acquire sufficient data by standard (staggered) design. Using a microsampling technique to reduce blood collection amount results in the reduction of animal numbers in adherence to the 3Rs. The purpose of this study was to evaluate microsampling technique in rats using

acetaminophen once daily via oral gavage. Microsampling and standard sampling were employed and clinical pathology and TK profile were compared. Three groups of SD rats were assigned to a 7-d repeated dose study. Animal numbers for control and microsampling group was 4 rats/sex/group (n=4) and standard sampling group was 8 rats/sex/group (n=8). For microsampling group, approximately 0.15 mL of blood was collected. For the standard sampling group, approximately 0.3 mL of blood was collected by staggered design. TK time points were conducted at 0, 30 min, 1, 2, 4, 6, 8, and 24 h postdose on day 1 and day 7. Clinical pathology was conducted on day 8 at endpoint. Although the total collection volume was lower than the recommended limit, slight anemia including decreases in RBC, HGB, HCT and physiological increases in RDW and reticulocyte were noted in both standard and microsampling groups caused by repeated blood collections. This confirmed that the TK animals had different physiological status in hematology and should be independent from systemic toxicology evaluation, despite the sampling techniques. TK profile was similar in 2 designs. In standard sampling design, samples were not collected from the same animals to the next time point and only 1 TK curve can be generated. In microsampling design, a single animal provided all-time point samples and single TK curve was available. Microsampling provided more information to evaluate the individual variation. Microsampling in rats reduced animal numbers, study cost, and refined the study design. The results also provide more valuable information for TK evaluation than traditional standard design.

#### **P7 Comparison of Housing Conditions to Ameliorate Aggression in Male Mice**

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Fighting among male laboratory mice is significant as it confounds scientific data, impacts animal welfare, and increases overall study costs. In particular, the C57BL/6 inbred strain is known to demonstrate especially aggressive behavior towards other male cage mates. Behavioral and physical observations were made with reference to aggressive behavior between male cage mates during subsequent studies that employed different housing and care standards. The parameters and procedures of each study were identical aside from the experimental compounds tested. All compounds were delivered orally as amalgamated in a Western diet. In a traditional set of conditions (TRAD), mice were housed 5 per cage, and facilities staff completed cage/water bottle changes. Enrichment included an igloo, intermittent chewing devices, cotton fiber nesting squares, and pouches of nesting material. In our hypothesized improved set of conditions (NON-TRAD), mice were housed 4 per cage, and research staff performed cage/water bottle changes. A dedicated research team member performed husbandry tasks simultaneously with daily observations, which reduced the number of cage disturbances as well as unfamiliar scents, thus presumably decreasing animal stress. Enrichment in NON-TRAD cages included gnawing sticks, cotton fiber nesting squares, and nesting material removed from its outer pouch. These changes were implemented as igloos and pouches were hypothesized to be territorial triggers for fighting behavior. Observations were recorded daily with respect to fighting, separations, and deaths as a result of fighting, for up to 433 d. Our results demonstrate that NON-TRAD groups yielded >65% reduction in fighting incidences, eliminated all deaths/euthanasia resultant of fight injuries, and nullified the need for single-housing. There were no effects of either sibling status or experimental treatment on aggression outcomes. Reducing the number and severity of fighting incidents can improve overall wellbeing, lower costs, and generate more reliable data. We conclude that our NON-TRAD conditions resulted in superior animal welfare, and side-by-side analysis additionally showed >17% decrease in overall costs.

### **P8 Development of a Laboratory Animal Individually Ventilated Cage Preference Test for Bedding and Enrichment Refinement**

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It has been shown that laboratory rodent housing environments can have a significant impact on the animals' welfare and affect the overall quality of research. There are several options for rodent cage types, bedding, and enrichment available on the market today. Previous studies have compared differences in housing environments with choice-based preference tests using static housing systems with either room- or cabinet-based ventilation. Many facilities have transitioned from static to individually ventilated caging (IVC), including this institute. A widely recommended bedding for IVC is corncob due to its reportedly lower absorption of moisture which leads to higher evaporation of urine and lower NH<sub>3</sub> levels. Reports by other laboratories have shown that cob-based beddings are not preferred by the animal when housed in static caging. The purpose of developing this preference-testing protocol was to evaluate which bedding and enrichment mice would prefer in an IVC. We developed an on-rack preference testing method using our IVC system. Two cages joined with a small length of PVC tubing are placed on the rack and monitored using behavioral analysis software to determine time spent in each environment. Animals are raised on paper bedding before being placed in the preference cages. To maintain consistency animals are placed on the same side and corncob or wood chip bedding is alternated between sides. Each cage receives the same amount of bedding, enrichment, food, and water. Monitoring of waste gasses CO<sub>2</sub> and NH<sub>3</sub>, light levels, nest scoring, and fecal accumulation can be done to determine any impact these may have on the animals housing preference. Establishing this protocol will allow us to evaluate current bedding and nesting options that maximize mouse wellbeing and welfare.

### **P9 Evaluation of Alternative Techniques for Blood Collection in Hamsters**

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Hamsters have historically been used in pharmacokinetic (PK) studies, often in research to study hepatic lipid metabolism. In these studies, retroorbital (RO) blood collection has been traditionally used, but this technique is limiting in both the number of samples and total volume that can be collected. This dramatically increases the number of animals needed to collect a full PK profile and can cause significant stress to the animals, adding to the financial and animal welfare cost of PK studies. Since the IACUC at our institution prohibits the use of RO blood collection (with rare exception), we needed to explore alternative methods for collecting blood in hamsters. We hypothesized that anesthetized jugular vein, saphenous vein, and tail nick methods of blood collection could be used for serial, moderate volume blood draws as alternatives to RO bleeds which would yield sufficient volume samples with lower stress levels and tissue damage. The study design involved a total of 56 animals, 7 groups (RO, jugular, saphenous, tail nick, isoflurane, and naïve) with 8 animals per group. Groups 1-5 were PO dosed with a known compound. One hundred microliters of blood were collected at 0 min, 30 min, 60 min, 120 min, 160 min, 240 min, and 1440 min. Group 1 RO had 2 nonterminal time points per animal collected; Group 2-5 had 7 nonterminal time points collected. Blood collection methods were compared to assess sample quality, drug concentration, and animal welfare concerns. Collection site evaluation and technical requirements along with a PK profile were collected. The PK curve was similar across collection sites to include the comparison of serial versus composite collections. Serial sampling from saphenous collection was difficult, leading to multiple attempts, an inability to collect all samples, and bruising at collection

site. Jugular collection required fewer attempts per sample with decreased bruising, while the novel tail collection method had the fewest attempts per sample, was reliable, and caused no bruising at the collection site. Based on the PK profile and experiential data collected, we conclude that jugular blood draws and the novel alternative of tail bleeding are acceptable approaches to serial blood collection in hamsters.

### **P10 Procedure for Repeated Ultrasound-guided Liver Biopsies in Nonhuman Primates**

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Safely collecting any biopsy sample from an internal organ is always a challenge; adding the need to make successive collections increases the difficulty. We chose ultrasound-guided biopsies as the collection method to monitor the changes in the livers of Cynomolgus monkeys (*Macaca fascicularis*) over several months. This method offered a minimal level of stress for the animal, a low chance of complications, and a short recovery period. With the animal under chemical anesthesia using a ketamine/xylazine mixture, the technician used the ultrasound system's 2D mode to visually identify the liver and gallbladder while inserting a 16-gauge biopsy needle. A percutaneous biopsy specimen was then collected from the right side of the liver. The procedure consistently yielded a 1.5cm length of useable liver tissue for analysis. Following the procedure, only minimal pain management was needed, with 0.1 mg/kg of meloxicam being administered for 3 consecutive d. After performing 311 ultrasound-guided biopsies across 119 animals, with most animals having received 3 biopsies over the course of 70 days d we did not have a single major issue during the biopsy or recovery. Ultrasound-guided liver biopsies have proven a minimally invasive, well tolerated, and safe method for repeated biopsy collection from the liver in nonhuman primates.

### **P11 Does Bedding Type Have an Effect on Breeding Performance in Mice?**

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Few studies have directly assessed the influence of different contact bedding types on mouse breeding performance and offspring survival and growth. We evaluated breeding performance of C57BL/6J mice by comparing 2 different contact bedding types: 40 IVC cages on aspen chip and 40 IVC cages on corn cob. We monitored the following variables over 6 consecutive mo: number of pups at birth, number of pups weaned per litter, average weight of weanlings at 21 d and the number of days between litters. The purpose of this study was to evaluate contact bedding types and assess if these conditions have an impact on breeding performance. This study supports that there were no significant differences for any of the study parameters between aspen chip and corn cob bedding. With these results, selection of bedding type may be based on factors other than breeding performance.

### **P12 Comparison of Wellbeing of ICR Mice Euthanized with CO<sub>2</sub> in an Induction Chamber as Compared to the Home Cage.**

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Based on literature that demonstrates a stress response associated in mice when placed in a novel environment, it is recommended that mice to be euthanized remain in their home cage to minimize distress.

However, when using an inhalant anesthetic to anesthetize mice for surgery, an induction chamber is recommended. Minimizing the distress of induction during anesthesia, whether for surgery or for euthanasia, is required to improve the wellbeing of mice used in research. For this reason, it is critical to determine which of these 2 scenarios, home cage versus induction chamber, is truly better at decreasing the potential distress experienced by the mouse. In this study, individually housed mice were euthanized with 30% volume displacement/min 100% CO<sub>2</sub> in either a home cage or an induction chamber. Behavioral assessment of jumping, digging, rearing, and sniffing at the gas entry point were compared between groups, as were noradrenaline, blood glucose, and corticosterone. There were no significant differences between the animals euthanized in the home cage as compared to those euthanized in the induction chamber in all measured parameters. The results of this study suggest that, from the perspective of the ICR mouse, the use of the home cage confers no significant advantage to the induction chamber when euthanizing with CO<sub>2</sub>.

### **P13 Habituation to Gentle Handling May Improve Success of Basic Rat Hands-on Training**

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Our institution established the Responsible Care and Use of Laboratory Animals (RCULA) training program with the aim to educate and certify personnel involved in using laboratory animals for scientific purposes. The species-specific online and hands-on training classes are designed to equip researchers with the basic skills required to work with research animals. This includes competency assessments for proper handling and restraint. In the past 6 y, we have seen approximately 1 rat bite incident a year, and excessive rat squeaking, struggling, and occasional porphyrin staining during the hands-on training. As part of improving occupational safety and reducing the risk of injury or distress to the rats, our goal is to identify measures that will help improve the interactions between rats and handlers. "Gentling" or gentle handling by personnel has been reported to reduce fear in rats. To accomplish this, we initiated 3 consecutive days of habituation by gently handling the rats before a hands-on class. The trainer started by touching the rat gently within the cage. Once accustomed, we placed it on the cage's wire bar. With 1 hand gripped firmly but gently around its chest, the rat was lifted off the wire bar and the other hand is used to support the lower body. This was done repeatedly until the rats were comfortable, as determined by the absence of squeaking, chattering, struggling, and porphyrin staining. After 3 d of interaction, if the rats still exhibited anxiety, they were not used for training; instead, they underwent another 3 d of handling before the next class. Since instituting the habituation process in February 2018, we noticed that the rats exhibited significantly less vocalization and struggling during the handling and restraint class session. Our rats' hands-on training has also maintained a 0 rat-bite incident record ever since. We will continue to monitor the changes in anxiety-related behavior in the rats as well as the number of bite incidents with this approach to quantify the success of this initiative.

### **P14 Use of a Postprocedural Veterinary Report for Postapproval Monitoring and Ongoing Program Review in a Mouse Facility**

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A part-time or contract attending veterinarian must be knowledgeable about and involved in many specific areas of the institutional animal care and use program, especially those areas with the potential for pain and distress to the animals. It is also important to document ongoing veterinary involvement for the IACUC and any appropriate regulatory or accrediting organizations. The use of a postprocedural veterinary

report in the mouse facility software program accomplishes these general goals as well as allowing regular review of individual invasive procedures. A surgical record accompanies each cage of mice for at least 10 d following any procedure requiring anesthesia. During weekly veterinary visits, these records are reviewed, the animals examined, and a veterinary assessment is added to the archival record. This allows specific veterinary review of anesthesia, analgesia, training and competence of surgical staff, assessment of newly implemented experimental techniques, as well as a variety of other possible assessments. A small-time investment provides an excellent opportunity for postapproval monitoring and ongoing communication between the veterinarian and technical staff.

### **P15 Harmonizing Global Training Using a Customizable Skill Assessment Document**

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How does a large diverse organization with a collaborative team of employees provide consistent and reliable data when working across multidisciplinary sites? Training has increased efficiencies in workflow, increasing self-worth and empowering employees for future advancement. This also ensures the consistent application of the *Guide* standards in promoting the 3Rs by decreasing the number of animals placed on studies. The challenge for a company with many global locations was to use a set of metrics to improve employee performance and to establish a standardized assessment process for competency and proficiency. Our competency model of Technical Assessment of Skills and Knowledge (TASK) was developed to meet this challenge. This documentation was used as a method to track and communicate with employees as they moved through the process. They were quickly identified with the appropriate skill sets so to be matched up to study needs or requirements. Based upon training and TASK documentation, our Discovery Services site was able to match skill sets to the appropriate study and were ultimately able to reduce the number of animals, decrease the variability in the data and obtain reliable and reproducible data.

### **P16 Retiring Research Companion Animals: What You Need to Know About the Laws and Partnering with a Rescue Organization**

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In 2014, Minnesota was the first state to pass the Beagle Freedom Bill, which requires publicly funded research institutions to offer adoptable research cats and dogs to nonprofit rescues. This began a movement of public participation in governing the disposition of research animals. Multiple states have passed similar laws, including New York and Connecticut, and many other states are submitting the Beagle Freedom Bill to their state legislation. Therefore, it is vital for research institutions to understand the state laws, communicate with nonprofit rescues, and realize the impact these laws will have on institutional policy. Our university has a rich history of placing adoptable research animals in private homes by using both private adoptions and partnering with local rescue organizations. The Beagle Freedom Bill emphasizes creating a partnership with rescue organizations. Therefore, we sought to gain a better understanding of how informed rescue organizations are of this legislation. Animal rescue groups were chosen based on proximity to the university and were asked a series of questions which included knowledge of the Bill, policies for the adoption of research animals, and their general perception of research animals. We provide education regarding the Beagle Freedom Bill, outline the responses collected from rescue organizations, and highlight the structure of our successful adoption program, which includes rescue organization partnership. Overall, our goal is to be a resource to institutions developing an adoption program and to

provide insight on what to expect when creating partnerships with the rescue organization community.

### **P17 An Electronic Animal Numbers Calculator: A Simple and Robust Protocol Tool**

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Per federal policies and regulations, IACUCs must review animal use numbers to ensure appropriate justification as well as accurate calculation. Investigators should describe the minimum number of animals required to accomplish study aims and achieve statistical significance. Often, calculations underlying this reported number are fraught with arithmetic and study design errors, which may result in animal use beyond what is necessary to achieve research objectives. Two such potential outcomes are overpowering a study and repeating a previously underpowered study. Further, determination of the correct allocation is intricate and time-consuming, depending on several variables including but not limited to numbers of experimental treatments, key time points for analysis, strain or lines under study, and the potential need for experimental replicates. To simplify and streamline the process, we have created an electronic calculator in Microsoft Excel for aggregating and totaling animals required per experimental aim as well as per overall animal use protocol. Following inputs, such as group sizes and genotypes of interest, the calculator instantly displays the animal counts required. All calculations may then be compared to requested totals in the protocol application as well as to initial study designs to ensure accuracy and feasibility. We encourage laboratories or animal resource centers to adopt this calculator or a similar tool to significantly reduce investigator calculation time, protocol review time, and likelihood for incongruence within protocols. In combination with sound power analyses, the calculator provides a robust verification method to simplify the critical question of how many animals should be used in a study.

### **P18 Pushing the Limits of Automated Blood Sampling Using Combined Technologies to Enhance Animal Welfare**

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Manual blood collection has historically been used for short-term pharmacokinetic (PK) studies. In these studies, it can be time-consuming and difficult for technicians to collect multiple samples back-to-back. In addition, the animal handling required during the collection periods can increase stress, introduce pathogens, or confound the study results. Several commercially available automated blood sampling (ABS) systems can be employed instead of manual sampling. Using these systems has been shown to reduce animal stress and can have a positive impact on animal usage and data collection. However, ABS systems have limitations when it comes to longer-term studies, such as a bioavailability study with a several day washout. We hoped to expand the length of time an animal could be sampled on an ABS system without having to increase animal numbers. To achieve this, we combined an ABS system with a miniaturized vascular access port (mVAP). This combination made it possible to collect blood samples from awake, freely moving Sprague-Dawley (n=6) rats over a 28-d period. Animals were monitored for patency, corticosterone levels, and blood chemistry to ensure that the study system did not cause adverse effects. Samples were collected throughout the 28-d study, and all 6 rats remained patent at the end of study. Combining the ABS and mVAP systems improved the study in the following ways: 1) animal handling for connection and dosing was quick and clean 2) reduced animal handling reduced the risk of accidents and bites 3) protective covers on the VB decreased the risk of catheter damage 4) animals were able to be group-housed during noncollection periods 4) catheter patency was maintained in a higher percentage of animals over the course of the study than was available with a standard catheter and

manual sampling, resulting in the need for fewer back-up animals. Combining 2 commercially available technologies helped us enact the 3Rs while improving study outcomes.

### **P19 IACLAM Review of the Diplomates' Survey and Identification of Emerging Global Issues Challenging the Specialty of Laboratory Animal Medicine**

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Laboratory animal medicine (LAM) has been a specialty for over 60 years, and now reflects the international nature of all animal-based research and science. IACLAM was founded in 2005 to facilitate LAM colleagues to communicate globally, exchange information and resources, and to define and harmonize the roles of LAM veterinary specialists across international boundaries. IACLAM helps represent over 1,400 LAM specialists worldwide in four colleges of LAM (ACLAM, ECLAM, JCLAM, KCLAM) to the scientific community, government bodies, and public. Previous mission-related projects included generating results from a survey of trends and practices by diplomates across the globe. Survey results defining the roles and responsibilities of specialists required training and skill sets, and other factors involving the LAM specialty will be presented. This survey identified challenges facing LAM such as euthanasia, transportation, cage space needs, rehoming research animals, and other issues such as addressing the need for specialists and microbiome research. These outcomes will be included. Ongoing projects for IACLAM include a meta-analysis of methods using carbon dioxide for euthanasia and a review of training and certification requirements for the member colleges with a goal of harmonization through resource sharing. New initiatives for IACLAM will be presented, such as development of a global strategic plan and development of position statements on topics for contemporary LAM specialists (such as reproducibility of research results and transportation). Resource sharing should lead to improved animal welfare policies and standards worldwide and enable development of the LAM specialty in countries in which it does not yet formally exist.

### **P20 Is that a Rat in your Pocket? A Novel Pocket Method for Rat Restraint**

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Providing safe and comfortable restraint for laboratory rodents is key to promoting animal welfare and is essential for efficient experimental manipulations. We have developed a sewn restrainer as an alternative for use in our facility. The sewn restrainer consists of a surgical towel that has been sewn into a pocket equipped with a solid base and an adjustable strap that can be secured behind the rear legs of the animal. All components of this restrainer are sanitizable. The base can be removed and cleaned with disinfectant and the cloth portion can be laundered. The premise of this restrainer is identical to that of an ordinary towel that has been folded into a pocket. We find that rats show preference for the dark pocket and acclimate quickly for routine procedures. Placing the animal into the restrainer takes very little effort as most of them willingly walk in. The sewn pocket restrainer, however, offers some advantages over a folded towel. The sewn pocket eliminates the need to refold the towel between animals. The addition of the strap behind the legs frees up a hand and allows for a single person to perform two-handed procedures as needed. The sewn restrainer provides restraint appropriate for procedures such as subcutaneous injections, tail vein injections/blood collections, intraperitoneal injections, and blood collections from the pedal or saphenous veins. We performed a small-scale comparison between using the sewn pocket restrainer and a plain towel for restraint. Briefly, 2 cages of 3 rats were sequentially restrained for 20 s each in each style

of restraint. The timer started as the restrainer was prepared and ended after the last rat was returned to the cage. Preliminary results demonstrated that the sewn pocket restrainer is more efficient. It took an average of approximately 30% longer to restrain 6 rats in the towel compared to the sewn restrainer.

### **P21 Novel Refinements to Improve Postoperative Outcomes in Nonhuman Primates Using Enrichment**

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We often manage nonhuman primate (NHP) studies with unique and varied postoperative challenges. Some NHPs start “exploring” the surgical site around day 5-7 when the healing is in progress and the tissue repair enters its wound healing phase. Local sensations such as pruritus, tingling, crawling, prickling, or numbness are all equally capable of inviting the host intervention in the form of touching, rubbing, or biting. The outcomes may include suture removal, local skin ulceration/damage, hemorrhage, dehiscence, infection, or necrosis and necessitate minor or major repairs. Traditional methods to reduce/eliminate host interference include wound care, analgesics, topical and systemic antibiotics and antihistamines, systemic sedatives and tranquilizers, and use of devices such as jackets, helmets, casting, dressing, or combinations. Some of these strategies have proven ineffective. We’ve developed complementary strategies used alongside the traditional ones and found them to be effective and often capable of replacing the traditional methods. These strategies include playing favorite videos several hours/day, pair housing (despite suture grooming concerns), painting finger and toenails with glitter nail polish, applying multiple paper or plastic stickers to the fur coat, and “gluing” seeds and nuts to the fur coat using honey. By evaluating these cases individually, we can then apply appropriate intervention strategies to improve animal welfare.

### **P22 Use of a Novel Simulation Device to Improve Caretaker Ability to Perform Accurate Rat Body Condition Scoring**

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Accurate body condition (BC) scoring requires understanding of the scoring system and practice. Under optimal conditions, the opportunity to palpate BCs which do not fall within the ideal range is usually not available, hindering one’s ability to gain experience with the entire range of the BC scale. Simulation is a technique that uses artificial situations or tools to provide learners with the opportunity to acquire skills that can later be applied to the real world. This study assessed the effectiveness of training care takers to BC score rats using a novel simulation device (SD) compared to lecture-only training. A within-subject design was used to test the effects of teaching style (lecture versus simulation) on the care taker’s ability to accurately perform rat BC scoring. Fourteen care takers were first given a lecture-based presentation which illustrated the landmarks and described the criteria for determining the BC score for rats. This lecture was then followed by a hands-on palpation exercise with live rats, in which they blindly assigned BC scores to rats, whose scores had been predetermined by a veterinarian. The same care takers were then trained on BC scoring using a SD constructed of a 3-D printed rat skeleton with removable padding. This exercise was then followed by hands-on palpation of the same rats previously described. In comparison to baseline score results (61% accuracy), the care taker’s ability to assign the correct BC scores significantly improved after simulation training in contrast to lecture-based training (88% vs 78% accuracy, respectively,  $P = 0.01$ ). A post-study survey was conducted to obtain the pros and cons of training with the SD. Learners commonly appreciated being able to see and feel the landmarks on an inanimate object, making it easier for them to learn how to perform BC scoring. Overarching negative attributes about the SD was that it was not real and the padding did not closely mimic the feel of actual soft tissues.

Our results show that BC scoring is a skill that is best learned using a hands-on approach. The described SD provides a training tool which allows learners to gain experience palpating the entire spectrum of the rat BC scale without the use of live rats.

### **P23 A Cage-base and Environmental Preference Test for Laboratory Rabbits**

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Measuring rabbit welfare in a research setting presents challenges. The housing is standardized and rabbits may appear to be sedentary and not show observable signs of anxiety. Caged laboratory rabbits normally spend most of their time on a perforated floor-base, or when housed in floor pens, on a wood substrate such as sawdust. However, without being able to ask the animals which resources they prefer, it is not easy to ascertain which environment will most closely meet their needs. Twelve male New Zealand White (NZW) rabbits were individually housed for the duration of the study in 3 connected cages. They were offered a choice from typical substrates available to laboratory rabbits: Aspen wood chips, sawdust, hay, and an empty cage. They were then able to access each substrate via a weighted entry door; the weight of which was increased every 24 h over a 5-d period. The exit door was unweighted. Time spent in each cage was recorded over 20 h, including the dark phase. As the cost of access increased rabbits made the fewest entries to the aspen resource ( $P = 0.004$ ) compared to the other options. However, time spent in the resource-cage decreased for bare (Pearsons,  $R = -0.662$ ,  $P = =0.224$ ), Cellu-dri (Pearsons,  $R = -0.868$ ,  $P = 0.056$ ), and significant for hay (Pearsons,  $R = -0.893$ ,  $P = 0.041$ ). Conversely for aspen, as the cost increased, the amount of time they spent in the resource-cage significantly increased (Pearsons,  $R = 0.946$ ,  $P = 0.015$ ). Results suggest that rabbits preferred the 3 alternatives over the aspen when the cost was low, but as cost increased the time spent with aspen increased indicating a need to access the aspen. Choice of floor types may therefore be more important than providing a single flooring to satisfy a variety of needs.

### **P24 Transparency and Openness in a Pharmaceutical Animal Facility**

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We are a signatory to the Concordant of Openness, an initiative from Understanding Animal Research (UAR) that over 120 UK facilities have joined. Each organization must demonstrate its commitment to transparency regarding the inclusion of animals in our research. Having signed up, we need to produce evidence of our commitment to being open about animal research, which includes raising awareness of the inclusion of animals to the public and our work colleagues and exchanging ideas and work practices across the industry. In order to do this, we highlight areas where we already demonstrate openness. For example, monthly facility tours are open to everyone who works in the company, including animal statistics in our responsible business supplementary report, having a page on our public facing website which focuses on animals in research, and hosting work experience students in the animal facility for a week. Recently, we have focused on a new initiative called the Live Virtual Tour, which enables us to show a larger audience of people around our facility in real time, opening our doors to a much wider internal audience from multiple sites. Part of the live tour initiative includes a pre-recorded virtual reality 3D immersive tour, whereby people can put a headset on and be “in the unit.” This tour has the potential to be taken to science fairs and conferences to showcase the great facilities we have, how we take care of the animals, the science we do, as well as increase our transparency and openness.

### **P25 Animal Research Strategy—Driving Change in Drug Discovery Using Novel Tools to Enhance 3Rs Application**

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Translational relevance of animal models used in drug discovery has come under increased scrutiny. In response, the animal research strategy (ARS) team drives strategic approaches to ensure use of translationally relevant models. Improved understanding of translational relevance of animal experiments a) positively impacts clinical attrition rates, b) impact cycle times, c) decreases animal numbers (eliminating low-value models), and d) creates 3Rs opportunities. ARS encompasses prospective strategy and <span style="background-color:rgb(246, 213, 217)">uses </span>iterative learnings from retrospective evaluations. This allows determination of best-quality models, driven by preclinical-clinical continuity and translational relevance and pathobiology of clinical disease. This strategy is complemented by 3 tools: animal model strategy teams (AMST), animal model quality assessments (AMQA), and after action reviews (AAR). AMSTs consisting of multidisciplinary scientists, providing capability, expertise, and experience leveraged to determine translational modeling pathways and specific scientific question(s) about proposed animal models. AMSTs are positioned in early stage of drug discovery programs to determine modeling strategy but can occur throughout a project as required. Supporting AMSTs, is a question-based assessment tool, AMQA. It is a structured spreadsheet defining translational relevance of models for specific questions. It is applied to mechanistic and disease models for qualitative assessments of the model. Strengths/weaknesses of models are highlighted, providing opportunities to refine or evidence for replacement. AMQAs can be done for all animal models at any point in the development cycle. AARs collate retrospective experiential and literature evidence for internal assets terminated for clinical efficacy. They are focused on animal and nonanimal models used preclinically to explore study design, results, and pathobiology to reveal areas of misalignment compared to the clinic. AARs are beginning implementation and will drive future modelling strategy by identifying non-concordant models, creating 3Rs opportunities. This strategy is applied across multiple therapy areas and forms part of our commitment to ensuring the judicious use of animals

### **P26 The Use of Analgesics Does Not Impact Growth of *Staphylococcus aureus* in a Göttingen Minipig Surgical Wound Infection**

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It is widely reported that the use of analgesics in infection models interferes with bacterial growth of many microorganisms. Some of these reports are in vitro studies or unrelated in vivo studies. Tramadol, a  $\mu$ -opioid receptor binding agent has been shown to have antibacterial activity against *E. coli*, *S. epidermidis*, *S. aureus*, and *P. aeruginosa*. Nonsteroidal anti-inflammatory agents (NSAIDs) have also been shown to inhibit the growth of bacteria. To determine if analgesics could be used without affecting the growth of *S. aureus* in a minipig surgical wound infection model, analgesics were administered to animals that exhibited postsurgical lameness and the effect on the growth of bacteria was evaluated at the end of the study. Male Göttingen minipigs underwent surgery on their left thigh and *S. aureus* was instilled into a surgical wound to the depth of the femur. Buprenorphine was administered preoperatively to all animals. Approximately 46% of animals were administered additional analgesics after surgery. Buprenorphine, carprofen, or a combination of these analgesics were administered until no signs of pain or distress were noted. At the end of the study (8-14 d postinfection) animals were euthanized and skin and muscle from the surgical site were evaluated

for bacterial burden. There were no statistically significant differences in bacterial burden between the group that received only buprenorphine preoperatively and the groups that received additional buprenorphine, carprofen, or a combination. Despite literature precedent contraindicating the use of analgesics with infection models, we have successfully shown that analgesics can be used and should be tested in specific animal models before withholding analgesics and assigning a protocol a USDA Category E status.

### **P27 Realignment and Redesign of a Training Program into the Digital Age**

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Over the past 2 y our facility has undergone a rapid expansion of existing research facilities resulting in 2 new geographically distinct vivaria. The rapid expansion combined with the geographical separation of animal care staff, veterinary technicians, and research staff has presented a challenging environment in which to efficiently and effectively disseminate training materials. Prior to the recent expansion, the training program was able to function on a smaller scale without a need for information standardization across facilities. Online resources were underused as there were fewer trainings and all trainings could be conducted in a live lecture format. As the research facilities expanded the existing training program design became limiting and in some cases the inability to ensure standardized information dissemination between facilities became problematic. As part of a 2017 training program initiative, we sought to use a holistic approach in the redesign of our training program. The redesign started with updating key standard operating procedures (SOPs). Following SOP updates the existing training program was comprehensively restructured in an effort to reinforce training material through the accommodation of different learning styles. Essential training materials were redesigned for efficient online distribution. Both supervisory and animal care staff were validated on standardized operational changes. Building signs and onboarding materials were modified to help reinforce training initiatives. The redesign and implementation process that has occurred over the past year will be discussed citing specific examples. A discussion of how the redesign of the training program has impacted the department over the past year will be included as well as future goals for the training program.

### **P28 Development and Establishment of an Interdisciplinary International Master Program for Laboratory Animal Science: A Contribution to the 3Rs**

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With the implementation of the EU Directive 2010/63 on the protection of laboratory animals, the principles of the 3Rs are for the first time incorporated into the animal welfare law and a declared goal. Therefore, the impartment of knowledge on 3Rs and alternatives to animal experiments has to be part of the qualification of personnel planning, performing, and evaluating animal experiments. Therefore, the RWTH Aachen started an executive master program in laboratory animal science. This program is unique in Germany and qualifies students as LAS specialist. The MLAS is designed as an international, English-language, part-time course. The blended learning concept incorporates e-learning complemented with attendance blocks for practical skills training. This media-supported education concept contains modular designed visualizations of knowledge integrated in the MLAS curriculum and are made available on the online learning platform. The curriculum contains modules addressing ethics and legislation in relation to the use of laboratory animals; biometry, statistics,

experimental design and facility management; and alternatives to animal experiments and laboratory animal science (including genetics, breeding, anatomy, physiology, pathology, and hematology). For the deeper knowledge courses in animal models, anesthesia, and experimental surgery as well as in vivo pharmacology, applied toxicology, microsurgery, and imaging are offered as compulsory and elective modules. In all the modules 3R principles are addressed and applied. Within the attendance block practical skills are taught by using toys, videos, and training models like the silicon ear in order to shorten the learning curve when finally trained on animals. In addition, in vitro and ex vivo techniques are taught during practical skill courses. Altogether, this should facilitate the qualification of persons responsible for directing animal experiments and should train the persons to use and advice other researchers in using alternative strategies.

### **P29 Refinement of Tail Vein Injections in Flank Tumor Xenograft Mouse Models**

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Tail vein injections are a standard way to deliver experimental drugs to laboratory mice. However, these injections not only require a high level of skill, but also proper restraint. Unfortunately, standard restraint devices may not work for all mice. In cancer research, mice are often implanted with tumors in the flank region, and drug treatment begins once tumors reach a defined volume (~100-200 mm<sup>3</sup>). Mice with flank tumors are difficult to secure properly in standard restraint devices and often get stuck in the device if it is too small. To complicate the situation further, the skin of nude mice often get stuck to the sides of plastic restrainers, which can result in skin irritation. We sought to find a simple solution that could be easily taught and used by lab personnel. Using the weighted cup and platform from a small animal tattoo machine, an empty mouse cage, and reusable gel heating pads, we are able to perform tail vein injections on any size mouse, with or without tumors. First, the heating pads are activated and are placed under the empty mouse cage and under the tattoo platform to create a slight angle. Then the mouse is placed into the warmed cage which dilates the lateral tail veins, aiding intravenous injection. After 3-5 min, the mouse is removed and gently placed under the cup on the platform, with the tail placed under the appropriate-sized notch in the lip of the cup for tail vein injection. This method is more cost-effective than having several different sizes of restrainers on hand, and by using a nontraditional restrainer, the mice are less confined and restrained for less time than with a traditional restrainer.

### **P30 Creating a Simulated Rodent Carcass for Euthanasia Training with Common Supplies**

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A standard practice is the training of animal care and research staff so they are experienced and competent when working with animals and in compliance with government and institutional policies. One of the procedures in which workers must be trained is on the euthanasia of research animals at the endpoint of a study. The person(s) conducting euthanasia must be well-informed on the proper technique. At our institution, this training requirement for rodents includes performing a secondary means of euthanasia by cervical dislocation or pneumothorax as formal euthanasia training classes designed to teach the skills needed to successfully perform these procedures. Euthanasia training performed on live rodents in a supervised setting is the historical default method of teaching these skills. However, for many reasons it is preferable to allow trainees, especially those who are novices, to learn and practice initially using nonanimal alternatives where they can grow in knowledge in a safe learning environment in a comfortable setting. Demonstrating how to carry out euthanasia techniques without an animal can pose challenges. We have overcome this obstacle by creating an alternative method that uses carcass and

common items that mimic and simulate performing a secondary means of euthanasia in rodents. Materials used to demonstrate this include a toy mouse and rat used as the carcass, rubber bands, super glue, scissors, flexible bendy straws, velcro, and a laminated printed picture of the thorax. The creation of the faux carcasses has been successful and achieve a less stressful environment for the trainers and trainees knowing there is an alternative available to using live animals.

### **P31 Rat IV Cannula Cather Tip Protection in an Enriched Environment**

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Veterinary technicians have several responsibilities in the laboratory animal field, one of which is using creativity to problem solve within a clinical environment while working closely with research labs. We describe an atypical approach to a challenge with catheter management that arose with socially housed rats for behavioral and addiction studies. For a portion of an experimental study, rats were surgically implanted with an intravenous catheter with cannula to prepare for a drug self-administration study, then subsequently group-housed. Initially, a protective cap made of plastic material was placed over the cannula tip to prevent contamination and provide protection. However, it was observed that the plastic cap endured damage as a result of the chewing behavior of cagemates as the material was destructible. For an expedient solution, we devised a method of covering the cannula tip with a cap made of metal instead. To create the improved cap, we used a Dremel tool to cut and remove sections of metal and shaped them to fit. After several prototype modifications, we arrived at a successful design. Despite the initial failure of the plastic cap, a new in-house cap was refined and implemented as a low-cost replacement to protect the cannula tip without further destruction from cagemates.

### **P32 Film Study and Performance Analysis Tools for Training and Skills Development in Germ-free/Gnotobiotic Mouse Husbandry**

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Teaching new users proper performance of aseptic practices can be challenging regardless of their previous level of experience. The challenge of learning these new skills is compounded when aseptic practices are being used in the complex context of maintaining germ-free/gnotobiotic mice. Adult learning theory suggests that learning is enhanced when the learner focuses on issues directly related to their work and when it is centered on problem-solving rather than content memorization. Therefore, during the establishment of our gnotobiotic caging facility we filmed technicians performing assigned tasks, such as aseptic cage change within a biosafety cabinet, and had them self-assess their performance through review of their own film. Facility supervisors also reviewed the technician's film. Supervisor-technician teams would then compare identified breaks in asepsis or high-risk behaviors that were deemed likely to lead to a break in asepsis they separately noted from films. Technicians found this film review more helpful than traditional methods of instruction such as SOP and guidance review. Film review as a performance analysis tool also led to rapid improvement in individual skill sets with technicians mastering aseptic cage change in only 1-2 film review sessions. Further the film analysis allowed early identification for improvement in engineering processes to reduce potential contamination events. Given the ease of video recording and the ability to securely share recordings within institutionally protected cloud-sharing services, film study is an excellent tool to quickly train new technicians to perform proper aseptic technique and other germ-free husbandry processes. Though we have yet to employ this in our own institution outside of the germ-free facility, we see the potential for this tool to be applied throughout our animal care and use program to enhance training and engagement of all of our husbandry personnel.



### **P33 The Cranium Challenge: Fueling Technicians to Acquire AALAS Certifications**

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The Cranium Challenge is a test preparative system intended to enhance skill, promote synergy, and most importantly, assist technicians in attaining certification exams. Three consecutive days each week, 2 questions are randomly selected from the exam prep materials of all AALAS certification levels. All elements necessary to participate (answer sheet, writing utensils, and answer-box) are provided and placed in a central location for convenience. Participants have until 1 pm each day to place their answer sheet into the designated answer box labeled Cranium Challenge. This affords the scorer time to tally up points and document them into the assigned spreadsheet before the end of the business day. At the end of every month, total points are calculated for all participants. The individuals with the top 3 scores are bestowed prizes that consist of an award certificate along with 3 different attractive selections for first place, 2 for second place and 1 for third place. All awarded prizes are unique to the staffs' preference and were preselected by majority vote prior to the program implementation. In summary, our facility has a surplus of technicians successfully achieving certifications beyond the requirements on the first try within their first 18 mo of employment. The moral towards obtaining the certifications beyond requirements has increased. Over 70% of the staff actively participate in the program at one point or another. Technicians' are pushed to be more competitive while being empowered to meet their goals and objectives for growth within the workplace. Fifteen out of 17 total technicians in our facility are certified through AALAS at the ALAT level or above.

### **P34 An Animal Program Administrative Electronic Management Tool**

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We have developed an electronic veterinary services website which helps us handle all of our animal program needs. Animal program staff, including veterinary staff, animal care staff, and animal researchers are able to access the website and gain information on a diverse set of topics, make technical requests, and initiate animal orders. For instance, animal users can access the website with a unique ID and password issued to them by the account manager. Following login, the animal user has access to all animal program standard operating procedures (SOPs) and guidelines. Links to required training are posted on the website. Completed training sessions are verified and this information stored. Registration in the occupational health and safety program is also stored. Writing and modification of the animal study proposal (ASP) is completed with the use of a standard ASP form template. The researcher can see animal usage for a specific protocol including specific strains used and animal orders that have been placed. This helps to eliminate the possibility of animal overages. The IACUC can review IACUC meeting agendas and meeting minutes, as well as review ASPs. Importantly, there is communication between the ASP and a separate animal ordering website. It is user-friendly, has 24-hour access, delivers updates in real-time, and is environment-friendly since the use of paper is eliminated.

### **P35 The 5 Es of Training: Improving Employee Engagement and Buy-in**

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One common, critically important challenge facing trainers is

motivating staff to take advantage of promotional opportunities within their company, which can help them to avoid burn-out (particularly long-term employees). The 5Es of training provides a framework for trainers, and when employing these 5Es (education, entertainment, encouragement, empowerment, and exposure) employees gain more interest in their roles expanding throughout the lab animal facilities. The 5Es focus on key developmental categories which aim to increase employee buy-in and motivation for achievement of certifications and promotions. Education would include daily duties, SOPs, continuing education, and new study protocols, for example. Entertainment is used with education, which helps employees retain information they are learning because the experience has been made memorable to them. Encouraging growth builds confidence in staff members, leading to an awareness of increased job satisfaction. Empowering employees allows them to take pride in and ownership of their responsibilities and completed tasks and projects. Exposing employees to other facilities, their SOPs, and policies and procedures, allows them to better understand the grand scope of the research industry. This also opens their eyes to the diversity between facilities and institutes, and gives them the ability to network with their colleagues. The 5Es training program provides employees with the tools necessary to become dynamic, indispensable assets. This training program has made a remarkable difference in employee buy-in, motivation, and attentiveness, thus lowering the amount of staff turnover and increasing internal promotions.

### **P36 Challenges Encountered in Implementation of IACUC Role in Developing Countries**

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Although the role of the IACUC is well established in developed countries and the laws that regulate their operation are under continuous review and updates, in developing countries the newly implemented IACUCs are encountering many obstacles and difficulties. At the start of the millennium in Egypt, research ethics committees in the different academic institutions aimed to enhance the research activities of both human and nonhuman animals in an ethical framework. In 2013, Cairo University established the first IACUC in Egypt in accordance to the World Organization for Animal Health (OIE) mandate and follows international guidelines in its review of animal protocols. Since then it has set an action plan to increase awareness of animal welfare principles with emphasis on the 3Rs, provide training resources to the researchers, design standard of operational procedure for its committee, and develop national guidelines. Training and education of researchers were integrated into the postgraduate educational program and in training program for faculty members' development. Egyptian constitution and the regulations of protocol submission as required by the university were among the opportunities that foster the development of animal care and use in research and education. Based on data collected over the last year some challenges that face the IACUC were elucidated. The dilemma of sample size calculation and the principle of reduction, the need to consider replacement before formulating a research question and designing a research plan, the concept of randomization and risk of bias, as well as pain relief in studies where analgesics may affect the outcome of the study are some of the difficulties encountered.

### **P37 Ultrasound to UltraSee: The Use of Ultrasound to Determine Pregnancy in Mice**

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In March 2017 we bought an ultrasound machine to assist in

determining pregnancy in time-mated mice. Prior to this, visual checks or palpation would be used to confirm pregnancy. There were limitations with these methods as mice generally are not visibly pregnant until E12.5 and in older females, it may still not be clear if they are pregnant or generally larger. Palpation is not possible before E9.5 and is not always accurate depending on the experience of the technician carrying out the technique and if done incorrectly palpating can cause discomfort to the female or in some cases hurt the pups. Using the ultrasound has meant checking for pregnancy is less stressful on the animal as they only need to be scruffed once for a few seconds while the scan is done with no need to anaesthetize or shave the mouse first. Before having the ultrasound, embryos required at earlier time points such as E8.5 meant females had to be used regardless as there was no way of confirming pregnancy. This led to animals being culled unnecessarily, experimental delays and time lost undertaking unnecessary dissections. There used to be possible waste of up to 45% for embryos before E12.5 but now using the ultrasound this has dropped to 7%. The training took about 6 mo to accurately see pregnancy at E9.5 with the help of a one-off training session from a specialist to get us started before training ourselves by working our way down gestation points. As confidence grew with the machine and images after another 2 mo pregnancy could be seen as low as E7.5.

### **P38 In-house Development and Use of a Large Animal Induction Chamber as a Refinement Method for Repeat Sedation in Miniature Swine**

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Swine are often considered the penultimate preclinical model for surgical or translational research. They are intelligent, making them impressionable with regards to both positive reinforcement training, as well as in their reactions to poor handling or stressful events. This is an important consideration in studies which require frequent sedation. Subcutaneous and intramuscular injections are frequently used methods of sedation in swine, and are considered as causing brief pain or distress. Despite positive reinforcement, over time we observed vocalizing, hiding, escape, and snapping behavior resulting from repeat injections in miniature swine requiring weekly sedation for study purposes. As a refinement to repeat injection, we designed and fabricated a large animal induction chamber in-house, similar in style and functionality to that used commonly for rodent anesthesia. Special consideration was given to sturdy construction, use of sanitizable materials, and the ability to sufficiently exhaust the chamber of waste anesthetic gases. Total fabrication costs totaled less than \$2,500, and technical time billed to the project decreased, as additional personnel were no longer required for restraint and sedation. Behavioral indicators of welfare improved, and animals continued to willingly enter the chamber biweekly for >6 mo. In addition, acclimation beyond our institutional standard of 7 d was not required in order to train to alternative methods, such as mask training. The use of the chamber also improved outcomes from an occupational health and safety perspective, particularly with regard to manual restraint or handling of mature miniature pigs weighing 30-50 kg. We have proven that the use of a large animal induction chamber created in-house for the sedation of miniature swine is a cost-effective and valid refinement strategy which improves animal welfare, requires minimal animal training, and adequately addresses occupational health and safety issues.

### **P39 Comparison of Male to Female Ratio for Fertilized Early Stage Embryo Production in the Mouse**

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Transgenic mouse founder programs require the use of fertilized

embryos. The male to female ratio is a crucial parameter used to optimize the number of fertilized embryos and the most efficient use of animals. Historically, the ratio of 1 male to 1 female has been used predominantly in the industry and this laboratory setting specifically. The aim of this study was to investigate whether using 1 male to 2 females would produce equivalent results while improving overall animal usage efficiency. The mouse strain CD1 was super-ovulated using a single injection of 5 IU PMSG, subsequently bred, and 8,158 embryos were collected 23-24 h post mating from 234 females upon confirmation a sperm plug was present. The embryo collection was performed by removing the oviduct and either rupturing the ampulla or flushing the oviduct with M2 media with hyaluronidase. The embryos remained in this media for approximately 0-5 min to remove the cumulus from embryos. The embryos were then counted, assessed, washed, and placed in M2 media. The mean ( $\pm$ standard deviation) number of embryos that were collected, fertile, infertile, and abnormal for 1-to-1 vs. 1-to-2 male to female ratio were 36 (23), 25 (17), 9 (17), 2 (3) and 33 (18), 23 (16), 8 (14), 2 (4), respectively. The results indicate there is no significant difference ( $p > 0.05$ ) between the one-to-one or the one-to-two male to female ratio. The use of one male to two females is equally as effective for fertilizing ova needed in a transgenic founder mouse program, while overall decreasing the number of male mice used and cage space occupied according to the principles of the 3Rs in an animal research setting.

### **P40 The Enrichment Club**

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The best enrichment and behavior management programs extract and implement novel ideas from other institutions. While we have forums that help us gain knowledge and exchange information exclusively on enrichment, we lacked in-person conversation about the challenges of implementing a successful enrichment program. This is why we started a New York-based Enrichment Club with the intent to expand it to the national level. The Enrichment Club started in 2016 by enrichment coordinators from NYU, Mount Sinai, and Cornell University. The club holds meetings, roundtables workshops, and presentations to provide a platform for interested people to discuss various issues. Meeting topics and discussions have included enrichment for aquatics, alopecia in regulated species, social housing in all species, positive reinforcement training in nonhuman primates. We also have had site visits to view various enrichment programs in other facilities, such as Princeton University. Our club is open to anyone in the lab animal community that has a passion for animal welfare. Our attendees include veterinary resident students, veterinarians, vet techs, facility managers, and principal investigators. Club members have spoken at LaGuardia Community College about the enrichment club. Through the implementation of the club, we've seen increased involvement from PIs and lab managers interested in new enrichment techniques and practices, as well as more husbandry technicians eager to learn about new enrichment practices. We offer tips on how to set up your interfacility Enrichment Committee as well as the benefits of having a statewide Enrichment Club.

### **P41 Improved Sensitivity of Continuous Animal Monitoring Increases Power and Reduces Numbers of Experimental Rats and Mice Required for Statistical Significance**

CH Ichim, J Mechanic, M Lim, M Rabe, L Schaevitz<sup>\*</sup>

Vium, San Mateo, CA

Reducing animal numbers used in research needs to be balanced with generating data that has sufficient statistical power. We hypothesize that continuous monitoring of behavioral, and physiological conditions (motion, breathing, etc.) could provide valuable insight into

disease process and increase statistical power and thereby reduce the number of animals required on study. Retrospective power analyses were conducted across a number of experimental models including liver and lung injury to determine the animal numbers needed to observe statistical significance, contrasting traditional measures with continuously recorded breathing and motion metrics. Analyses were conducted with power set at 0.95 and an alpha at 0.05 in all animal models using G\*Power. In a mouse Concanavalin A-induced liver toxicity model, ALT and AST levels gave effect sizes of 1.40 and 1.31, with sample sizes of n=15 and 17, respectively. In contrast, continuous animal monitoring gave effect sizes of 4.01 and 1.67, with sample sizes of n=4 and n=11 for motion and breathing metrics, respectively. In a rat paraquat-induced lung injury model, temperature, body weight, and lung weight gave effect sizes of 2.23, 1.40 and 3.94 with sample sizes of n=7, 15 and 4, respectively. In contrast continuous animal monitoring gave effect sizes of 5.33 and 9.48, with sample sizes of n=3 and n=2 for motion and breathing metrics respectively. Hydroxyproline assay gave an effect size of 1.47 with sample sizes of n=11 in a mouse bleomycin-induced lung-injury model. In contrast, continuous animal monitoring gave an effect size of 4.3, with sample sizes of n=3 for breathing-based metrics. Overall, we show that continuously monitored motion and breathing rate were able to reduce the overall number of animals needed for an experiment without compromising data integrity.

#### **P42 Social Media as a Tool for Informing the Public about Animal Science and Biomedical Research**

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There is a continued lack of positive information available to the public regarding animal science and biomedical research. The public is commonly misinformed by animal rights activists, advocates, and public figures who preach against the accomplishments derived from animal science and research. This is accomplished by using celebrity-filled television commercials, advertisements, community events, and/or social media to spread their misconceptions. We inform members of the animal science and biomedical research communities that they can use social media in response to a public figure's post or paid advertisement without a conflict of interest to one's institute or the science being conducted. We educate our colleagues on how to use social media, such as Facebook and Instagram, in a transparent and constructive manner to share positive insights about the science that is being investigated and the long-lasting benefits of scientific research, not only for humans but for animals as well. Using social media to share this information will encourage and motivate others in our industry to start and/or continue to use social media to enlighten the public of our advancements and subsequently modify negative public opinions and misconceptions toward animal science and the biomedical research profession.

#### **P43 Comparison of Tail Vein Blood Collection Techniques in Mice**

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Blood collection via the tail vein snip technique has historically been used in a number of studies that require pharmacokinetic profiles in mice. This method requires the initial snip of the distal end of the tail (1-2 mm) with subsequent samples collected by dislodging the crust to refresh the blood flow. It is reported that this method is favored over alternative tail vein methods (such as the tail vein nick or tail vein puncture) due to its efficiency and that it does not require a supplemental heat source for vasodilation. There were concerns regarding whether or not an alternative method would be a better option. We hypothesized that the tail vein snip method would cause the least trauma to the tail and be the most efficient method of blood collection when compared to the tail vein puncture and tail vein nick. The mice used were C57BL/6NCRl, male, 6-7 weeks old. Fifty-four animals total were used. All 3 blood collection methods were

compared against a commonly used PK profile, to assess sample quality, collection time, and animal welfare concerns. Each technique had 3 groups, 1 without a heat source, 1 with a heat lamp, and 1 using a slide warmer to aid vasodilation. Collection site evaluation, time deviation, and tissue for histopathology were collected. No analgesia was used. Plasma samples were assessed for hemolysis using a simple visual inspection against a white background. Visual discoloration of each sample was scored from 0 (non-hemolyzed plasma) to 5 (100% hemolyzed plasma). The 3 groups that had tail snips performed showed no deviation in time and plasma samples had a score of less than 2. There was no bruising of the tails observed. In contrast, the groups that had the tail vein nick or puncture technique performed had deviations in time and poor quality plasma samples, with many scoring above a 3. Additionally, the groups that received the tail vein nick had visible tail bruising. Based on data collected, we conclude that the tail snip technique in mice is an acceptable and reliable technique when performing serial sampling in mice. In comparison to alternative methods, the tail snip technique produces high-quality plasma samples, without the need of supplemental heat, with minimal effect to the animal's overall wellbeing.

#### **P44 Development of Improved Telemetry Device for Use in Assessment of Rodent Activity as an Indirect Measurement of Animal Wellbeing**

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In preclinical animal studies, rodent activity can be used as a general measure of wellbeing. Healthy mice will engage in active behaviors including nesting and grooming, while mice experiencing distress or pain will be less active. Measuring rodent activity historically relied on imprecise observational and discontinuous methods. The increased use of wireless telemetry units has allowed for transmission of real-time information to a sensor. While measurement of physiological parameters such as heart rate and blood pressure have been widely used in research, development of a unit that can accurately and rapidly measure mouse activity has been limited. We describe the design and development of an embedded system providing a quantitative, real-time measure of rodent activity. A 10x10mm micro-device capable of gathering and transmitting data concerning the acceleration of rodents in 3 dimensions was manufactured and a variety of intelligent signal processing algorithms were engineered to transform the data into a meaningful measure of activity change over time. The final iteration is highly sensitive to rodent movements, 86% smaller than many leading telemetry units in the Internet of Things (IoT), and records data 300 times longer than comparable behavior-tracking solutions at only 3.5% of the cost. The device was used at a major research university to evaluate the effects of single housing and cage change on mouse activity. Activity levels were also measured following treatment with a variety of analgesics and anesthetics. The resulting activity values over time were compared to baseline values from untreated mice and published time response curves, demonstrating both accuracy and precision in the solution. Additional testing will further validate the device's ability to report long-term rodent activity, offering greater insight into rodent wellbeing following experimental manipulation.

#### **P45 Evaluation of a Novel Urine Collection Apparatus in Rodents**

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Urine is a commonly collected sample used to assess a variety of drug/therapy combinations, as well as evaluate disease progression in the animal model. In order to meet our researchers need of large volume collected over time without contaminants or expensive metabolic caging systems, we have developed a novel collection apparatus from common veterinary and laboratory supplies. We used mice as our model because their small size often makes urine collection by standard methods difficult. Often researchers may need to collect smaller serial samples of 10-20 $\mu$ l of urine for studies, which does not warrant investment in metabolic caging as it can be free caught, but our greater target volume of 150 $\mu$ l is more than a mouse will voluntarily micturate. Even with metabolic caging, urine often dries on the apparatus walls or in the tubing, evaporates while awaiting collection, or is contaminated by feed, dander, or fecal particulates, therefore our focus was on nonsurgical bladder catheterization coupled with appropriate tubing and collection reservoir. Currently, we have evaluated the methods and final materials in over 50 female mice. The animals evaluated all appeared to tolerate the procedure and catheterization well and on average samples ranging between 50 $\mu$ l to 150 $\mu$ l were collected into 1.5ml Eppendorf tubes. Because of the size of our collection aliquots we needed to change them out every 12 h at a minimum. Thus far, this system has proved effective in urine collection without causing undue health concerns out to 72 h. The ability to collect a noncontaminated sample via a nonsurgical method has resulted in reducing the overall number of animals used by our researcher, as well as met our original aim.

#### **P46 Impact of Identification Methods on Animal Behavior and Physiology**

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Vium, San Mateo, CA

Identification of experimental subjects is necessary to ensure the overall success of a preclinical study. Many different methods such as tail-tattooing, ear-tagging, ear notching, and implantation of radio-frequency identification (RFID) transponders exist. These procedures are considered to be relatively noninvasive and therefore not typically taken into consideration in the overall experimental design. Our goal was to understand the duration and magnitude of impact of animal identification (ID) on animal behavior and physiology. Using a continuous monitoring platform that automatically assesses motion and breathing rate 24/7, we performed retrospective analysis on data gathered during the acclimation period (3-14 d prior to study start) of independent mouse studies (n=10-174 mice/ study). We found that several animal ID procedures, such as tail-tattooing, ear-tagging, and RFID implantation, resulted in distinct changes in activity and breathing rate. More specifically, we observed a <50% decrease in daytime motion and <15% decrease in night-time motion post-tail-tattooing and ear-tagging, as well as <60% decrease in motion post-RFID implantation. Tail-tattooing also resulted in a 20% increase in breathing rate. These behavioral and physiological changes lasted 2 to 3 d post-procedure. Consistent elevations in breathing rate post-tattooing compared to post-ear-tagging may suggest that the former is more invasive of the 2 animal ID methods. Animal identification procedures, especially when combined with other procedures (such as blood collections, dosing, and handling), can have acute or longterm effects on animals that are likely to contribute to higher inter- and intra-animal variability on study, thereby necessitating the need for larger study sizes. Insights from this study can guide researchers to make more informed decisions around the choice and timing of animal identification, specifically in choosing a method and a time with the least impact on the animal while still achieving study aims.

#### **P47 The Use of Novel Epiglottic Manipulation Device for Swine Endotracheal Intubation**

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Endotracheal intubation in swine is technically difficult as a result of the pig's oral anatomy and presence of excess tissue in the oropharyngeal region. Typically, a clear view of the laryngeal-tracheal pathway is obstructed by the thick base of the tongue and entrapment of the epiglottis by the soft palate. Standard intubation techniques involve using the laryngoscope blade to position the epiglottis such that the vocal cords can be visualized. This technique is often complicated by the pig's long oropharynx and can result in laryngeal trauma. In order to reduce intubation times and the number of attempts needed for successful intubation, we used a 25 cm epoxy tool with a 150-degree angle to easily, quickly, and safely manipulate the epiglottis and provide an unobstructed view of the glottis and vocal folds. Compared to using a laryngoscope blade alone we found that use of this device reduces intubation time by close to 50% (preliminary data: 40.4  $\pm$  3.39 s with device vs 74.5  $\pm$  28.7 s without device). Staff members training on swine intubation also reported greater comfort when using the device versus traditional methods for manipulating the epiglottis. We believe that our device results in faster intubation times, less attempts needed for successful intubation, and increased comfort with more rapidly acquired proficiency for those acquiring swine intubation skills. Future work will be aimed at demonstrating that this device will also reduce laryngeal trauma (decreased laryngeal edema, hemorrhage, and cellular infiltrate).

#### **P48 A Guide to Postapproval Monitoring via Annual Protocol Review**

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Continuing IACUC oversight of animal activities is required by federal laws, regulations, and policies. Mandatory semiannual program reviews alone may not be adequate, especially considering that, on occasion, the drive for scientific advancement and the need for regulatory processes may not sufficiently connect. Therefore, it can be challenging to assure all aspects of regulatory requirements beyond the initial IACUC protocol approval. To prevent unintended missteps, that could cause serious programmatic and research consequences, Postapproval monitoring (PAM) in form of an annual protocol review can mitigate noncompliance on the part of the institution and the principal investigator (PI). When commercially available electronic systems are not in place, manual means to facilitate PAM can be burdensome and cause excessive paper trails. Having an efficient PAM process in place encourages PI engagement and is crucial to assure ongoing IACUC oversight. Ultimately, the goal of PAM should be of supportive nature and presented to PIs as a valuable and mutually beneficial tool to improve research outcomes in a regulatory compliant manner and perhaps the reliability of research data. To streamline the PAM process, a standardized electronic postapproval monitoring form (ePAM) was created. This simple PDF form can easily be customized to any site-specific needs and processed via any email provider or even integrated into a content management system, which enables additional features on workflow management. The ePAM is completed by the IACUC designee (such as the compliance coordinator) during an annual administrative protocol review and critical sections reflected for review. Upon completion, the IACUC designee submits ePAM to PI/designee for review and arranges PAM meeting. During the meeting, ePAM serves as protocol review agenda. Post meeting, IACUC designee reflects PAM results on ePAM and resubmits to PI/designee to initiate any required protocol amendments. Upon implementation, the PAM process flows with greater ease for the IACUC office. The results are (1) simplified documentation, (2) faster completion time, (3) increased PI engagement, and most importantly, (4) a reduction in identified protocol inconsistencies.

### **P49 Sex Balance in Preclinical Animal Models: Have We Made Progress?**

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Taconic Biosciences, Hudson, NY

The NIH announced new policies in 2014 aimed at requiring NIH-funded researchers to address sex balance in preclinical cell and rodent models. Although exclusion of women in clinical research was identified as problematic and addressed decades ago, the preclinical research world has not followed suit. The NIH identified this continued reliance of animal studies on a single sex, often male, as inappropriate: "consideration of sex is a critical component of rigorous experimental design." These changes took effect for the fiscal year 2016 grant applications for projects funded in fiscal year 2017. Sales data from commercial animal vendors is a unique source of information to judge whether the new NIH policies are affecting animal usage. We analyzed Taconic Biosciences mouse and rat sales data for nonprofit institutions in the United States. For the most common research models, there was little to no movement towards equal usage of males and females. These common strains and stocks all display bias towards a particular sex which has remained unchanged from 2013 through 2018. For transgenic models, most of which have more narrowly defined uses compared to standard inbred strains and outbred stocks, the results are similar. Each model maintains a similar sex bias over time, with some fluctuation seen by year. From an animal welfare perspective, a move towards sex parity in rodent research would reduce the numbers of animals produced but not put to research use. The nature of breeding is that generally equal numbers of males and females are produced for each line, but sex bias in usage means a large percentage of one sex may not be used experimentally. No strong evidence for movement towards parity of rodent usage by sex is evident in Taconic's sales data. Research thus continues to potentially overlook the influence of sex in many experiments, and vendors continue to produce animals of the nonpreferred sex which may not be used experimentally.

### **P50 Continuous Improvements to Infusion Systems in Rats: Refinements to Enhance Animal Welfare**

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Dosing via continuous infusion using a tethered system in rats presents many challenges. Our facility continues to refine infusion models to improve animal welfare and gain workflow efficiencies. Tether systems using a rodent jacket/harness are labor intensive to maintain. They require constant monitoring and adjustments to ensure proper fit. An improper fit may cause irritation/abrasions that can result in lacerations or conversely allow the animal to remove the system resulting in catheter complication which could lead to infection or patency issues. A tether system that requires no jacket/harness was evaluated in an attempt to mitigate these issues. The tether connects magnetically to an externalized port at the dorsal scapular region. Thirty rats were surgically implanted with a femoral vein catheter connected to the externalized port and were attached to a 10" tether/swivel system. Normal saline solution was infused at a continuous rate of 0.3 mL/hr for approximately 12 wk. While this system eliminated contact irritation previously associated with the jacket/harness and decreased labor associated with system maintenance, there was notable tension on the skin around the port caused by the tether and how it was mounted to the caging. This tension caused the port to tilt and the skin around the port to be pulled as the animal moved around the cage. A more flexible tether at different lengths (8, 9, and 10") was compared and a standard tether mount was compared to a spring tether mount in an attempt to decrease the tension on the skin. Six rats, (1 rat for each length of tether and mounting style) were tethered for 5 wk. The animals were observed twice daily and the port sites were evaluated and scored for the amount of tilt/tension on the

port. There was no apparent difference between the 2 swivel mounts, and the 8" and 10" tether lengths had similar tilt/tension scores. The 9" tether on both swivel mounts had higher scores for severe tilt on the port. Based on this evaluation, the 8" flexible tether was selected for use with our existing swivel mounts. The elimination of the harness/jackets and the use of appropriately sized, more flexible tethers will improve both animal welfare and efficiency during rat continuous infusion studies.

### **P51 Holistic Approach to Animal Procurement in the United States**

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An important aspect of an animal welfare compliance program is animal procurement. Animal procurement can be complex, based on requirements and regulations such as the Animal Welfare Act. We focus on U.S. sourcing of animals for research purposes only, as well as the legal and ethical responsibility for the purchasing company and investigator. Additionally, we emphasize a company's need to enact via its own internal corporate policies numerous safeguards in areas, such as risk, transportation, legal agreements, payments, audits, and cross-divisional impact. We also detail how the animal procurement process can be effectively managed with an electronic database that simplifies the attending veterinarian's role to approve animal vendors and ensures alignment of animal requests with the approved animal use protocol, which in turn, enables the IACUC to keep current on animal usage. The electronic database also enables IACUC administrators to easily pull census information for facility planning, AAALAC, and USDA annual reports. Lastly, we cover the importance of building and maintaining a strong partnership with the vendor in regards to communication and forecasting requirements, especially when unexpected events occur. A multiteam approach with clearly defined roles can simplify the animal procurement process while still maintaining the highest of standards.

### **P52 Problem Solving for Positive Reinforcement Training Challenges**

MM Dragon\*

Comparative Medicine, Pfizer, Groton, CT

At some point during positive reinforcement training (PRT) of laboratory animals, all trainers whether novice or experienced will experience roadblocks. Common issues include animals not participating in training events, performing inconsistently, or performing the wrong behavior. Our organization has PRT programs at several sites across the country. Due to geographical distance and a large number of animals, it is not possible to provide daily oversight to all new animal trainers regularly. Several ways to further the education of new trainers and help with problem-solving include mentoring, brainstorming meetings, videotaping sessions, and keeping a training journal. Problem solving involves determining the root cause of the problem, evaluating alternatives and implementing solutions, which can be challenging for someone that is new to PRT. Regularly scheduled teleconferences bring together trainers of all skill levels to share ideas on challenges and solutions. Several themes have emerged when discussing challenges, including animals that won't participate, inconsistent response, regression, failure to transfer behavior with another trainer, and fear of new objects. From these, the group brainstorms to evaluate the root cause. While the issue is most often a result of an overly ambitious shaping plan or trainer, other common causes are motivation, inconsistent cueing, and environmental distractions. Trainers are able to share ideas and offer suggestions that have worked in different scenarios. Often the cause and steps for remediation are more apparent to another member not directly involved with the specific challenge. These problems, causes, and solutions are captured in a spreadsheet and available for trainers to consult as they advance their PRT skills. This global approach to

problem-solving for PRT challenges has resulted in a forum for trainers with various experience and knowledge to freely share information, provided a network of colleagues to communicate with, and facilitates faster resolution when roadblocks occur during the training process.

### **P53 The Cat Conundrum: Improving Welfare and Reducing Stress for Cats and Their Handlers through Primary Enclosure Refinement**

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Every species presents unique challenges for handling and care in the laboratory environment, but perhaps none more so than the cat. Cats are independent. They do not require humans to thrive in the natural environment, and they have a tendency to develop large solitary territories even in a home. Social housing is a challenge due to the close proximity to other members of their species made necessary by the relatively small size of our standard caging. Handling is a challenge as human injuries, due to bites and scratches, often result when the cat feels itself cornered, an unavoidable situation with standard tiered caging. The vendor supplying our cats solves these issues by group-housing the animals free in a room. Due to the design of our facility, specifically the lack of anterooms and procedure rooms, some form of primary enclosure within the room is required. In order to improve social housing success, decrease human injuries, and increase the welfare of both the cats and the handlers, we introduced an alternative housing method to our cat colony by using European style nonhuman primate caging. Simple modifications to the pens allowed for vertical climbing opportunities, hiding opportunities, and structures allowing for establishing mini-territories if the cats so chose. Observations after transfer to the caging included full use of the pen structure by the cats, no apparent social conflict or sickness behaviors, increased ability on the part of the handler to capture the cats, less aggression from the cats toward the handlers, fewer injuries to the handlers during both receipt and handling procedures, increased handling acclimation success, and increased willingness of the techs to perform handling activities. Due to the group sizes permitted by the pens, no reduction in room capacity was necessary. Therefore, due to increased animal welfare, reduction in human injuries, increased human welfare, and lack of impact on study design and business needs, this caging will be recommended as standard for cats at our facility in the future.

### **P54 Pathology Associated with Repeated Submental Blood Collections**

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Repeated blood collections over multiple time points is commonly performed in pharmacokinetic (PK) studies. In order to determine the fate of a chemical from the time it is administered to the time that it is eliminated from the body, multiple blood collections are taken minutes to hours apart. PK studies conducted with mice must consider blood volume removed at each time point as well as trauma associated with the blood collection site in order to minimize animal welfare concerns. Recently, a new technique called the submental blood collection method has been developed and reviewed in *JAALAS*. This method was described to have less trauma associated with it than the submandibular blood collection method. Our goal was to determine the gross and histopathology associated with the tissue surrounding the collection sites using both the submandibular and submental technique. We hypothesize that the submental (chin) blood collection method will have lower histopathology scores than submandibular (cheek) and would provide a refined method of blood collection when multiple collections are needed. We used 28 NU/J HOM male mice split into groups based on blood collection method. With each technique mice were either bled repeatedly on the right side of their cheek or chin or on alternating sides of the cheek or chin. Blood was

collected at the following times: 0, 30 min, 1 h, 4 h, 8 h, and at 24 h. Necropsy was performed 24 h after the final time point and gross pathology scores, as well as histopathology scores, were recorded. Gross necropsy scores for the chin method were significantly ( $P < 0.01$ ) lower compared to the cheek method in both the alternating and same side collection groups. Within collection methods there were no significant differences in gross necropsy scores between alternating sides or same side collections. Histopathology scores correlated with gross necropsy scores where the chin method had less adverse observations. The alternating cheek group had the most adverse observations both in number and in severity. In conclusion, blood collected from the same side or from alternating sides with the submental method is a refinement over repeated blood collections with the submandibular method.

### **P55 Tracking Animal Health, Teaching, and Research Usage within an Agricultural Program**

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Oversight for agricultural and veterinary programs, which include multiple large animal and farm species, can be complex and multifaceted. At our institution, where farm animal species are involved in biomedical and agricultural teaching and research, concerted efforts were undertaken to harmonize veterinary case monitoring, medical records, and tracking of animal use in advance of applying for AAALAC accreditation. Following the award of accreditation, attempts were made to focus on the quality of medical reports across farms and tracking of animal reuse within educational courses. Initially, weekly farm rounds were conducted by a dedicated veterinarian and a licensed veterinary technologist (LVT) from the laboratory animal care team. Farm rounds included reviewing medical records and discussing clinical concerns with farm staff. A spreadsheet was used to record once weekly summaries, but ultimately became cumbersome, with a limited scope of content and lacking details of case concerns. To improve our approach, the LVT was trained in database design and created a records database which has been used now for more than 10 y. Cases are entered into the database as they are reported, with the ability to download at-will reports by species, investigator, or clinical issue. A related database was created to accurately track teaching and research use, especially repeated involvement of individual animals on study. These databases provide a centralized repository of animal treatments, health, and usage. To date, greater than 10,000 medical records and more than 8,000 reports of animal usage in courses and research have been documented. Case summaries are reported weekly and delivered to veterinary staff, IACUC, and researchers for transparency of animal care. To strengthen interactions between farm personnel and veterinary staff, farm rounds are now held twice weekly and have improved communications between the agricultural and animal care programs. In summary, this internal database system has been a cost-effective solution to document caseload within the farm animal program. Overall, the database and dedicated farm rounds have enabled our institution to solidify improved care, management, and oversight of agricultural species.

### **P56 Standardizing the New Hire Training Process**

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Our comparative medicine department has nearly 200 animal caregivers providing daily care for research animals housed in nine vivaria, on approximately 600 protocols. To enhance and standardize new hire training, we developed a comprehensive training platform that ensures consistent, effective onboarding for new employees hired

work in any of the 9 vivaria. A binder was created by the training department that contains SOPs and other educational material regarding human relations, occupational health and safety, and other job-related tasks. The binder, which includes checklists, accompanies all new hires to their assigned facility. Each manager is responsible for ensuring the employee is trained on the material in the binder and completes the checklist, which is submitted to the training department along with the signed training documentation. This has proven to be an effective means for onboarding new employees and ensuring that each new hire is receiving the comprehensive, consistent training necessary to care for animals properly. The managers and trainers also find it convenient to have all the training materials needed at their fingertips.

#### **P57 Incorporating Adult Education Concepts into Laboratory Animal Training Programs**

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Providing adequate, effective training is a constant area needing improvement in most laboratory animal training programs. Often training sessions are mandatory and repetitive due to the regulatory nature of the subject matter. Employees regularly approach these sessions with perceived knowledge, thinking there is nothing further they can learn and begin shutting down to learning before the training even begins. Incorporating elements of formal adult education can improve training quality overall and also contributes to greater learner receptiveness. Adult education, formally known as andragogy, is the study of teaching adults and offers valuable concepts and tools to greatly enhance training programs. Acknowledging that adult training needs are unique is a key concept to consider when developing training for adult learners. Using simple concepts can improve employee participation and receptiveness to learning. Seeking to improve training quality, we introduced several basic concepts of adult education to existing training, such as providing training outlines and using needs assessments and providing staff with the opportunity to be in charge of their own learning. We discuss tips and tools to incorporate adult education concepts and techniques into existing training programs to improve employee participation and receptiveness.

#### **P58 Development of an Experimental Device for Needle Oscillation to Reduce Insertion Force and Tissue Trauma during Rabbit Serial Blood Collections**

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Needle insertions performed on animal subjects can potentially cause discomfort, increase stress hormone levels, and cause bruising. Previous rodent studies demonstrated that an oscillated needle yielded blood samples with lower plasma corticosterone levels, improved sampling success, and reduced tissue trauma. This original device uses oscillating needles to pierce superficial vessels for low volume ( $\leq 125$   $\mu$ l) sampling collected via a separate collection device. To leverage the benefits of oscillating needles for injections and larger volume blood collections, an experimental device (ED) was developed. The ED applies longitudinal microoscillations to a needle that is in fluid communication with a standard syringe, enabling larger volume blood collections and injections, while reducing needle insertion force, tissue damage, and potentially animal distress. The ED was evaluated using both in vitro models and in vivo serial sampling. The ED reduced ( $>50\%$ ) the maximum insertion forces for oscillating needle entry (23G and 25G) compared to control ( $P < 0.0005$ ) in a bench model (orange skin), and for angled ( $<30^\circ$ , 25G) entry into ex vivo porcine ears. Male New Zealand White rabbits ( $N=24$ ;  $n=8$ /group) underwent serial sampling from the marginal ear vein over 3 wk using a single method

(control, ED, or control + ED noise). Samples were collected from alternating marginal ear veins, every 60 min for a total of 3 collections/day (3 collection days total at 1-wk intervals) with bruising assessed the day following collections. Mean blood glucose levels were reduced by the ED compared to control ( $P < 0.05$ ), and total procedure time was reduced during the ED collections ( $P < 0.05$ ). The ED group had significantly less bruising (blinded visual scoring, 0-5 scale) compared to other groups ( $P \leq 0.01$ ); 62.3% of ED collections caused minimal tissue damage (scores 0-2), while only 31.2% and 37.5% of control and noise collections, respectively, were in this range. No statistical differences were detected between sample blood mass, plasma corticosterone level, or required number of needle insertions. This pilot study supports the use of the ED as a refinement tool for blood collections. ED studies for rodent injection and nonhuman primate blood collections are currently planned.

#### **P59 Welfare Issues Associated with Carbon Dioxide Euthanasia in Laboratory Mice and Rats: Results of a Systematic Review**

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Concerns have been raised as to whether carbon dioxide is a humane method of euthanasia for laboratory mice and rats. The International Association of Colleges of Laboratory Animal Medicine (IACLAM) convened a task force to examine the evidence for adverse welfare indicators in laboratory rats and mice undergoing CO<sub>2</sub> euthanasia using a SYRCLE-registered systematic review protocol. Of 3,747 potential papers identified through a database search (PubMed, Web of Science, CAB Direct, Agricola, and grey literature) from 1920 to 2017. Thirty-seven studies were identified for detailed review, including 5 in neonatal rodent pups, 14 in adult mice, and 21 in adult rats. Experiments or reports assessing other parameters during CO<sub>2</sub> induction and/or euthanasia unrelated to animal welfare were excluded. Study design and outcome measures were highly variable and there was an unclear or high risk of bias in many of the published studies. Of 18 outcomes evaluated, including both continuous and dichotomous outcome data, changes were inconsistent or poorly differentiated between treatments. It is likely that repeated exposures to carbon dioxide inhalation are aversive to adult rats and mice, based on avoidance behavior evidence; however, this effect is largely indistinguishable from aversion induced by repeated exposures to inhalant anesthetics. In conclusion, there is insufficient evidence to permit an unbiased assessment of the impact of a single exposure to CO<sub>2</sub> inhalation for euthanasia on welfare indicators in laboratory rodents. Additional well-designed, adequately powered studies are needed to accurately assess the impact of this euthanasia method.

#### **P60 Cardiovascular Assessment during Paired Feeding in Telemetry Implanted Cynomolgus Macaques**

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In accordance with the requirements of the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals*, we socially house and feed our cynomolgus macaques unless separation is justified and approved for scientific or veterinary reasons. Historically, our safety pharmacology colony of telemetry implanted, male cynomolgus macaques have been fed separately and telemetry data has been collected during studies with animals singly housed. Newer telemetry devices allow data to be collected while animals are socially housed without confounding signal interference (or cross-talk). In an effort to provide a 24/7 social environment while meeting study needs, we acclimated this colony to social feeding and assessed their cardiovascular parameters as an indicator of stress to evaluate the feasibility of using this husbandry paradigm during safety

pharmacology studies. Compatible pairs of macaques were gradually acclimated from unpaired to paired feeding. This was successfully accomplished in approximately 3 wk by gradually decreasing the time of unpaired food access until the animals were no longer separated during feeding. Heart rate and blood pressure data were collected via telemetry prior to and following paired feeding acclimation, and statistical comparisons were conducted between the unpaired/paired sessions. In addition, the macaques were weighed weekly and observed closely for any signs of aggressive behavior during the acclimation process. There were minimal differences in heart rate or blood pressure between unpaired feeding and paired feeding following the acclimation period. No aggressive behavior was observed during the paired feeding acclimation period, and all macaques gained an average of 0.4 kg (range 0.2-0.7 kg) over the 6-wk study period. In conclusion, cynomolgus macaques can be successfully acclimated to paired feeding with minimal aggressive behavior and no marked effects on heart rate or blood pressure as measured by telemetry, suggesting stress was limited. Furthermore, we demonstrated that 24/7 paired housing and feeding can be used during safety pharmacology studies using telemetry data collection with no impact to the data while simultaneously providing a substantial improvement to animal welfare.

### P61 Optimizing Isoflurane Induction in Mice

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Isoflurane, a fluorinated hydrocarbon, is commonly used in animal anesthesia for its effective properties of producing rapid unconsciousness and central nervous system depression and is considered nonirritating. However, induction with isoflurane is considered somewhat aversive as isoflurane can cause neuronal excitability during induction which can result in distress-like behaviors. The goal of this study is to establish a refined protocol for inducing mice with isoflurane to minimize distress. Four groups of 6 adult, female, ICR mice were placed in a chamber either that was or was not pre-filled with isoflurane. The isoflurane gas with 100% oxygen was then introduced at 2 different flow rates. Group A, 2% isoflurane with 0.5L/min oxygen; B, 4% isoflurane with 4L/min oxygen; C, pre-filled induction chamber with the rate of group A; and D, pre-filled induction chamber with the rate of group B. Time to induction and anxious behaviors were scored. Groups A and B were re-exposed to isoflurane a second time 1 wk later with their respective settings and scored again for anxiety behaviors. Group D animals had significantly the quickest time to induction compared to all groups ( $P < 0.05$ ); groups C and D animals had significantly less anxious behaviors scored compared to groups A and B ( $P < 0.05$ ). There was no significant difference in anxiety behaviors scored between the first and second exposures in group A and B. We summarize that an induction chamber pre-filled with isoflurane at a high oxygen flow rate and high vaporizer percentage rate resulted in a fast induction with minimal to no anxious behaviors exhibited.

### P62 Trial for Adequate Environmental Enrichment

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Enrichment is vital for animal health but it may also create problems. Too much bedding and/or enrichment material in a standard mouse cage can lead to flooding of the cage floor, dehydration of the animal, difficulty in observing sick or injured animals, and failure to record accurate dates of birth for new pups. Alternatively, providing ample amounts of environmental enrichment has been linked to improved animal health in regards to aggressive behavior and metabolic-based illness such as dermatitis. As our own rodent colonies, *Mus musculus*, were noting an increase in dermatitis, our IACUC raised concern on the current standard of enrichment provided to a typical mouse cage. In order to address this, we ran a trial study over 9 d to observe 10

alternative options for enrichment. Three primary types of malleable enrichment were used: tissues, crinkle paper discs, and enrichment squares. From these 3 options, each of the 10 groups was assigned a combination of 1 or more of the materials. Two cages composed a group, each with a mating pair, of BL6 strains. The first cage of the group contained a male/female pair while the second cage in the group contained a male/female pair with pups. Observations were made for percentage of enrichment used, visibility of the animals (1-5 scale), and other significant notations such as cage flooding. We saw that groups with the first material had more frequent flooding, groups with the second material used their enrichment faster than other materials, and comparisons in visibility across groups gave us the ability to identify preferences in combinations of material, which allow full use by the animal but also ease of observations for the caregiver. Through consultation of these results and other references, our IACUC updated the policy for providing nesting material for mice to include 6-10g of environmental enrichment provided by each facility as being adequate.

### P63 So You Want to Retire your Nonhuman Primates? How to Choose a Reliable Sanctuary

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Deciding to retire your institution's nonhuman primates after the study is completed is a difficult and multifaceted decision. There are many factors to consider such as cost, transportation, and identifying a sanctuary. The latter is certainly one of the most difficult. The need to locate a sanctuary that will not only continue to take exceptional care of the animals but also displays a tactful dialog on social media and/or their website regarding the intake of animals from research facilities and animal research in general. Confidence in the sanctuaries ability to keep confidentiality (if requested) is of utmost importance to the research facility. It is a difficult first step for an institution to take the risk of providing information about the animal, its care, and perhaps past surgical procedures or medicines it has been treated with to an entity the institution is not familiar with. It is likely too that the sanctuary will disagree with the work that we do. A second major concern is the quality of care the animal will receive at the sanctuary. Research environments have full-time veterinarians, access to multiple diagnostic tools, and medications. One may be skeptical that sanctuaries have the resources to provide this quality of care. We aim to describe how to vet proper sanctuaries to address these particular concerns.

### P64 Keeping Annual Refresher Trainings Creative and Engaging

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Designing and delivering engaging and creative annual refresher training to a large department can be a challenge. To prevent the audience from having to endure the stale slideshow presentation or the 'same old' discussion, we have devised some unique and creative ways to deliver training topics to engage the staff while encouraging collaboration. Refresher training topics may include tumor identification and tracking, cage density and overcrowded policies and procedures, safety training, sentinel exposure training, and animal health checks. Prior to training on a designated topic, the training team kicks off a design phase with a brainstorm. Six Sigma brainstorming rules are followed to gain as many ideas as possible. An effort is made to incorporate multiple learning styles into the training. We discuss and present examples of various trainings which involve games, food, case studies, and more. Active learning, which encourages participation and increases retention, is now a staple in our trainings. Training knowledge is measured through post-training surveys, assessments, and feedback. In addition to receiving positive feedback and seeing more consistent and efficient task performance across all staff, we deem the training a



success when previously unengaged, unreachable staff request more training and encourage others to attend.

#### **P65 Keepers of the Dirty Cages**

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Dirty bedding sentinels were used as a primary component of the rodent health surveillance monitoring program. Preparation of the sentinel cage requires attention to detail to ensure that the health surveillance program is effective. A grading scale was developed for sentinel cages to provide a guideline for training and set a consistent standard for animal care staff working with sentinel cages. Serial photographs of a sentinel cage containing 2 mice were taken over a 14-d period starting from completely clean as an example for baseline. Photographs were taken as examples of "excellent" (dirty) or "poor" (clean) sentinel cages for training purposes. Sentinel checks were instituted to score sentinel cages according to the grading scale which included number of cages contributing to the sentinel cage and how long ago the cage was created (days since change out). The sentinel superstar club was created to motivate staff to create excellent sentinel cage scores. Individuals that received excellent scores for sentinel cages in all of their areas for 4 consecutive quarters was named a Sentinel Superstar. Superstars received a certificate, a striving for excellence lanyard, and a gray mouse pin. A different colored mouse pin was given for each year that staff remains a sentinel superstar. Scores for sentinel cages have improved through increased training and motivation. The goal of the program was to encourage individuals to excel when making sentinel cages through recognition and provide confidence when staff performs their duties.

#### **P66 Impact of Implementation of a Veterinary Verification and Consultation Policy on IACUC Resources**

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Safety assessment research in a contract research organization setting is dynamic and ever-changing. Modifications of study design including additional in-life procedures are often requested to better assess responses to test article and to monitor the health of the animals. Many of these changes met the OLAW definition of a significant change and were time sensitive which resulted in frequent full committee review meetings for our IACUC. With an increasing number of modifications required that affected our animal use protocols, the IACUC was challenged to find a solution to reduce the regulatory review burden of the committee, while maintaining the high standards of compliance and animal welfare required by our program. Using the OLAW guidance document NOT-OD-14-126 for reference, we reviewed prior modification proposals and identified 8 significant changes that could be reviewed and verified by consultation with staff veterinarians as adhering to an IACUC-approved policy. The results of implementing the Veterinary Verification and Consultation (VVC) policy were immediate and exceeded our expectations, reducing the number of modification submissions handled by designated member or full committee review by 67 percent. Each submission handled under the VVC policy saves approximately 2.75 review hours equating to a \$40,000 savings in the last 6 mo in 2017. In addition, implementation of this policy has allowed us to reduce the number of full committee meetings held for the review of protocol modifications and has allowed committee members to focus more of their review time on the significant change proposals. Based on these metrics, implementation of a VVC policy at our institution has yielded both cost and time savings and has increased efficiency in the IACUC review process which has been well received by both the IACUC and our investigators.

#### **P67 Myocardial Intercalated Discs Defects May Lead to Dilatative Cardiomyopathy in Laboratory Mice**

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An increased incidence of atrial thrombosis with dilatative cardiomyopathy was noted in a genetically modified mouse breeding colony. The condition affected several strains of both sexes and different ages. Extensive testing failed to ascertain the cause. Further research showed the affected strains were on the same Balb/c background strain. Heart tissues submitted for transmission electron microscopy (TEM) examination revealed defects in the myocardial intercalated discs characterized by unorganized and heavily convoluted arrangement, desmosomes that are less dense and prominent, and dilated adherens junctions. In addition, the cardiomyocytes of the affected animals presented myofibrillar lysis adjacent to intercalated discs, dense myelin figures, fatty lysosomes, and occasional myofiber degeneration. The intercalated disc cell adhesion molecules form cell junctions which coordinate muscle contraction by coupling the electrical and mechanical connection between cardiac fibers. Defects at this level result in poor myocardial contraction, intracardiac blood stagnation, and, consequently, cardiac dilation ultimately resulting in clinical signs of heart failure. In humans, these defects have been associated with mutations of desmosomal and alpha-catenin genes. Both genes are found at high levels in myocardial tissues and contribute to strong cell-to-cell adhesion. Electrical coupling of cardiac muscle can also be affected by intercalated disc defects. Dilatative cardiomyopathy and atrial thrombosis have been reported in Balb/c mice, particularly older animals, but to our knowledge the cause is undetermined. How much the background strain used to create these genetically modified mice contributed to the elevated incidence of cardiac dilatation found in this colony is unknown. This report underscores the importance of TEM for an accurate diagnosis in routine phenotyping of new genetically modified mice strains. This unexpected gene modification that introduced a defect to the intercalated discs could be a mouse model to study the human disease.

#### **P68 Facial Mass in a Cynomolgus Macaque (*Macaca fascicularis*)**

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A naive, 4-y-old, 5 kg female cynomolgus macaque (*Macaca fascicularis*) was found to have a focal, spherical, firm, subcutaneous mass on the proximal aspect of the nasal bone slightly right of midline during routine semiannual physical examination. Radiographs of the skull did not identify abnormal pathology and CBC/chemistry were within normal reference intervals. Initially it was thought to be a blockage in the nasolacrimal duct and several attempts to flush the duct were made and treatment with heat packs was initiated. After several weeks of heat therapy, it was decided that the mass was not responding and a more aggressive approach was initiated. Attempts to lance and drain the mass were unsuccessful, and aspiration of the contents revealed a slightly red, stringy, viscous material. In the cytologic evaluation of this material occasionally cells with neoplastic features and pinkish extracellular materials were noted, suggestive of mesenchymal cell neoplasia, most likely osteosarcoma or chondrosarcoma. Bloodwork continued to be within normal reference intervals and repeat radiographs did not show abnormalities. An MRI scan revealed a contrast enhancing, soft-tissue mass that was slightly smaller in volume than the right eyeball. It was determined that this NHP would not be a good candidate for future drug studies and the decision to euthanize was made. Necropsy revealed a focal 2 cm x 2 cm raised, spherical, semi-firm, tan nodule on the proximal aspect of the nasal bone slightly right of midline that was solid on cut-section. No further macroscopic lesions were observed and the mass did not appear to be invading into the nasal turbinates, orbit, or cranium. Histologic

appearance of the mass revealed a disorganized, hypocellular matrix of collagen and spindle cells with the presence of blood vessels and chondrocytes, suggesting a tumor of mesenchymal cell origin. To our knowledge, this is the first report of a mesenchymal cell neoplasia in the nasal bone of a cynomolgus monkey.

#### P69 *Streptococcus thoralensis*: Commensal or Pathogen of Mice?

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Over a 2-y-period, 6 female mice from multiple strains and genetic lines presented for abdominal distention, palpable abdominal masses, hunched posture, lethargy, or dystocia. Five of the mice were housed in the same room and belonged to a single investigator, whereas the other mouse was from a different investigator in a separate room in the same facility. On gross necropsy, the abdominal masses were found to be large, multiple to coalescing uterine abscesses often associated with mummified fetal pups. Cocci were abundant on histology within the abscesses. Histology of the animal in dystocia revealed placental necrosis with hemorrhage, fibrin exudation, and cocci bacteria with neutrophilic inflammation extending into the amnion. Cocci bacteria were also noted in the fetal lung suggesting antemortem infection. All abscesses cultured *Streptococcus thoralensis*, which was confirmed by 16S rRNA sequencing, however, additional bacterial species were also present in some cases. While *S. thoralensis* has been shown to be a commensal bacterium of the vaginal flora of swine, it has not yet been determined to be a commensal of the murine reproductive tract. Given the number of cases seen with acute or chronic reproductive pathology, there is significant concern that *S. thoralensis* may be an opportunistic pathogen in the mouse reproductive tract.

#### P70 Eradicating Mouse Norovirus through Disinfection Procedures and Cross-foster Rederivation

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Mouse norovirus (MNV) is the most prevalent viral pathogen of laboratory mouse colonies. In immunocompromised mice, it is known to cause clinical disease including peritonitis, pneumonia, and hepatitis. It can also alter the immune response, which may confound research results. After the discovery of MNV in a colony of IFN $\gamma$ KO and IL17 KO mice, we attempted to eliminate MNV through disinfection procedures and cross-foster rederivation. Newborn pups were disinfected with chlorine dioxide and cross-fostered to CD-1 females that were confirmed to be free of MNV. These pups were then tested 3-4 wk later to evaluate efficacy of the rederivation process. Disinfection and cross-fostering was successful in 100% of the litters as determined by fecal PCR for MNV. Our results suggest that cross-fostering, along with detailed sanitation procedures, can be effective in eliminating mouse norovirus from mouse colonies.

#### P71 withdrawn

#### P72 Marmoset Cross-fostering: An Attempt to Preserve the Life of the Weakest Infant and Enhance the Growth of the Breeding Colony

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Although twinning is the most frequent reproductive pattern seen within a common marmoset breeding colony, it is not uncommon to witness single births and triplets in a family group. In the event of

triplets, the burden on the female frequently proves too difficult and the smallest of the litter is unable to survive due to weight-bearing and lactation restrictions of the mother. Rather than allowing inevitable suffering, it is often necessary to euthanize the smallest or most feeble infant(s) to limit the number of offspring to 2. Cross-fostering any offspring to another female marmoset who has given birth to a singlet within a close time frame, or when a lactating female suddenly loses 1 or more of her twins, if done carefully, has proven to be an effective alternative to euthanasia. Our fostering case began when surrogate gave birth to 2 stillborn infants and 1 surviving infant: baby A. Two days later, another female gave birth to surviving triplets: baby B, baby C, and baby D. That morning the smallest surviving triplet, baby D, and baby A were rubbed together to transfer scents and placed in a separate small cage in a quiet room with female marmoset 1. After some time had passed, and baby D had not yet been accepted by its surrogate, the hammock from the surrogate's original cage was placed on the bottom of the small cage with baby D to aid in scent transfer. This proved successful, and after a small amount of time surrogate mom picked up baby D and allowed him to nurse. Today, this marmoset family unit continues to thrive and both infants are now healthy active juveniles. Cross-fostering can both preserve life and successfully enhance the growth of a captive marmoset breeding colony.

#### P73 Diagnosis and Surveillance of *Streptococcus Equi* Subspecies *Zooepidemicus* Infections in Chinchillas (*Chinchilla lanigera*)

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Within a 6-mo-period, two 6-mo-old, female chinchillas (*Chinchilla lanigera*) presented with large, round, firm, nonmobile, subcutaneous masses. The first chinchilla being experimentally naïve had a mass in the left submandibular region approximately 3.5 cm in diameter. The second chinchilla which had undergone several weeks of acoustic behavioral training had 2 masses in the left axillary region both approximately 3 cm in diameter. At the time of arrival, there were no abnormalities noted on physical examination and these masses were observed almost 2 mo later. Due to being poor research candidates, euthanasia was elected in both cases. Blood was collected for a CBC, and a necropsy was performed which confirmed abscesses, followed by bacterial culture, antibiotic sensitivity testing, and histologic assessment. CBC results for both chinchillas showed mild anemia, likely due to an inflammatory response, lymphopenia with a large number of toxic neutrophils, and monocytes. Histology of the lungs, liver, spleen, and kidneys showed a marked increase of polymorphic neutrophils and lymphocytes. *Streptococcus equi* subspecies *zooepidemicus* was isolated in pure culture from the abscesses. To aid in diagnosis and surveillance, a qPCR assay was developed. Oral and nasal swabs were collected from the remaining 11 chinchillas in the original cohort. Two of 11 (18.2%) tested positive for *S. zooepidemicus* by oral swab only, suggesting exposure but no evidence of infection. Guinea pigs housed in the same room were all negative for *S. zooepidemicus* by both oral and conjunctival swabs. In total, 2 of 26 (7.7%) chinchillas developed abscesses after arriving at the institution which was confirmed to be caused by *S. zooepidemicus*. *S. zooepidemicus* is an opportunistic commensal organism found in the upper respiratory tract of horses and the endometrium of mares. It is known to cause disease in several species and is zoonotic. While documented infections in humans are rare, they can cause life-threatening illness. Once diagnosed, precautions were taken to

decrease the risk of transmission to staff and the rest of the chinchillas in the colony. To date, these are the first documented cases of *Streptococcus equi* subspecies *zooepidemicus* in chinchillas.

#### **P74 Cystic Adenoid Carcinoma of the Minor Salivary Glands in a Geriatric Pigtail Macaque (*Macaca nemestrina*)**

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A 22-y-old male castrated pigtail macaque (*Macaca nemestrina*) presented with focal swelling on the left side of the face on routine physical exam. The swelling was located 1 cm lateral to the nasal bridge and 2 cm ventral to the orbit, was firm on palpation, and measured 4 x 3.5 x 1.5 cm. Airflow through the left nostril was occluded. On oral exam, there was moderate firm swelling of the left hard palate. Radiographs revealed a radiopaque lesion in the nasal cavity adjacent to the apex of the maxillary canine tooth. Surgical extraction of the left maxillary canine tooth was scheduled due to suspect tooth root abscess. Preoperative bloodwork was unremarkable. During surgery, 4 additional teeth in the left maxillary quadrant were found to be mobile due to compromised alveolar bone. Upon removal of the teeth and surrounding bone, a soft, irregular space-occupying mass was noted at the floor of the canine socket extending into the maxillary sinus. The mass was debulked and the oral-antral fistula was closed with a buccal mucoperiosteal flap. Biopsy of teeth, alveolar bone, and the mass revealed an adenocarcinoma of either salivary or lacrimal origin. An MRI was performed 1 wk later to determine the extent of the mass. MRI identified a septated, expansile, avidly contrast-enhancing, osteolytic lesion associated with the left ventral aspect of the maxilla estimated to be 4.6 x 2.7 x 3.5 cm in size. The mass extended from the left incisive bone ventrally to involve the hard palate and caudally to involve the entire alveolar bone of the left maxilla. Dorsomedially, the mass extended into the inferior meatus of the left nasal passage and the ventral aspect of the left paranasal sinus. Due to the locally extensive nature of the mass, euthanasia was elected. Following consultation with specialists in bone pathology and head and neck tumors, final diagnosis was a cystic adenoid carcinoma of the minor salivary glands. To our knowledge, this is the first report of this tumor type in a macaque.

#### **P75 Canine Hemorrhagic Pneumonia Caused by Extraintestinal *Escherichia coli* Infection**

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On receipt of a shipment of 40 purpose-bred beagles, 8 mo in age, into an indoor facility, one 8 kg female presented laterally recumbent in the shipping crate with vomitus and mucoid feces. Immediate veterinary examination identified hunched posture, reluctance to ambulate, mild responsiveness, quiet mentation, tense with vocalization on abdominal palpation, tachypnea (90 breaths/min), tachycardia, and fecal staining with blood and mucus. Following buprenorphine administration, the heart rate decreased to 162 beats/min with diminished heart sounds and a weak peripheral pulse. Clinical condition rapidly deteriorated to unresponsiveness, absent withdrawal reflexes, and visible nictitating membranes. Radiographs identified no notable findings. Euthanasia and a diagnostic necropsy were conducted. Eight days post-arrival, a second female from the same shipment was euthanized and diagnostic necropsy performed due to similar clinical signs, including wheezing inspiratory sounds in the lungs. Necropsy of the first female showed hemorrhage of the right middle lung lobe and a darkened mediastinal lymph node. Histologic evaluation identified acute, marked diffuse fibrinohemorrhagic suppurative pneumonia with edema and red blood cells present within the mediastinal lymph node. Necropsy of the second female revealed bilateral pulmonary hemorrhage and exudative pleural effusion. Swab cultures were collected,

with *Escherichia coli* identified from all samples. Both cases were diagnosed as acute hemorrhagic pneumonia with edema caused by an extra-intestinal *E. coli* infection. This condition is rare and 2 cases within a single shipment have not previously been reported in the literature. These cases emphasize possible effects of shipment stress and continued efforts to mitigate.

#### **P76 Use of Plastic Food Wrap to Maintain Core Body Temperature during Surgical Procedures in New Zealand White Rabbits**

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Maintaining appropriate body temperature of an animal going under anesthesia is important to minimize intra- and postoperative complications. This is especially true with smaller patients like New Zealand White rabbits where it can be increasingly difficult to maintain adequate body temperature in a surgical setting. It has been observed that it only takes on average 10 min for a rabbit to lose 1-2°F of their body temperature intraoperatively. This pattern continues even with the rabbit fully draped by 2 layers of surgical drapes and a recirculating warm water blanket. Our hypothesis was that wrapping the nonsurgical area of the rabbit with an impermeable plastic food wrap would result in maintenance of a more stable and adequate core body temperature throughout surgery. Each rabbit was sedated and aseptically prepared for each procedure, which takes approximately 30 min. Once monitoring equipment was properly placed, the rabbit was covered in plastic food wrap over the abdomen and thorax securing it to the edges of the metal surgery table. Body temperature was monitored via a rectal probe throughout the procedure. Each procedure lasted on average 120 min. Our study had 76 rabbits in total, 38 rabbits in each group. Group 1 had plastic food wrap, and group 2 did not have plastic food wrap. The rabbits in group 1 had an overall higher average body temperature than those in group 2. The rabbits in group 1 had an average temperature at sterile prep time of 101.0°F, at surgical position change the average was 100.3°F and the average temperature at the end of surgery was 99.7°F. In group 2, the average temperature at sterile prep time was 101.8°F, at surgical position change the average was 100.2°F and the average temperature at the end of surgery was 99.4°F. The average decrease in body temperature for group 1 was 1.3°F and the average decrease in body temperature for group 2 was 2.4°F. Our study provides a simple and cost-effective means to improve core body temperature in New Zealand White Rabbits undergoing surgical procedures.

#### **P77 Rat Polyomavirus 2 Infection in Immune Competent Rats**

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Rat Polyomavirus 2 (RatPyV2) was first identified in rats with severe combined immunodeficiency. Screening of immunocompetent rat samples with RatPyV2-specific tests revealed a 32% seroprevalence and a 0.7% prevalence by fecal PCR which suggested transient viral shedding. To assess disease in immunocompetent rats, SD rats were administered an intranasal dose of 10E5 copies of RatPyV2 purified from feces from infected nude rats. At 12 wk postinoculation, 11 of 12 rats seroconverted and no virus was detected by PCR in feces or multiple target tissues. These findings confirmed subclinical disease with seroconversion and limited fecal shedding in immunocompetent rats. Transfer of virus by soiled bedding was verified using SD rats exposed weekly to soiled bedding from cages housing RatPyV2-infected nude rats. After 12 wk of exposure, all SD rats (n=4) were seropositive. Since polyomaviruses can persist in tissues of seropositive hosts, persistence of RatPyV2 was evaluated by evoking viral replication via immunosuppression of seropositive rats. SD and nude heterozygous rats, 7-12 wk of age, from infected breeding colonies were bled at time 0. Rats were assigned to seronegative or seropositive groups, and within each group, to a sham (PBS) or

immunosuppression (methylprednisolone acetate) treatment protocol. Doses were administered subcutaneously at weekly intervals for 8 wk. At study end, target tissues were tested by PCR. No RatPyV2 was detected in any sample from seronegative rats from either treatment group or from seropositive rats in the sham group. RatPyV2 was detected in 25% of tracheal and 6% of nasopharyngeal samples in the seropositive group receiving immunosuppressive treatment. In summary, RatPyV2 infection in immunocompetent rats is subclinical with seroconversion and transient virus shedding. Virus can be transmitted to naïve rats through contact with soiled bedding. There is preliminary evidence of viral persistence and the potential for reactivation in seropositive rats receiving prolonged immunosuppressive therapy.

**P78 A Comparison of the Effects of a Single Subcutaneous Injection of Buprenorphine, Sustained Release Buprenorphine, and Simbadol in Rabbits**

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Pain management in rabbits is a difficult and complex task, and many analgesic regimens are ill-defined in this species. As a prey species, rabbits are adept at hiding pain and can be stressed by handling and restraint for injection. The use of drugs with longer durations can be beneficial by reducing the number of times the animal needs to be restrained and potentially less stressed. However, there are several negative side effects of opioid or sustained-release opioid use in this species. Gastrointestinal ileus and inappetence are 2 major side effects which can be measured by food consumption as well as feces production. A possible negative side effect documented in literature when using sustained-release opioids is tissue reactions. We compared the effects on injections site reaction, food consumption, and elimination following single injections of buprenorphine HCl (BupHCl) (n=7), buprenorphine sustained-release (BupSR) (n=8), and Simbadol (n=7) for the control of pain associated with minor survival surgery. Data employed in this study included gross tissue reactions at the site of injection, changes in feed consumption, and feces production in the 3 d following surgery. No injection site reactions were visible grossly in any rabbit. Relative to baseline measures, food consumption was suppressed to a greater degree in BupSR subjects than in controls on day 1 ( $P < 0.05$ ), day 2 ( $P < 0.001$ ), and day 3 ( $P < 0.001$ ). In the subjects receiving Simbadol, the same patterns were seen on days 2 and 3 ( $P < 0.001$  and  $P < 0.05$ , respectively). Feces production was reduced relative to baseline values to a greater extent in BupSR animals than control animals on days 2 ( $P < 0.05$ ) and 3 ( $P < 0.05$ ). These side effects should be taken into consideration when choosing a long-lasting opioid to manage pain in rabbits.

**P79 Monodiscoid Placenta and Severe Placental Abruption in a Rhesus Macaque (*Macaca mulatta*)**

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A 12-y-old, female, SPF rhesus macaque (*Macaca mulatta*) housed in an indoor-outdoor breeding group presented to the clinic for complete right arm lameness of 3-d duration. During morning observations the subsequent day, the animal was noted with clinical signs indicative of labor. Despite conservative monitoring, no clinical progress or parturition was noted. Physical examination revealed hypothermia, pale mucous membranes, poor capillary refill time, mild dehydration, fresh blood perivaginally, and third trimester pregnancy with semi-firm uterus and fetal head positioned near the pelvis. iSTAT values revealed azotemia, anemia, and hyperlactatemia. Pelvic ultrasound revealed no discernible fetal heartbeat with a single placental disc on the ventral aspect unattached and separated from the uterine wall by a large hypochoic space. Due to the patient's clinical status and a high clinical suspicion of placental abruption, emergent cesarean section

was elected. Diffuse petechiation and ecchymoses covered approximately 50% of the uterine surface with a small blood clot and serosal tear on the ventral uterine surface. Immediately after a horizontal fundic uterine incision was made, the amniotic membrane and a large blood clot bulged from cut surface. The blood clot was gently removed via digital manipulation, and the fetus and placenta were removed en bloc. The placenta was monodiscoid with a diffusely thickened yellow friable material at the margin of the disk and placental membrane, which corresponded to regions of hemorrhage, fibrin, and coagulative necrosis (infarction), histologically. The patient recovered from anesthesia without complication after routine closure methods. Monodiscoid placentae have been reported in up to 9.5% of rhesus macaque births, and is generally considered an incidental finding. While a relatively rare condition, placental abruption is a leading cause of maternal morbidity and perinatal mortality in human medicine and requires emergent management. Placental abruption has been reported in multiple nonhuman primate species, and should be considered a differential for any pregnant female presenting with reproductive complications.

**P80 Surgical Loupes: A Useful Tool for Small Target Rodent Procedures**

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Performing technical and surgical procedures on mice demands precision and accuracy. The need for accuracy further increases when working with more costly animals, such as humanized mice, which continue to be used in preclinical research studies. To perform such tasks requires the technicians to be highly trained and skilled. An average technician can perform 50-150 IV injections a day, in veins not much bigger than a 27 gauge needle. With such repetition, musculoskeletal injuries, such as chronic neck and back pain problems and/or eye strain can occur. Aesthenopia (eye strain) can result from focusing on a fixed point for an extended period of time. Symptoms can emerge over time as headaches, blurred or double vision, fatigue, trouble focusing the eyes on an object, or pain around the eyes. Problems with eye strain can be further exacerbated by poor posture and incorrect ergonomic position as technicians' hunch over to move closer to their mark to enhance visual acuity and perform their work. Although many have often viewed magnification as an aid to failing vision for older staff members, the benefits can be significant by increasing visual acuity and accuracy reducing musculoskeletal stress by promoting good posture. In the past many technicians have turned to using a head-mounted magnifier, frequently used by jewelers to enhance magnification. Unfortunately, these are heavy, ill-fitting, and require the user to move closer to the target because of a limited focal distance and do not solve the ergonomic issues. We have found that using surgical loupes resolves both the musculoskeletal and eye strain issues. Loupes can increase target size up to 8 times and provide visual focus such that performing technical and surgical procedures can be performed in a normal ergonomically correct sitting position. Loupes can be individually custom-fitted, including ocular prescriptions and provide for mountable light sources for optimal illumination. They are lightweight and comfortable to wear, and portable, replacing costlier, less mobile surgical microscopes. Most important, loupes have greatly enhanced visibility for veins that have been injected repeatedly. The condition of the tail declines with each injection, and magnifying the vein helps increase accuracy.

**P81 Pulmonary Edema following the Systemic Absorption of Topical 10% Phenylephrine Hydrochloride Ophthalmic Solution in the African Green Monkey (*Chlorocebus sabaeus*)**

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Phenylephrine is an  $\alpha_1$ -adrenergic receptor agonist frequently used as a mydriatic for ophthalmic examinations and procedures. Systemic absorption of 10% topical phenylephrine solution has previously been reported in human pediatric patients. We report 3 cases of pulmonary edema in the African green monkey (*Chlorocebus sabaeus*) following administration of topical 10% phenylephrine HCl solution. These clinical signs observed include blood-stained foamy fluid exuding from the mouth and nares, elevated respiratory effort and rates, and grey to purple mucous membranes. Thoracic auscultation revealed bounding, elevated heart rates, and significant bilateral crepitations. The animals were immediately placed in the prone position and eyes were rinsed with 0.9% saline to reduce further exposure to phenylephrine. The foam in the oral cavity and throat was repeatedly removed with gauze, and supplemental 100% oxygen was delivered via oxygen mask. Hydralazine (5-8 mg) was administered intramuscularly to ameliorate suspect hypertension. After 20-40 min, the monkeys' mucous membrane color, heart rates, and respiratory efforts improved and the foaming ceased. The animals were monitored over the following days and their respiratory rates and efforts gradually returned to normal. Veterinary and research staff should be aware of the cardiovascular impact of systemic absorption of phenylephrine ophthalmic solution. Individual sensitivity to the compound, preexisting cardiovascular abnormalities, and repeat topical dosing of higher concentrations of phenylephrine (e.g. 10%) may increase the likelihood of these cardiovascular side effects. To decrease the incidence of this complication, staff should be trained to identify early signs of adverse phenylephrine reactions, ensure appropriate administration of the compound, and incorporate substitute or supplemental mydriatics, including 1% tropicamide and 1% cyclopentolate hydrochloride ophthalmic solutions or lower concentration phenylephrine.

#### **P82 Serum Matrix Metalloproteinase 9 (mmp9) as Useful Biochemical Marker and Molecular Candidate for Treatment of Wasting Marmoset Syndrome**

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Use of the common marmoset (*Callithrix jacchus*) as a nonhuman primate experimental animal has increased in recent years. Although wasting marmoset syndrome (WMS) is one of the biggest problems in captive marmoset colonies, the molecular mechanisms, biochemical markers for accurate diagnosis, and a reliable treatment remain unknown. In this study, as a first step to finding biochemical marker(s) for the accurate diagnosis of WMS, we conducted blood cell counts, including hematocrit, hemoglobin and platelets, and examined serum chemistry values, including albumin, calcium, and levels of serum matrix metalloproteinase 9 (MMP9), using a colony of marmosets with (WMS group; n=7) and without weight loss (control group; n=7). MMP9 is thought to be an enzyme responsible for the degradation of extracellular matrix components and participates in the pathogenesis of inflammatory conditions, such as human and murine inflammatory bowel disease (IBD), which, like WMS, are characterized histologically by inflammatory cell infiltrations in the intestines. The values of hematocrit and hemoglobin and levels of serum albumin and calcium in the WMS group were significantly decreased versus the control group. The platelet values and serum MMP9 concentrations were increased significantly in the WMS group compared with the control group. MMP9 could be a new and useful marker for the diagnosis of WMS in addition to hematocrit, hemoglobin, serum albumin, and calcium. Our results also indicate that MMP9 could be a useful molecular candidate for treatment. MMP9 is activated by plasmin, a fibrinolytic factor. In mice, it was reported that inhibition of plasmin using an antibody prevented the progression of IBD. Next, we examined the efficacy of tranexamic acid, a commonly used plasmin inhibitor, for the treatment of WMS, with supportive measures including amino acid and iron formulations. Six marmosets among WMS-affected animals received tranexamic acid therapy with supportive measures for 8 wk. The body weight, hematocrit values,

and serum albumin levels of the 6 marmosets in this study significantly increased, while serum MMP-9 levels significantly decreased after tranexamic acid therapy with supportive measures. Thus, tranexamic acid therapy may be a new and useful treatment for WMS.

#### **P83 An Alternative to Hysterotomy for Cerebral Injection and Electroporation in a Ferret Model**

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Ferrets are commonly used in research for their short gestational age of 41-42 d and their ability to carry a fetus with little resorption and high yields. Young ferrets have a comparable cerebral development to humans and are an ideal species for the study of early cortical development. Our goal was to design an effective delivery method of DNA plasmid to fetal cerebral ventricles followed by electroporation. Traditionally, fetal surgery occurs via a single or multiple hysterotomy sites. The potential risks of hysterotomy present a risk to both fetus and jill. In previous studies, complications observed included, but are not limited to fetal re-absorption, facial malformation, uterine necrosis, and wound dehiscence. An alternative method to hysterotomy was performed and examined to minimize risks and to preserve fetal viability. A group of n=5 wild-type ferrets at a known gestational age (31 d) underwent a laparotomy. Each uterine horn was exposed, allowing complete visualization of the number of embryos present. Each fetus was individually isolated with careful palpation to locate the fetal head, with respect to laterality. The fetus underwent intraventricular injection through the uterine wall, followed by electroporation. The jill was allowed to recover and deliver kits vaginally. There was no loss of jill or kits due to surgical complications. This approach allows for an unlimited number of fetal kits to undergo injection, as well as increase kit viability and minimize cranial and facial deformities. Closed uterine fetal surgery also presents the possibility for the jill to be rebred to maintain a breeding colony and conservation of an animal.

#### **P84 A Comparison of Catheterization Methods for the Preterm Piglet (*Sus scrofa domestica*)**

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Piglets of varying gestational ages (105-107 d) underwent venous catheterization. A retrospective review was conducted to determine the best catheterization methods in preterm piglets. Three litters were delivered via c-section with a total of 33 piglets. The piglets were delivered and immediately placed under isoflurane. Piglets underwent right external jugular catheterization via cut down method. The catheters were secured in place using 2-0 PDS suture for the duration of the study. Comparatively, 10 piglets presented at smaller weights, making jugular catheterization more difficult or impossible. Alternative to jugular cannulation, umbilical vein catheters were placed using aseptic technique temporarily the day of c-section for administration of 0.45% NaCl with 5% dextrose, B12 supplement, blood collection, and maternal plasma transfusion. This method was highly favorable and more successful because of the multiple implications presented while using long-term central lines. Umbilical catheterization did not require anesthesia, did not require alterations or surgical replacement during the study, was least invasive and did not present the risk for irritation or patency issues as there was no central line in place for the entire duration of the study.

### P85 Use of an Electronic Anesthesia Recording System to Aid in Anesthetic Monitoring and Recordkeeping in a Laboratory Animal Medicine Setting

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The American College of Laboratory Animal Medicine (ACLAM) has extensive recommendations for anesthesia records, which include identification of individual animals or groups of animals, documentation of drugs administered, description of the surgical procedure, and monitoring parameters and ongoing intraoperative findings. Currently, electronic programs for monitoring and tracking anesthesia records in the laboratory animal medicine field are lacking. We investigated the utility of an electronic veterinary anesthetic record system in a laboratory animal medicine setting. We used a commercial veterinary anesthetic record program to aid in anesthetic monitoring and recordkeeping. According to the American College of Veterinary Anesthesia and Analgesia (ACVAA), monitoring variables should be recorded at a minimum of every 5 min during anesthesia. The program was used to record anesthetic parameters for ~160 anesthetic procedures, involving multiple species, over the course of 5 y. Recorded parameters included notations regarding preoperative evaluation, anesthetic plan, routine vital signs, complications noted during the procedure, and recovery quality. Formalized records were generated at the end of each anesthetic procedure, and stored electronically. We identified many advantages with the electronic record system, including visual and audible prompting to record vital parameters at the appropriate time intervals, convenient tracking and graphing of changes in intraoperative vital signs, and drug dosing support as the software calculates doses for some laboratory animal species. Use of the program also aided billing accuracy and efficiency. Electronic records were organized by investigators, which allowed rapid referencing of specialized, research associated anesthetic procedures. Challenges included difficulties amending incorrectly entered values, and defining when specific procedures had begun or ended. However, both challenges were resolved by individual instruction. We found that the electronic anesthetic monitoring program functioned well and complied with the recommendations set by ACLAM and ACVAA.

### P86 Treatment of Chytridiomycosis in Laboratory-maintained Axolotls (*Ambystoma mexicanum*) and Wild-caught Rough Skinned Newts (*Taricha granulosa*)

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Axolotls (n=12) in a laboratory-reared colony developed multifocal erythematous dermatitis over 12 mo, mainly on the distal limbs and tails. Wild-caught newts handled by the same lab personnel were housed in an adjacent room and occasionally presented with abdominal bloating and lethargy. Diagnostic approaches included assessments for issues with water quality, pathogen infection, parasitic infestation, and trauma. Water analysis found no mineral abnormalities. Aerobic bacteria cultured from tank water and skin or liver samples from affected animals (3 axolotls, 1 newt) included *Aeromonas*, *Microbacterium*, *Pseudomonas*, and *Shewanella* spp., while fungal culture from an axolotl skin lesion found no growth. Skin histology revealed heterophilic and lymphocytic erosive or ulcerative dermatitis from 2 axolotls and severe lymphohistiocytic cellulitis from 1 newt. Based on sensitivity, axolotls (n=3) were treated with enrofloxacin (10 mg/kg IM, SID x 7 d), with limited efficacy. Subsequent skin swabs from affected axolotls (n=2), 1 previously

treated with enrofloxacin, were positive for *Batrachochytrium dendrobatidis* on a novel PCR panel. Additional testing noted positive results with PCR and DNA nucleotide sequencing in clinically normal group-housed newt tanks, and suspect-positives for additional animals. Of note, *B. dendrobatidis* is a reportable disease in Michigan; collaboration with the Michigan Department of Agriculture and Rural Development (MDARD) was initiated and all animals officially quarantined. Axolotls with skin lesions (n=2) and newts with bloating and lethargy (n=2) were selected for experimental treatment with itraconazole baths (0.0025%; 5 min SID x 10 d), which were well tolerated and led to clinical resolution. Itraconazole treatment of the entire colony followed, with regrowth of extremities and restoration of normal coloration seen among many axolotls during treatment. PCR results after treatments (at 1 wk and 1 mo post) were negative. Therefore, this itraconazole submersion protocol appears to be a successful treatment for *B. dendrobatidis* in laboratory-maintained colonies of *A. mexicanum* and *T. granulosa*.

### P87 Evaluation of Incidence and Risk Factors for Surgical Glove Perforation in Nonhuman Primate Surgery

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Surgical gloves act as a physical barrier to protect the surgeon from potential exposure to bloodborne and skin pathogens. Gloves also protect the surgical wound from contact with microorganisms on the surgeon's hands. Perforation of surgical gloves is common and well documented in human and veterinary literature with perforation rates ranging from 2–50% of gloves evaluated. Nonhuman primate surgical procedures are of interest due to the potential exposure of surgeons to bloodborne pathogens and potential pathogenic bacterial colonizing chronic implants or infected wounds. We sought to evaluate the incidence of surgical glove perforation and potential risk factors for glove perforation in nonhuman primate surgical procedures. Surgical gloves were collected from operative personnel at the end of surgery and putative risk factors were recorded. Gloves were evaluated for visible perforations followed by testing using the water-leak test as described by the American Society for Testing and Materials International. Associations between categorical variables were determined with Fisher's exact tests. Surgical glove perforations occurred in 37% (11/30) of surgeries and perforations were present in 10.5% (19/180) of surgical gloves evaluated. Perforations primarily occurred on the thumb (47%) and index finger (42%) with no difference in perforation rate between the dominant and nondominant hand. The intraoperative detection of perforation by the surgeon was poor, being present in only 5% (1/19) perforations. The use of a surgical drill significantly increased the risk for glove perforation ( $P = 0.03$ ). The results of this study highlight the need for investigator training regarding the potential for surgical glove perforation in nonhuman primate surgical procedures, as well as the importance of postoperative hand cleansing.

### P88 Bilateral Auricular Erythema and Necrosis in a Male Gottingen Minipig

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A 6-mo-old, intact, single-housed, 10 kg male Gottingen minipig presented with bilateral ear erythema at the apical margins 4 h post repetitive auricular venipuncture. The pig had open cage bar communication with conspecifics. The pig had a jugular venous access port and telemetry implanted 2 mo prior to presentation. The pig was enrolled in a pharmacokinetics study 1 mo prior and received 2 doses scopolamine. On exam, the pig was bright, alert, responsive (BAR) with 10 needle punctures per ear with bruising and blood extravasation. At the 24 h recheck, pig was BAR but the erythema expanded the entire pinnae with ulceration, crusting, and epidermal thickening with necrosis at the apical margins. Aerobic culture

revealed *Staphylococcus aureus* and povidone-iodine scrub with silver sulfadiazine therapy was initiated. Primary differentials included research/conspicuous-induced trauma, bacterial, and/or viral systemic disease. At the 48 h recheck the pig presented with depression, inappetence, dyspnea, cold extremities, petechiation on snout, lips, rectum, and prepuce, and red-purple discoloration on all limbs distal to hock/elbow. Clinical pathology revealed a regenerative anemia, low hemoglobin, marked thrombocytopenia, and elevated ALT, AST, GGT, and albumin. A differential count supported thrombocytopenia with schistocytosis, nucleated RBCs, acanthocytes, and target cells. The pig was euthanized due to poor study candidacy and disease severity. Gross necropsy noted hemorrhage, necrosis, and degeneration in multiple organs. Histopathology showed vascular thrombi and arteriosclerotic changes in the arterioles and small arteries of the heart, kidney, lung, GI tract, liver, pancreas, and diaphragm. Microscopic findings included lymph node erythrophagocytosis, membranoproliferative glomerulonephritis, bone marrow megakaryocytic hyperplasia and extramedullary hematopoiesis. Necropsy finalized a diagnosis of thrombocytopenia purpura syndrome of Gottingen minipigs. Pathogenesis is unknown but suspected to be a Type 3 hypersensitivity reaction causing degenerative vasculopathy with arteriosclerosis as a distinctive feature. This case warrants inclusion of thrombocytopenia purpura syndrome as a differential for acute auricular hemorrhagic necrosis in the Gottingen minipig.

#### **P89 Xenogeneic Graft-versus-Host Disease (GvHD) in Humanized Mice**

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Fundamental differences between mouse and human biological systems result in significant limitations in mouse models of human disease. Yet mice remain an important tool for understanding basic and translational aspects of the human immune system. Recent advances in mouse technologies, including the development of humanized mice, have allowed for new studies of primary human immune responses. Humanized mice are immunodeficient mice engrafted with human cells, tissues, or most commonly, human immune systems. Compared to traditional mouse models, they better recapitulate some aspects of human immune response in the context of several diseases, and have been used to study human-specific responses to infectious agents, drug metabolism, cancer biology, regenerative medicine, and transplantation. The different factors involved in creating humanized mice (immunodeficient mouse strain, method of human immune system engraftment, knock-in or knock-out of additional genes) also affects the onset and development of xenogeneic graft-versus-host disease (GvHD). However, little is known about the impact of age on xenogeneic GvHD in humanized mice. At our facility, a cohort of 8 mice engrafted with human hematopoietic stem cells at 1 mo of age developed eventual lethargy, weight loss, and anemia. The onset of clinical signs ranged from arrival to our institution at 8 mo of age, to 6 mo later at 14 mo of age. Gross necropsy performed on 3 of these animals revealed tan discoloration of the liver and generalized emaciation. Histopathologic findings included lymphoplasmacytic infiltrates associated with cellular degeneration in multiple organs including the kidney, spleen, lungs, bone marrow, and brain. The clinical presentation, gross necropsy, and histopathological findings were characteristic of chronic xenogenic GvHD. We summarize the different humanized mouse models and associated presentations of xenogeneic GvHD centered around our experiences with the human CD34+ hematopoietic stem cell-engrafted NSG mice.

#### **P90 Systemic Hypereosinophilic Syndrome in a Double Knockout Mouse**

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A 3-mo-old, group-housed, experimentally naïve female Casp8 (-/-) Rip3 (-/-) mouse (*Mus musculus*) presented with dermatitis. This mouse was housed on an autowater rack with ad libitum access to standard rodent chow in ventilated caging with 1/8 in corncob bedding in a room with a 12:12 light:dark cycle. No other mice in the cage were affected. Notable physical examination findings were skin lesions characterized by dorsal erythema with pinpoint scabbing and dorsal and ventral alopecia, in addition to hyperkeratosis and crusting of the dorsum, anogenital area, and both hindlimbs at the level of the tarsus. Possible differentials included ulcerative dermatitis, acariasis, staphylococcal infection, *Corynebacterium bovis*, an immune-mediated inflammatory process, underlying endocrinopathy, or neoplasia. Due to the atypical presentation, the mouse was recommended for necropsy which revealed multiple enlarged organs, including the liver, cecum, and spleen with congestion of the liver and spleen. The salivary and submandibular lymph nodes were also enlarged, as documented for the phenotype. All other organs appeared grossly normal. Diagnostics included histopathology, infectious disease PCR testing, including mite screening, and *C. bovis* testing, and aerobic culture of lung, spleen, and liver. Histopathology revealed diffuse eosinophilic inflammation in the skin, lymphoid tissue, bone marrow, lung, heart, spleen, liver, and kidneys. All other diagnostic tests were negative. Based on these findings, this mouse was diagnosed with systemic hypereosinophilic syndrome which is likely strain-related due to the inflammatory pathways that are disrupted by the Casp8 and Rip3 mutations. There are induced models of hypereosinophilic syndrome of mice, but this is the first known reported incidence of spontaneous disease. In summary, this case is an abnormal presentation of a common clinical problem due to genetic manipulation.

#### **P91 Periocular Swelling in Immunocompetent Mice**

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Two of 5, 25-d-old male, mixed 129/B6 background mice were examined antemortem for periocular swelling. Differential diagnoses for periocular swelling include a harderian/lacrimal gland abscess or neoplasia. On postmortem gross examination, there were multiple tan and opaque supraorbital masses. On microscopic examination, there were multiple coalescing subcutaneous periocular abscesses and moderate bilateral corneal edema. Aerobic culture of the masses grew *Pasteurella pneumotropica*. Clinically affected animals were treated with a fluoroquinolone antibiotic in the drinking water at 85 mg/kg for 14 d with water changes after 7 d. Over a 1-mo period, this infection spread throughout the room to several other unrelated cages with the involvement of animals from multiple principal investigators. Once this outbreak was appreciated, a room cage-changing order protocol was put into place and the entire room went on treatment with a fluoroquinolone antibiotic. The Hey1 biotype was identified by PCR testing of oropharyngeal swabs taken from a 10-d-old, female pup presenting with a closed right eye and ocular discharge. This presentation of *P. pneumotropica* is atypical due to its effect on immunocompetent individuals and historically clinical signs only present in immunocompromised.

#### **P92 Variability of Injectable Anesthesia in Germ-free Mice: Evaluation of Ketamine Cocktails**

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Tribromoethanol (TBE, 100-300 mg/kg i.p.) is used in our gnotobiotic facility as an injectable anesthetic for germ-free (GF) and defined flora (DF) Swiss Webster mice undergoing embryo transfer (ET) and vasectomy procedures. Due to the unavailability of pharmaceutical grade TBE as well as its variable anesthetic efficacy, we performed a study to evaluate use of a ketamine/xylazine/acepromazine cocktail (65-100 mg/kg ketamine, 13-20 mg/kg xylazine, 2-3 mg/kg

acepromazine i.p.) as an alternative for use during ET. Data collected included weight of animal, volume/dose given, time to induction of a surgical plane of anesthesia, and time to recovery, as well as whether animals received an additional dose of anesthetic, and whether any adverse or unusual events occurred during anesthesia or after recovery. Spontaneous mortality during use of this cocktail was unacceptably high (13 of 40 [32.5%] GF animals and 4 out of 35 [11.4%] DF animals anesthetized with the cocktail) compared to 113 out of 4741 [2.4%, combined GF and DF] during 9 mo use of TBE at our facility. Furthermore, a higher percentage of animals (4 out of 40 [10%] GF and 2 out of 35 [5.7%] DF) failed to achieve a plane of surgical anesthesia than is typical with TBE at our facility (0.34%). Provision of analgesia in the study group was unchanged from our usual procedure (buprenorphine 0.01-0.05 mg/kg i.p. administered after induction of anesthesia). These results demonstrate variable effects of different injectable agents for anesthesia of germ-free mice and highlight the need for evaluation and comparison of different injectable anesthetic protocols.

### P93 Ibuprofen in Drinking Water Reduces Paw Swelling and Improves Wellbeing in a Mouse Model of Postsurgical Pain

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Responsible, ethical standards in animal research require effective management of postoperative pain, whenever possible. Current postoperative medications include anti-inflammatory drugs such as meloxicam (acute or slow-release), opioids (buprenorphine), and topical local anesthetics (lidocaine). However, there is limited data about how efficacious these compounds and doses are for regular postsurgery pain routinely used by research laboratories. In addition, compliance with IACUC regulations may present problems since different treatments require different programs and strict timings for treatments postsurgery may be missed leading to regulatory noncompliance and suboptimal welfare management of the experimental animals. Our aim was to test if ibuprofen administered (0.4mg/ml) in the drinking water could represent an efficacious, easy-to-implement strategy to provide postoperative care to mice by reducing inflammation, pain hypersensitivity, and associated discomfort. We used the plantar incision model of postsurgical pain in mice and measured the flinch response to a heat stimulus (nociceptive reflex), running wheel activity (operant wellbeing assay) and swelling of the injured paw (local inflammatory oedema measured using a plethysmometer) 24, 48 and 72h post-incision). Ibuprofen (0.4mg/mL; n=11) compared with vehicle (n=10) resulted in approximately 100 mg/kg/day ibuprofen imbibed by a 30 g mouse. Postincision paw swelling increased ~50% at 24 and 48 h, running wheel reduced ~20% at 24 and 48 h and heat hypersensitivity increased ~30% at 24 and 48 h. Ibuprofen treatment reduced swelling and ameliorated reduced wheel running at 24 h and 48 h postincision but did not inhibit heat hyperalgesia at any time point. We conclude that administration of ibuprofen (0.4mg/ml) in the drinking water reduces local inflammation induced by surgical incision and normalizes a measure of wellbeing in mice and is, therefore, an appropriate postoperative treatment for mice undergoing minor surgical procedures.

### P94 Multiple Malformations of the Axial Skeleton in a *Xenopus laevis*

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A 1-y-old, experimentally naive, wild-caught, female African clawed frog (*Xenopus laevis*), co-housed with other females in a recirculating housing system, was identified with a ~5 mm<sup>3</sup> firm subcutaneous papule on the left side of the dorsum ~1 cm cranial to the pelvis. The frog was otherwise normal and anesthetized with MS222 for further assessment. On examination, the swelling appeared to be the cranial end of an approximately 2 x 0.25 cm, smooth, firm, oval mass, which could be moved slightly in both the cranial-caudal and dorsal-ventral directions. The frog was subsequently euthanized for oocyte harvest and complete necropsy. Careful dissection revealed the swelling to be a dorsal elevation of the cranial extremity of the left ilial shaft. Scoliosis of the spine between the 5th and 9th vertebra was also evident. Faxitron radiography and CT 3D reconstructions on the formalin-fixed axial skeleton were performed to further characterize the anomalies. A normal frog was imaged for comparison. The radiologic findings included diffuse hypodensity of the axial skeleton, abnormal dorso-ventral orientation and cartilage hypoplasia of the left sacral wing, incomplete ossification of the right sacral wing, and asymmetry of ilial shaft length with a left ilial-sacral disarticulation. CT imaging revealed mineralized intervertebral disks between the 6th and 9th vertebrae as well as multiple irregular hyperdense nodules (hyperostosis) along the dorsal, ventral, and right lateral surfaces of the sacral and presacral vertebrae, as well as along the left ilial shaft. Individual variation and sacral abnormalities have been described in laboratory-reared *X. laevis* with an unusually high prevalence relative to other species in the order Anura, while the description of ilial malformations is rare. It is unknown if these abnormalities have any association with their unique ilial-sacral anatomy in which the sacral wings move cranial to caudal with flexion and extension of their hind-limbs during propulsion. The skeletal abnormalities in this frog were likely congenital.

### P95 Vasectomy in the Owl Monkey (*Aotus nancymaae*)

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Owl monkeys, *Aotus* spp., form monogamous pairs that live in small family groups with their subadult offspring. Accordingly, owl monkeys housed for research are often maintained in male-female pairs or small families. Our institution housed a small colony of owl monkeys, *Aotus nancymaae*, for ophthalmology-focused research studies in previously established male-female pairs. Contraception was achieved with quarterly medroxyprogesterone acetate injections provided to colony females. When the studies concluded, a family unit containing 1 male, 1 female, and their approximately 5-mo-old female offspring retired to a primate sanctuary. Vasectomy surgery was performed on the male monkey as a permanent birth control solution prior to shipping the family. This procedure was elected over orchietomy or ovariectomy due to its minimal invasiveness and the benefits of maintaining primary sex hormone-producing organs. A no-scalpel vasectomy technique using electrosurgery was performed under general anesthesia with isoflurane gas. The monkey recovered without complication and meloxicam and buprenorphine was administered for postoperative pain management. Vasectomy proved to be a fast and effective surgical sterilization procedure for owl monkeys; thus, the surgery details may be useful for other institutions housing pair-bonded owl monkeys or other similarly sized primates.

### P96 Acute Morbidity and Mortality in Severely Immunocompromised Mouse Strains Due to an Enteroinvasive *Escherichia coli*

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Acute diarrhea and death were noted in a SPF barrier maintained colony of young adult NSGS (NOD.Cg-Prkd<sup>scid</sup> I12rg<sup>tm1Wj</sup>/Tg(CMV-IL3, CSF2, KITLG)1Eav/MloySzl) and NSG (NOD.Cg-Prkd<sup>scid</sup> I12rg<sup>tm1Wj</sup>/Szl) mice. Sterile housing, autoclaved water, and



irradiated food were provided. Mice presented moribund or dead, frequently with evidence of diarrhea. Initially, only mice that had been irradiated with 1 Gy, followed with engraftment of human leukemia cell lines were affected, however, PDX implanted NSG mice within the room, and a separate room of NSGS weanlings were subsequently affected. Fecal samples were negative for common murine pathogens when tested by PCR and culture (aerobic and anaerobic). Necropsy revealed a distended, ingesta-filled stomach; red-brown, dilated duodenum and proximal jejunum; dilated, gas- and fluid-filled cecum and colons with scant soft/unformed fecal matter. Segmental necrotizing enteritis with blunting and fusion of villi was noted along with catarrhal typhlocolitis with crypt abscesses, superficial enterocyte necrosis, and apically adherent coccobacilli. Following histologic recognition of colibacillosis-like lesions, targeted PCR for attaching and effacing, enteroinvasive, enteroaggregative, and Shiga-like toxins VT1, VT2, and VT2e were performed. PCR identified enteroinvasive (Einv) toxin, believed to represent *Escherichia coli*, from small intestine and colon in 3 of 3 NSGS mice tested, and 2 of 3 NSG mice. NSGS mice were depopulated, while the NSG mice were placed on enrofloxacin and allowed to complete the experiment with in-room decontamination. Additional protective measures included: use of additional PPE (synthetic material sleeves, double gloves), prolific use of a reduced contact timed disinfectant, replacement of cage-top filters, retraining of personnel on microisolation technique, and the use of autoclaved diet in the cage (previously used irradiated food in a common bin for the room). The source of the enteroinvasive *E. coli* was not determined, however, this outbreak highlights the importance of the stringent environmental standards required to successfully maintain severely immunocompromised mouse strains where germ-free animal facilities are unavailable.

#### **P97 Infant Pants Used as Refinement for Postsurgical Protection in Male New Zealand White Rabbits**

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Elizabethan collars, also known as e-collars, are the most common form of postoperative protection in all laboratory animals, including New Zealand white rabbits. In this retrospective study, we examined a novel postsurgical protection alternative which allowed us to increase social housing success in male uncastrated New Zealand White rabbits. Historically, rabbits were separated for surgery and unsuccessfully reintroduced to each other 10 d postoperatively. We introduced a new method of 2 groups, using a different method for each group to increase social housing success. Group 1 consisted of 80 rabbits that were separated for surgery then reintroduced when animals were off clinical rounds, using e-collars solely as postsurgical protection. Group 2 consisted of 82 rabbits, which were not separated for surgery, and wore 0 to 3-month-old sized human infant pants solely as postsurgical protection. When the infant pants were destroyed or fell off, they were replaced with replacement human infant pants or an e-collar. While investigating these methods, we found that using infant pants as postsurgical protection for left and right femoral angioplasties incision sites increases pair housing success in adult male uncastrated New Zealand white rabbits significantly. Group 2 had 98% social housing success with a total of 42 pairs of rabbits socially housed postsurgically, and group 1 had no success pair housing postsurgically. Group 1 rabbits were singly housed after their first and second surgeries and for the duration of the study. These animals remained paired for the duration of the study, which varied from 120 to 180 d. Factors such as self-mutilation rate, rate of replacement of infant pants, and rate of failure of the infant pants were also examined. We suggest that human infant pants be used as an alternative to e-collar to increase social housing success in male New Zealand uncastrated rabbits.

#### **P98 Consumption of a Breakfast Cereal as a Pain Assessment Tool in Rats**

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Analgesia is an important component of animal use in laboratory animal medicine, particularly for rats that are commonly used in research studies involving pain. There are few easy, reliable, and objective pain assessment tools for assessing pain in rats that can be used cageside in a timely manner. Rats are typically motivated to consume a commercially available breakfast cereal, and we evaluated the time it takes for rats to consume the cereal following ovariectomy for its use as a proxy indicator of pain. Baseline time to consumption (TTC) was recorded in 36 rats prior to surgery. They were then ovariectomized (n=27) or received anesthesia only (n=9) and assigned 1 of 3 groups: subcutaneous treatment with 2 mg/kg meloxicam, 4 mg/kg sustained release SR-meloxicam, or 1 mL saline. After surgery, the rats were given 1 h for recovery before 4 pieces of cereal were dropped into the 4 corners of the cage and TTC was recorded at 3 and 24 h postoperatively. Additional behavioral pain assessments (activity, grooming, wound licking, orbital tightening) were recorded for 5 min at 1, 3, 6, 12, 24, 48 h postoperatively. The baseline TTC was inconsistent among the treatment groups and varied from 175-300 s. After surgery, there was no difference in the TTC in the rats regardless of treatment, although all treatment groups tended to have reduced TTC at 3 and 24 h postoperatively. This included the rats that were anesthetized only. Rats in the saline-treated and meloxicam-treated group had higher orbital tightening scores than those treated with SR-meloxicam at 1 h postoperatively. There was no difference in the frequency of arched postures in the treatment groups at 1, 3, or 6 h postoperatively. Rats that were anesthetized only had no orbital tightening or arching postures. The cereal consumption time did not correlate with the treatment groups, indicating that it is not a sufficient proxy indicator of pain in the rat ovariectomy model.

#### **P99 Effective Treatment of Pododermatitis in Rats**

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Animal experimentation uses laboratory animals as models of human diseases to better understand their pathophysiology and treatment. With the human population aging, there is a need for aging rats used in research studies. Unfortunately, older rats tend to develop pododermatitis which can be difficult to treat efficiently to humanely maintain the animals until reaching the target age for the experimental endpoint. Facing this problem in an aging colony of a rat model of Alzheimer's disease, we have developed a multimodal approach to prevent and treat pododermatitis. Seventy-two rats received the following treatments: as soon as an animal was observed with minor pododermatitis, the corncob bedding was replaced with a commercially available softer substrate for the paws. When inflammation, swelling and/or presence of a blister were visible under the paw, we provided hydrotherapy for 5 min, followed with green clay topically on the lesion, and a bandage. Hydrotherapy was provided every 12-24 or 48 h depending on the severity of the lesion. The bandage was changed daily or every other day. Within a few days of treatment, the pododermatitis was almost healed with less inflammation, erythema, and no noticeable blister or ulceration. The animals were able to regain a better quality of life until the end of their experiment. In conclusion, the multimodal therapy was effective to prevent severe lesions and heal pododermatitis in aging rats.

**P100 Use of Intramuscular Meloxicam in Zebra Finches (*Taeniopygia guttata*)**KA Miller<sup>1</sup>, N Hill<sup>2</sup>, MM Patterson<sup>3</sup>

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Zebra finches (*Taeniopygia guttata*) often undergo surgical procedures as part of animal use protocols looking into learning and memory. Individual birds are typically given a local anesthetic and a systemic opioid preoperatively, with the opioid, continued postoperatively for 2-3 d. More recently, the possibility of giving intramuscular meloxicam (a selective COX-2-inhibiting NSAID) for enhanced multimodal analgesia has been suggested; however, it is unknown what meloxicam dosage regimen would be appropriate for zebra finches. Based on previous publications looking at flamingos, hawks, owls, and parakeets, among others, the pharmacokinetic parameters of meloxicam (with doses ranging from 0.5-3.0 mg/kg by various routes) can differ widely among bird species, thus highlighting the need for species-specific analyses; indeed, zebra finches are passerines, in contrast to the types of birds that have already been investigated. In the current study, adult zebra finches are dosed with 1 or 3 mg/kg (approximately, and based on exact weight of each bird) of meloxicam intramuscularly. At 5 time points after dosing (ranging from 0.5 to 24 h), plasma is collected from the birds during a terminal cardiac blood draw under isoflurane anesthesia, and the plasma samples are analyzed using HPLC. The data show that a target plasma concentration of 3.5 ug/mL, which has been used in other studies involving birds, is sustained for approximately 9 h at 1 mg/kg of meloxicam, and for over 12 h at 3 mg/kg. In addition, maximum plasma concentrations of meloxicam are achieved at both dosages by 0.5-1 h after an intramuscular injection, but the 3 mg/kg dose results in an extremely high peak concentration level of over 35 ug/mL followed by a sharp decline. Meloxicam given intramuscularly is metabolized quickly by zebra finches, and a currently recommended dose of 1 mg/kg will need to be administered more frequently than once a day to maintain optimal plasma concentrations.

**P101 Behavioral Validation of Analgesia from 3 Formulations of Carprofen in a Mouse Laparotomy Model**M Walker<sup>\*1</sup>, KE Brannick<sup>1</sup>, CL Freed<sup>1</sup>, C DeVries<sup>2</sup>

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Empirically based investigation of both methodology and dosing is needed to refine pain control in rodent models. Injectable administration of nonsteroidal anti-inflammatory drugs (NSAIDs) requires repeated restraint and may increase stress, while oral formulations may result in analgesic inconsistency due to variable consumption. Our goal was to confirm postlaparotomy analgesic efficacy of 2 oral Carprofen options relative to injectable. Twelve-wk-old C57BL/6Cr1 male mice (n=27) housed 3 per cage were provided Carprofen via water bottle (0.02mg/mL), gel (0.025 mg/g), or daily injection (0.22 mg). The target dose was 5 mg/kg/day for a 25-gm mouse. Carprofen was provided 24 h prior to laparotomy through 72 h postop, and each animal received buprenorphine HCL (0.1 mg/kg) at surgery. Under isoflurane anesthesia, a 1-1.5 cm-midline incision was created and a retractor placed for 3 min prior to closure. Individual animal weights and cage level food, gel, and water intake were measured each morning. Analgesic efficacy was evaluated each afternoon using open field testing, mouse grimace scale (MGS), time-to-integrate-to-nest test (TINT), and via fecal corticosterone (CORT) assessment known to parallel behavioral responses to pain. Provision of medicated gel resulted in the lowest average mouse dose per cage

(2.9 mg/kg), which was significantly lower than the water group (5.93 mg/kg) and target dose on day 1 only. Although no differences in behavior tests were noted between groups, relative to baseline all groups displayed significantly higher MGS scores (day 0), decreased rearing motion (day 0), and total locomotion (day 1). Fecal CORT was not significantly different from baseline for any group, but a significant increase was noted for the injectable group relative to the gel group only (day 3). Despite significant variability in average dose, a lack of behavioral testing differences among treatment groups supports that both methods of oral Carprofen dosing resulted in postsurgical analgesia comparable to daily injections.

**P102 A Practical Reference Chart for Selection of Endotracheal Tubes for Swine Intubation**MA Card<sup>\*1</sup>, CA Manuel<sup>1</sup>, B Davenport<sup>3</sup>, S Osorio Lujan<sup>2</sup>

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Swine are commonly used in biomedical research for surgical procedures that require endotracheal intubation and general anesthesia. In swine, endotracheal tube (ET) size in comparison to the size of the animal is much smaller than ET tube sizes commonly used in other domestic animals. Proper ET tube selection can be challenging for the novice technician since selection of the most effective endotracheal tube size remains poorly defined. Although general guidance has been published for ET size selection for swine, a direct comparison of body weight and appropriate ET tube size has not been developed. We hypothesize that body weight can accurately be used to predict ET tube size for the intubation of domestic swine used in biomedical research. To develop a comprehensive weight-based chart, retrospective data regarding weight and ET tube size was collected from 97 swine over a course of 3 y. In a prospective cohort of 53 swine, weight (kg), endotracheal tube sizes, intubation attempts, positioning for intubation (dorsal, lateral, ventral), and SpO<sub>2</sub> (%) measurements were collected from each swine during intubation. The swine used in both cohorts were SPF Yorkshire crosses and standard farm pigs of various breeds such as Yorkshire, Landrace, Hampshire, and Duroc. All swine experienced a similar injectable pre-med sedation protocol followed by mask inhalation of isoflurane for induction and intubation. We used the data collected from both cohorts to perform a correlative analysis, which showed significant correlation ( $P < 0.005$ ) between weight and ET tube size. Using linear regression, we developed an equation along with a comprehensive reference chart providing suggested ET tube size based on weight. In the prospective cohort we also observed trends in decreased SpO<sub>2</sub> levels during intubation that were prolonged due to repeated failed attempts. SpO<sub>2</sub> was higher in the animals placed in lateral or ventral position when compared to those who had to be placed in different positions. When training technicians on swine intubation our reference chart will be a useful tool to improve proper ET size selection. These findings also reinforce the need to monitor SpO<sub>2</sub> during intubation to prevent extended periods of hypoxia which can cause more severe complications.

**P103 BAM! A Safe Approach to Rapid Sedation in Swine**M McIntyre<sup>\*</sup>, C Cain

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Veterinary staff was tasked with sedating large (80+ kg) Sinclair swine for biweekly blood draws. Initially, we used a cocktail of tiletamine/zolazepam + xylazine (0.04 ml/kg) and a reversed with yohimbine (0.2 mg/kg). Recovery from this combination took approximately 1-1.5 h. Therefore, we searched for a safe drug that could produce adequate sedation with a more rapid recovery. We switched to a patented combination of butorphanol tartrate, azaperone tartrate, and medetomidine hydrochloride (BAM). BAM was originally developed

in 2003 to provide veterinarians and wildlife biologists with a formulation that was effective in a wide variety of species, safe, reversible, has a small volume, and a low DEA classification. Over the course of 6 mo, we documented the amount of time from injection to immobilization and time from reversal to recovery of 3 Sinclair swine. Each animal was given 0.02 ml/kg of BAM IM. After completion of the procedure, the butorphanol tartrate was reversed with 0.5 mL of naltrexone and the medetomidine was reversed with atipamezole IM. BAM provided analgesia, sedation, and muscle relaxation for up to 45 min. Average time to immobilization was within 10 min. Average recovery time was approximately 15 min. Vital signs (heart rate, respiration rate, SpO<sub>2</sub>, and core body temperature) remained within normal limits throughout sedation. This data demonstrates BAM can be a safe, effective, and time-saving option for sedation in swine.

#### **P104 Psoriasiform Dermatitis in Rhesus Macaques (*Macaca mulatta*)**

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Dermatitis characterized by chronic ulceration, hyperkeratosis, and xerosis of the ischial callosities and the palmar and plantar surfaces of the hands and feet was diagnosed in 17 colony rhesus macaques (*Macaca mulatta*). Indoor-housed males were preferentially affected and the age at diagnosis ranged from 3 to 10 y with an average onset of 6 y. A psoriasiform pattern was evident on microscopic examination with acanthosis, rete ridge elongation, and parakeratotic hyperkeratosis. Other features were perivascular, pleocellular inflammatory infiltrates, and spongiosis. The differential diagnosis was allergic contact dermatitis and was favored given the lesion distribution and the character of the inflammatory infiltrate. Pathogenesis was thought to involve subsequent exposure to an allergen following initial sensitization. Dermatitis is a common, often clinically frustrating disease to manage in nonhuman primates. Identification of the underlying etiology and topical treatment can be challenging. Treatment of contact allergic dermatitis involves a 3-step approach: identification and removal of the allergen, reduction in inflammation, and restoration and protection of the skin barrier. Epicutaneous patch testing is the gold standard noninvasive method for identification of inciting allergens. Potential allergens are placed in patches on the back and removed in 48-72 h. The underlying skin is then observed for signs of irritation. Despite testing, an inciting antigen has not yet been identified in our colony. Treatment of inflammation can be achieved using various strengths of immune modulating drugs. First line of treatment involves topical corticosteroids including triamcinolone and hydrocortisone. Oral corticosteroids are reserved for lesions covering greater than 20% of the body. Restoration of the skin barrier can be achieved through topical application of barrier creams or petroleum-based emollients. The greatest success in treating animals has been achieved through elimination of contact with wet caging, topical or oral corticosteroids, and cageside application of barrier cream to affected areas.

#### **P105 Retroperitoneal Cystic Teratoma in a Cynomolgus Macaque (*Macaca fascicularis*)**

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A 22-mo-old, 2.1-kg female cynomolgus macaque (*Macaca fascicularis*) socially housed in an outdoor same-sex peer group presented with an irregular soft tissue mass in the right cranial abdomen palpated during routine semiannual physical exam. Ultrasonography revealed a multilocular mass with compartments of hypoechoic (fluid) or mixed echogenicity contents. Multiple irregular heterogeneous (mineral) opacities within the cranial abdomen were observed on radiograph, and a barium series confirmed the mass was extragastrintestinal and

suggested adjacent organ displacement. During an exploratory laparotomy, a large multiloculated cystic mass was adhered to the right dorsolateral peritoneum and diaphragm, compressing the lateral margin of the right kidney medially, and displacing the entire abdominal viscera to the left. The mass did not appear to be associated with either ovary. After aspiration of approximately 100 mL of clear straw-colored fluid from one compartment of the mass for decompression and improved access, it was excised. The mass had an irregular surface, weighed 139 g, and measured 8 x 10 cm. The mass contents varied by compartment: clear straw yellow fluid, caseous white material mixed with clear yellow fluid, turbid yellow fluid with high viscosity, flat irregular cartilage- and bone-like structures, dry caseous white material with pieces of hair, and tissue resembling skin. Cytology of the turbid yellow fluid diagnosed a squamous-lined keratin-producing cystic mass; scant growth of a coagulase-negative *Staphylococcus* sp. was present after 72 h of culture from the same compartment. There are few reports of teratoma in cynomolgus macaques, but prevalence is likely higher than reported because smaller masses may not be detected grossly.

#### **P106 It's Not a Tumor: Abdominal Mass in Ferret**

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A 6.5-y-old, 830 g, spayed, trio-housed female laboratory ferret was evaluated with a reported history of decreased activity level. The animal had been employed in neurobehavioral studies, where it served to provoke predator-based stress in a rodent model of posttraumatic stress disorder and was eating, drinking, urinating, and defecating normally. Physical exam revealed alopecia on the dorsal tail base, vulvar enlargement, and a large, firm, palpable abdominal mass. Heart rate, respiratory rate, and temperature were within normal ranges, and the remainder of exam was unremarkable. An unsedated abdominal ultrasound confirmed the presence of a large left-sided abdominal mass. Exploratory laparotomy was considered, but due to the ferret's age, intensive postoperative care requirements, and limited potential use after surgery, euthanasia was elected. After heavy sedation with ketamine/acepromazine, blood was collected from the jugular vein, and euthanasia was performed via intracardiac injection of a barbiturate. Bloodwork revealed mild hypoglycemia and mild thrombocytopenia. Gross postmortem examination showed marked splenomegaly, gastric and mesenteric lymphadenomegaly, and a 2 mm-diameter focal nodular mass in the left adrenal cortex. Histology confirmed that the splenomegaly was attributable to red pulp extramedullary hematopoiesis (EMH), and that the adrenal mass was a cortical adenoma. Histology also revealed chronic gastritis with rare spirochetes consistent with *Helicobacter* spp., a common and natural infection in ferrets; the gastric and mesenteric lymphadenopathy was due to lymphoid hyperplasia, likely secondary to chronic gastritis. Splenic EMH is a common finding in ferrets, yet its underlying pathogenesis is not fully understood.

#### **P107 Efficacious Single-dose, Multimodal, Long-acting Parenteral and Local Analgesia for Femur Fracture and Sciatic Nerve Injury in Rat Model**

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Femur fracture and sciatic nerve injury produce severe pain in animals. An effective pain management regimen is critical for relieving pain in animals and the outcome of research. An analgesic protocol should include a multimodal approach that incorporates analgesics from various classes such as opioids and nonsteroidal anti-inflammatory drugs (NSAID). In this study, thirteen Sprague Dawley, 300 g male rats had both a femur fracture and sciatic nerve injury. Six animals had

internal fixation of the femur. We used a single dose of preemptive multimodal long-acting opioid and NSAID (duration of 72 h) subcutaneously 30 min before the surgery. A local anesthetic was also applied at the incision site. Animals received postoperative fluid (5 cc of saline) and a nutrient-rich viscous paste diet for 3 d after the surgery. Animals were monitored for signs of pain at 1-h intervals for the first 12 h, 2-h intervals for the second 12 h, then 24-h intervals thereafter, for a total of 4 to 6 wk. Multiple parameters were observed to determine the rat's health and pain status: activity level, posture, food and water intake, hydration, fecal output, incision site inspection, autophagy of their injury/wound, chromodacryorrhea, grimace scale, body conditioning score (BCS), lethargy, piloerection, and body weight measurement at 2 wk after surgery. The animals did not show signs of pain during the 3 d of analgesic treatment and after the termination of analgesic drugs. The animals gained body weight and showed mobility within 3 d after the operation. Based on the behavioral assessment for pain and increases in body weight, the pharmaceutical and nonpharmaceutical analgesic regimen used in this study showed effective pain management.

#### P108 Cryptorchidism in 2 Male Castrated Pigs (*Sus scrofa domestica*)

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Six Yorkshire-cross barrows, each weighing 55-61 kg, were received as part of an acute, cardiovascular study. One of the 6 barrows exhibited mounting behavior toward other pigs in the group, as well as aggression toward animal care staff. This animal was separated and individually housed due to this behavior. Following the acute cardiovascular study, a complete autopsy was done. Grossly, an intraabdominal testis was identified and tissue collected for histologic analysis. Seminiferous tubules were diffusely atrophied, with irregular, undulant basement membranes, lined by a single layer of Sertoli cells, devoid of germ cells, spermatocytes and spermatids, containing variable amounts of fibrillar to vacuolated eosinophilic material. Subsequent autopsy of the remaining cohort found a second barrow with an intraabdominal testis; this animal did not manifest any of the behavioral changes seen in the first pig. Cryptorchidism is the failure of 1 or both testes to descend normally from within the abdomen through the inguinal canal into the scrotum. Both of our cases were monolateral cryptorchidism, where 1 testicle descended and was removed during castration, but the second testicle remained in the abdomen. Cryptorchidism is a hereditary anomaly and is one of the most frequent congenital defects in pigs. These findings were communicated with the vendor and they were advised to cull this breeding line.

#### P109 Palpable Abdominal Mass in a Rat

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A 2-y-old female Chat-Cre transgenic Long Evans rat used for handling training was noted to have developed acute ataxia. During physical examination, the rat was tachypneic, lethargic, and a 4 cm cranial abdominal mass was palpable. On gross necropsy, the abdominal cavity was expanded by 2 large adherent masses along the lesser curvature of the stomach, spread into the peritoneum, and displaced the diaphragm cranially. The larger mesenteric mass measured 4 x 6 x 2 cm and was dark red-brown with multiple fluid-filled cysts. The smaller mass measuring 2 x 4 cm was firm and adherent to the gastric cardia and fundic wall along the lesser curvature. Histopathologically, these expansile masses had a moderately pleomorphic appearance comprising of a solid, moderately cellular neoplastic spindle cell population with abundant stroma that was admixed with large psuedocystic and necrotic foci. In 1 segment,

the neoplastic spindle cells were located within the submucosa of the gastric cardia and infiltrated into the gastric wall with expansion into the peritoneum. On the basis of the histopathological features, the palpable abdominal mass was diagnosed as regionally metastatic gastrointestinal stromal tumor (GIST) originating from the stomach. On immunohistochemistry, the neoplasm was negative for pancytokeratin and positive for vimentin. Gastrointestinal stromal tumors are mesenchymal gastrointestinal tumors that arise from the interstitial cells of Cajal. Spontaneous GIST are rarely reported in rats, but as noted in this case, can result in significant clinical signs and a poor prognosis.

#### P110 Unexpected Weight Loss and Decreased Oocyte Production in a *Xenopus laevis*

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A pair-housed African clawed frog (*Xenopus laevis*) of unknown age presented for weight loss and decreased oocyte production. The frog had undergone 3 surgical laparotomies for oocyte harvest prior to the onset of clinical signs. On physical exam, aside from weight loss, no other abnormalities were noted, and previous incision sites had healed without complications. Differential diagnoses for weight loss include parasites, mycobacterium-associated granulomatous intestinal disease, nitrogenous or heavy metal toxicosis, neoplasia, and viral infection. The animal was removed from the recirculating system and placed in static housing for closer monitoring; however, within 1 wk the frog died. On gross necropsy, it had a low body condition score, no external signs of trauma or infection, and within the body cavity, 2 coiled nodules were adhered to the serosal surface of the stomach. Vendor history revealed they test and exclude for *Batrachochytrium dendrobatidis* and ranavirus. The frog was assumed to be laboratory-reared, as health records did not indicate otherwise. Bacterial culture of the skin and coelomic cavity grew *Aeromonas veronii*, *Pseudomonas* sp., and *Shewanella putrefaciens*. Heart, kidney, liver, lung, spleen, and GI system were submitted for histopathologic evaluation. Results revealed coiled adult nematodes attached to the serosal surface of the stomach and nematode larvae within the stomach wall. Additionally, the stomach mucosa had multifocal vascular congestion and sloughing of the surface epithelium, which was associated with moderate numbers of bacteria. The characteristic findings of these nematodes were consistent with *Contracaecum* spp., which have been identified in wild-caught *Xenopus laevis*. *Contracaecum* nematodes have a complex life cycle and amphibians act as an intermediate host. Upon further consultation with the vendor, it was discovered that a wild-caught frog from Chile was distributed to our facility. Heavy parasite infestation can lead to malabsorption, weight loss, and increase an animal's susceptibility to bacterial infection. This case report illustrates the importance of periodic review of vendor practices, and the potential impact intestinal parasites may have in *Xenopus laevis* including oocyte production and quality.

#### P111 Comparison of Hematology and Serum Chemistry Parameters in F1 And F2 Captive-bred Mauritius-origin Cynomolgus Macaques

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Normal reference ranges are used for purposes including releasing incoming animals from quarantine and interpreting study data. Hematology and serum chemistry reference ranges have been published for Mauritius-origin cynomolgus macaques that are wild-caught or first-generation captive bred (F1). This report compares blood values for F1 animals and animals bred in captivity for 2

generations (F2). A retrospective analysis was performed on baseline clinical chemistry and hematology (42 parameters) obtained from 64 naïve purpose-bred 2.5-3.6-y-old macaques 3 wk after arrival. All data are from animals deemed clinically normal at a concurrent physical exam. Parametric and nonparametric statistical tests were used to determine if there were differences related to generation or sex. There were no biologically significant generational differences in hematological or serum chemistry parameters in F1 versus F2 animals. While a significant  $P$  value  $< 0.01$  was observed for 3 parameters (red cell distribution width in males, mean cell volume when pooled sexes were compared, and basophil percentage in females), the lack of corroborating data from the other sex and from the basophil count suggests that these were not truly significant. ALT, GGT, red cell count, hematocrit, hemoglobin, and reticulocyte count differed in males and females. In conclusion, F1 and F2 cynomolgus macaques do not have important differences in baseline blood values.

#### **P112 Spontaneous Encephalomalacia in a Rhesus Macaque (*Macaca mulatta*)**

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A 5-y-old male rhesus macaque (*Macaca mulatta*) presented for acute visual impairment. The animal was assigned to a neuroscience study which included noninvasive behavior and imaging procedures. Cage-side observations showed bilateral pupillary dilation, reduced light perception, and visual acuity. Physical examination revealed absent palpebral and pupillary light reflexes, and all other findings were unremarkable. Initial diagnostics, including complete blood count, serum chemistry, urinalysis, and cerebrospinal fluid (CSF) analysis, were within normal limits. Supplemental supportive therapy was elected, with initial improvement of clinical signs, followed by subsequent progression of abnormal mobility and behavior a week later. At this time, CSF analysis revealed evidence of a central nervous system insult characterized by erythrocytosis and marked pleocytosis. Magnetic resonance imaging detected a large circumscribed lesion in the right occipital lobe including the primary visual cortex. The primary differentials for a focal brain lesion were neoplasia or ischemic cerebral stroke. Due to a poor prognosis, the animal was euthanized and submitted for a postmortem examination. Pathological findings revealed a large focal area of severe encephalomalacia of the right occipital lobe. Additional diagnostics including bacterial cultures and histopathology showed no evidence of other systemic, such as viral, bacterial, or parasitic causation. A diagnosis of spontaneous ischemic cerebral stroke leading to severe encephalomalacia is consistent with this animal's clinical signs and diagnostic tests. In humans, cerebral strokes are the fifth leading cause of death and the primary cause of long-term disabilities for adults in the United States. Due to anatomical and physiological similarities of nonhuman primates (NHPs), they have proven to be a quintessential cerebral stroke model. However, naturally occurring neurological diseases and more specifically spontaneous ischemic strokes are rarely reported in NHPs. Although rare, ischemic cerebral stroke should be considered as a differential for NHPs presenting with acute neurological signs or ocular deficits.

#### **P113 Fluid-filled Swelling on the Snout of a Yorkshire Pig (*Sus scrofa*)**

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A 5-mo-old 54 kg female Yorkshire swine presented with an approximately 1.0 cm diameter swelling on the dorsolateral surface of the snout. The swelling was soft, fluctuant, and appeared to contain straw-colored fluid. The animal was bright, alert, and had a normal appetite. A complete physical exam revealed no additional

abnormalities. The fluid-filled swelling on the snout of this swine was consistent with a vesicular lesion. Differential diagnoses included physical trauma, chemical insult, and viral diseases including, foot and mouth disease, swine vesicular disease, vesicular exanthema of swine, vesicular stomatitis, and Seneca virus A. Since viral causes of vesicular disease are clinically indistinguishable from each other and from physical, thermal, or chemical injury, prompt reporting to USDA and molecular diagnostics are the only means to confidently rule out a foreign animal disease. The regional USDA office was contacted and vesicular fluid and tissue, as well as a blood sample, were collected for diagnostic testing. Strict quarantine procedures were put in place for the housing room containing this animal pending test results. Serology by ELISA and RT-PCR of vesicular fluid and tissue were negative for foot and mouth disease. Viral isolation results were also negative. Although the cause of the vesicle in this swine was not determined, this report highlights the importance for laboratory animal professionals working with agricultural species to be vigilant for reportable diseases.

#### **P114 Investigating a Multiuse Approach to Single-use Cefazolin: Extended Life and Safety of the Drug against Bugs**

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Antibiotic compliance is a matter of considerable significance for treating nonhuman primate patients, often necessitating injectable cefazolin. Cefazolin is labeled as a single-use vial, which can be cost prohibitive and can lead to waste of unused portions. We hypothesized the life of cefazolin can be safely and effectively extended for multiuse purposes for a 10-d period. Five bottles of cefazolin were reconstituted with bacteriostatic water and assigned variables to isolate the effects of temperature, lighting, and contamination over the span of 10 d. Temperatures were recorded daily for both the refrigerator and hospital counter where vials were maintained. Two bottles received multiple needle punctures to simulate multiuse withdrawals. Cefazolin samples were collected every third day for pH, turbidity, coloration, and antibiotic susceptibility testing (AST). Sterile assay discs were infused with our cefazolin for AST against pure *Staphylococcus aureus* and compared to a commercially prepared disc. On day 10, the contents of each bottle were plated on sheep blood agar to test for the growth of bacterial contaminants. The results for AST and bacterial contamination plating were provided by a diagnostic laboratory, allowing for an objective evaluation. No bacterial contaminants were detected and the AST results performed well above standard requirements throughout the 10-d investigation. Our hypothesis was supported by the results; a multiuse approach is possible without sacrificing efficacy or patient safety.

#### **P115 Aggressive Fibrosarcoma Secondary to Microchip Implantation in a Feline**

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A 6-y-old obese female spayed Abyssinian presented for a nonpainful firm subcutaneous growth on the left interscapular region that was 2.5 cm in diameter. Vaccines were administered regularly and were up to date according to historical records on presentation. The cat repeatedly scanned for a microchip (implanted in 2012) in the region of the mass. Preoperative CBC, chemistry, and electrolytes were unremarkable. While under general anesthesia, a dental prophylaxis was performed prior to excision of the mass. The skin was shaved and sterile prepped. An elliptical incision was made around the mass with wide margins. The excised mass positively scanned for the microchip. Histologic examination of the mass revealed high-grade fibrosarcoma with margins  $< 1$  mm. Three months later, the cat presented again for a regrowth of the mass in the same region as previously excised. Excision was again performed under general anesthesia with 3 cm margins laterally and extended deep into the muscle. Histopathology

revealed that the mass had advanced into an anaplastic sarcoma with >1 cm margins laterally and 0.5 cm margins deep. The incision healed without incident or regrowth in 3 mo. No imaging or oncology consults were obtained. Thousands of microchips are currently implanted in a wide variety of species, including laboratory animals, for identification purposes each year. While these devices are designed to be a benign foreign object in the body, adverse reactions to the microchips occasionally occur, including migration, postinjection swelling, infection, functional failure, or even complete loss from the body. Even more rare are neoplastic growths secondary to microchip implantation. To the author's knowledge, this is the second report of a tumor arising post-microchip implantation in a feline. Since 1996, microchip associated tumors have been described in dogs, a cat, mice, rats, a fruit bat, shrew, and mole rats. While microchips are a competent form of long-term identification, further study on materials used and other forms of identification should be researched to further reduce risks of neoplastic reactions. The potential for adverse reactions to microchips should be considered when used in laboratory animals, especially for those on long studies.

#### **P116 Bringing Wildlife Indoors: Conditioning of a Laboratory-housed Cowbird Population**

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Research using wild-caught cowbirds introduces numerous health issues that we, as veterinary staff, are not commonly exposed to with our specific pathogen-free, purpose-bred animals. Over 1 mo post-capture on an IACUC-approved protocol, 3 cowbirds were found dead within their primary enclosures and submitted for post-mortem examination. Poor body condition and a heavy parasite burden of mites, nematodes, and multiple protozoans were appreciated. These findings prompted treatment of the entire colony of indoor-housed cowbirds. Fecal testing for endoparasites was performed both before and after treatment with parasiticides. Fecal samples were pooled from 9 cages housing a total of 18 birds over a defined time period. Topical permethrin powder was used for the ectoparasites, and endoparasite treatment with rounds of levamisole, sulfadimethoxine, and amprolium was provided in the drinking water. This combination of treatments was successful in eliminating ectoparasites and nematodes, but only reduced the burden of coccidia. The decreased parasite burden likely improved colony health, as there were no further deaths within the colony post-medication. The result of this treatment regimen demonstrates that while it can be labor intensive, conditioning provides for greater animal welfare and a higher quality of research.

#### **P117 A Retrospective Study of Age-related Conditions of the Meadow Jumping Mouse (*Zapus hudsonius*)**

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The meadow jumping mouse (*Zapus hudsonius*), an uncommon research model, is currently used to study metabolism during periods of hibernation and very cold temperatures at our institution. This species is not commercially available nor bred by other researchers at other institutions. As such, all of the founding individuals in both our breeding colony and study group are wild caught. As these mice are so infrequently maintained in captivity, their husbandry and veterinary care were all developed de novo. Mice are conventionally housed in large, filter-top polycarbonate shoebox cages on wood bedding, bedding with brown crinkle paper, an igloo, and rotating chewing substrate (i.e. wood blocks) for environmental enrichment on a 16:8 hour light cycle. Mice are fed a high-protein standard rodent diet as well as a myriad of food enrichment such as mealworms and sunflower seeds to simulate their natural foraging-based diet. Animals

are housed individually as they are solitary in the wild and extremely aggressive towards other mice. All animal handling/cage change occurs within a deep plastic bin due to their ability to leap 2-3 ft. There is little information available addressing their disease and mortality, so a retrospective analysis of clinical cases and necropsy reports have been compiled for the previous 14 mo in an effort to better understand the species and its particular veterinary health challenges. Clinically, the overall health of the colony is robust with traumatic foot and/or tail injuries constituting most acute cases. However, a myriad of age-related health issues, primarily cancers, have been observed due to their longevity in captivity versus wild meadow mice.

#### **P118 Evaluating Collection Methods for Gut Microbiome Analysis in the Common Marmoset (*Callithrix jacchus*)**

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The common marmoset (*Callithrix jacchus*) is increasingly used as an animal model for biomedical research. However gastrointestinal diseases are endemic in many captive marmoset colonies. Perturbations in the gut microbiome have been associated with disease states in humans and play a role in observed phenotypes in animal models. An understanding of the gut microbiome patterns in a marmoset colony may aid in clinical decision-making and model reproducibility. However, fecal collection in marmosets remains a sporadic and unreliable method of sample collection. A standardized method of sample collection and storage is essential for proper interpretation of microbiome data. The goal of this study was to determine whether the microbiome profile from a rectal swab performed on a sedated animal is comparable to the fecal microbiome profile. During routine physical exams, 2 samples were collected from each of 23 marmosets (11 males and 12 females, 6 mo to 8 y of age). Feces were sampled from transport cages with a sterile swab, and paired rectal swabs were obtained from the sedated animal. Paired fecal and rectal swab samples were processed with the same DNA extraction protocol and prepared for 16S rRNA sequencing. Comparing relative abundances at the phylum level between fecal-rectal pairs, the R<sup>2</sup>-value was 0.68% with a standard error of regression (S) of 0.09%. Alpha diversity metrics showed no significant differences between fecal-rectal pairs but there were differences in beta diversity. Further analysis revealed 5 discordant fecal-rectal pairs which corresponded with the only 5 rectal swabs that were classified as clear (free of visible fecal matter) during collection. Removal of these 5 pairs resulted in a better fit of the data as evidenced by a R<sup>2</sup>-value of 0.86% with S of 0.05%. Reanalysis showed no significant differences in alpha and beta diversity metrics. These results demonstrate that nonclear rectal swabs are a reliable method for sample collection for gut microbiome studies in marmosets. This study highlights the importance of standardized sample collection methods and exclusion of inadequate samples. Ongoing longitudinal studies will provide information on stability of the gut microbiome in healthy and clinically affected marmosets.

#### **P119 Managing Pododermatitis in an Aging Colony of F344 Transgenic Rats**

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Pododermatitis (PD), also known as bumblefoot or sore hocks, is a common condition encountered in rodents. It also occurs in rabbits and guinea pigs. PD encompasses a range of clinical presentations that, in our experience, include mild erythema with superficial to deep dermal lesions with or without swelling and/or lameness. PD is a common health condition in aging rats used for behavioral studies at our institution. While there is no definitive treatment for PD, in order to maintain health and welfare of study animals, we have employed several novel treatment modalities. Treatments employed included nail

trims (NTs), with or without multi-wave locked system (MLS) laser therapy, and/or placement on a commercially available variable paper-free bedding. MLS laser therapy is reported to induce strong anti-inflammatory, antiedema, and analgesic effects simultaneously and within a short period of time. It has also been shown to accelerate wound healing in several species. It does, however, require purchase of the equipment. Performing NTs reduces pressure to the skin on the hind feet by allowing proper placement of the foot and even distribution of weight on all parts of the foot. Placement on the paper-free bedding is expected to provide a more absorbent and softer surface compared to wood chip bedding. Groups of 8-9-y-old rats with PD lesions were randomly assigned to 4 different treatment groups. All groups received NTs with and without MLS laser therapy and/or paper-free bedding. Weekly observations with scoring (severity of dermal lesion, erythema/swelling) were performed for each treatment option. No lameness was noted during this study. When PD lesions were small ( $\leq 3$  mm, including size of dermal lesion and/or swelling), the MLS laser therapy and/or NTs worked equally well. For larger lesions, the MLS laser therapy promoted increased erythema/swelling and sometimes reduced the overall size of the wound, especially when combined with placement on paper-free bedding. MLS laser therapy offers similar advantage to placement on paper-free bedding for less severe PD lesions ( $\leq 3$  mm dermal lesion and/or erythema/swelling). Size and severity of the PD lesions along with placement on the paper-free bedding were the most predictive measures of treatment success.

#### **P120 Severe Periocular Edema Subsequent to Intraarterial Carboplatin Chemotherapy for Retinoblastoma in a Rabbit (*Oryctolagus cuniculus*) Model**

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A rabbit model was used to assess pharmacokinetics and ocular and systemic toxicity following endovascular, microcatheter-based intraarterial (ophthalmic artery) carboplatin infusion. Following unilateral intraarterial administration of carboplatin, severe uni- or bilateral periocular edema was reported in 6 adult male New Zealand White rabbits. Time to onset varied from less than 4 h post-administration (n=3, 50 mg) to approximately 24 h postadministration (n=3, 25 mg). Upon becoming symptomatic, 5/6 animals were euthanized, and 1 (25 mg treatment) was medically managed for 4 d before being euthanized due to intractable edema-related lagophthalmos. Globes and orbits from all 6 euthanized rabbits were harvested en bloc and whole mount sections were prepared and stained with H&E for histologic evaluation, revealing drug-induced vasogenic edema in confined spaces as the main underlying pathogenesis. Transient and self-limiting periocular edema is a common finding seen with intraarterial chemotherapy but is thought to predominantly occur with melphalan and carboplatin combination therapy, or with melphalan monotherapy. Carboplatin itself can cause orbital fibrosis following direct periocular injection. However, the severity of this adverse consequence in rabbits was unexpected and its use in the study was subsequently discontinued. While the definitive cause for this vasotoxicity and striking clinical presentation is unknown, we suspect species-specific features, such as the retrobulbar venous plexus, dual ophthalmic arterial supply, and carotid artery anastomoses might have contributed to amplified complications following intraarterial carboplatin chemotherapy of the eye. In addition, 25 mg may represent an undercorrection (relative to human infants) for vascular supply to the rabbit eye.

#### **P121 Protective Effect of Molecular Hydrogen on Oxidative Stress-induced Impairment in Mouse Sperm Motility**

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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 sperm and subsequent male infertility. It was previously reported that molecular hydrogen (H<sub>2</sub>) acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. To investigate the effects of H<sub>2</sub> on damaged sperm, we prepared oxidative stress-induced low motility sperm. Suspension of fresh B6D2F1/Crlj mouse sperm, in which motility rate was 82.4%, were treated with 0.3 mM hydrogen peroxide for 30 min, resulting in damaged sperm with low motility rate (14.6%). We further incubated the suspension for 20 min with or without H<sub>2</sub>, and found that H<sub>2</sub> significantly increased the motility rate (63.9%) accompanied by improvement of intrasperm ATP content. To investigate fertilizability of H<sub>2</sub>-treated sperm, we used them for in vitro fertilization and found that H<sub>2</sub> markedly improved the fertilization rate (59.2%). Transfer of 2-cell stage embryos to pseudopregnant ICR mouse showed normal ontogeny (94.6%). Because of the rapid diffusion and high membrane permeability, H<sub>2</sub> can reach and react with intrasperm ROS, including hydroxyl radical, and improve low sperm motility. Our results strongly suggest that H<sub>2</sub> is a new promising tool for male infertility treatment.

#### **P122 Development of a Comprehensive Content Management System to Simplify the Maintenance and Dissemination of Animal Program Guidance Documents**

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Our institution's animal care and use program includes over 500 PIs and 4,000 individuals that work with animals in a decentralized administration and communications infrastructure. Finding an efficient, systematic way to disseminate complex information of the type(s) contained within animal care and use policies, guidelines, and standard operating procedures (SOPs), has proved difficult. Prior to the implementation of our new system, documents were housed on three different platforms and maintained by different departments; consequently, there was no way to ensure that documents were uniformly updated, meaningfully categorized, or appropriately versioned. Our benchmarking of 25 peer institutions' websites showed that most programs' animal care documents are maintained as PDFs linked on static web pages, and hosted on separate websites. To enhance user experience, minimize redundancies, and streamline processes, we developed a custom Drupal-based website to publish, maintain, and track forms, guidelines, SOPs, policies, and resource documents. The website offers a user-friendly interface to allow for navigation of our 300+ documents. Users can browse content across the entire site via text search, document type, species referenced, and/or topic; most viewed, or recently updated documents. Each document is broken down into collapsible accordion list(s) for easier navigation, includes contact and approval information, and an auto-populated list of related documents from across the program (such as animal care, IACUC, OSEH, etc.). Researchers are able to log in to the website to access protected content, save custom searches, and bookmark documents—features that allow a more tailored experience. Our program's transition to a dynamic web-based content management system has not only improved our service to researchers, it has reduced administrative redundancies and simplified document management processes. Program documents are now tracked and maintained in one system, changes are coordinated simultaneously across documents, and manual processes such as hard-coding and linking related documents have been automated.

### **P123 Labor Comparison of Whole Blood Microsamplers to a Conventional Serum Collection Method for Murine Health Surveillance**

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Murine health surveillance at our institution's comparative medicine department consists of testing sentinel mice for serology. Our current practice includes a time-consuming, multistep approach for processing samples after collection prior to shipping to an external diagnostic laboratory for analysis. Recently several whole blood collection options for rodents have become available that require less blood volume and do not require additional processing thus cutting overall time from collection to shipment. We selected one of these, a micro sampler onto which 20µL of blood is collected, to compare method times. We anticipated that labor time would be reduced without compromising result accuracy. During routine sentinel testing, blood was collected and both processing methods were simultaneously performed on randomly selected Swiss-Webster adult female mice of various ages over 3 sentinel collection days (n=33). Process times were recorded. The microsampler was introduced to the blood, which was absorbed onto their tips, and were placed back into their packaging. After the remaining blood clotted, it was centrifuged to obtain the serum, which was then diluted and heat inactivated. The microsampler and serum were then sent out for analysis. The time from blood collection to being ready for shipment decreased substantially with the microsampler. With the current process, each group of serum averaged 1 h and 44 min of additional processing, while each group of microsampler averaged 3 min and 13 s (17.5 s per microsampler); a time savings of approximately 1 h and 41 min. Based on our current testing schedule, this translates to a time savings of over 3 h per sentinel week just from additional processing. Along with the significant time savings, there were no differences in the diagnostic results.

### **P124 Merging Wireless Technology with Cage Washing Systems: A Proposed Means to Validate and Monitor Performance**

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Common conventional methods of validating cage washer performance use temperature-specific and heat-activated devices that qualitatively demonstrate a given temperature was achieved. However, these devices may not yield definitive and conclusive results, nor do they necessarily reflect the actual duration of exposure of the target temperature. Although under many circumstances this method is an acceptable and long-standing performance standard, limited information on true washer performance is gained through its use. Lost temperature indicators, ambiguous results, indirect communication of temperature validation failure, and other interrelated issues represent some of this process's challenges. To address these and other issues and to gain a more comprehensive insight of washer performance, an alternative approach to consider is the use of commercially available wireless technologies, particularly those that offer remote water temperature monitoring coupled to a notification system for reporting out of range temperature conditions or deviations. For the past 5 y, the comparative medicine program husbandry management team (HMT) has used a wireless environmental monitoring system for all animal housing and critical support areas. The HMT explored on a trial basis, monitoring cage washer performance using a water temperature sensor compatible with the environmental monitoring system. Testing of this approach revealed that not only can any management team member view real-time washer water temperatures but can also view the temperature history. The coupled notification system was implemented and established thresholds for wash cycle initiation as well as achieving set

point temperatures. Subsequently, establishing a new performance standard that documents washer performance was established. This new process gives HMT a means of evaluating and validating washer performance from any desktop computer or mobile device and negating the need to document this aspect of the animal care program by the aforementioned means. Furthermore, establishing temperature notification thresholds enhances this aspect of the program and allows for more immediate means of evaluation and remediation of departures from temperature set points.

### **P125 Nesting Enrichment and Shredding Prevention for Mice**

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In our facility, we have 1 mouse colony (CD-1 mice) that has historically exhibited significant levels of shredding of their pelleted feed. Our program uses a commercially available cotton product as the standard nesting material in each cage, and we hypothesized that a variation and or mixture of different types of nesting material would prevent the amount of pelleted feed being shredded on a weekly basis. To test our hypothesis, we rotated 6 different types of nesting material between 36 cages over 6 wk. The nesting material types included 1) 8 g 2 x 2 in cotton fiber piece, 2) 8 g 1 x 1 in cotton fiber piece, 3) 8 g of paper roll, 4) 8 g of brown crinkled paper (BCP), 5) BCP with one 2 x 2 in cotton fiber piece to equal 8 g, or 6) 4 g BCP with 4 g paper roll. Each cage was given the nesting material for 1 wk, and at the end of the week, a photo was taken to score the nest building and amount of shredding. Observations of fighting, health reports, flooding events, and extra cage changes were also recorded throughout the 6-wk period. There were no health reports, and no fighting was observed over the 6 wk. Extra cage changes were not necessary; however, some of the shredding was significant by the end of the 7 d. Nesting scores were significantly higher for mice given BCP or any combination including BCP, while shredding scores were significantly lower for mice given BCP alone. In conclusion, we would recommend that strains prone to shredding be given brown crinkled paper as a nesting material with or without other nesting materials. The decreased shredding maintains a cleaner cage environment and may lead to a decrease in cage changing frequency, a less stressful experience for the mice, and better research results.

### **P126 How to Plan, Design, and Fit a New Research Animal Facility: Animal Care Staff Contributions**

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In 2015, the expansion of a start-up contract research organization to include an additional animal facility led to the evaluation of animal housing needs with the commitment towards optimization of health status for novel severe combined immunodeficient (SCID) rat models while reducing the ergonomic risk for animal care staff and supporting existing standards of ethics and regulations. The failure to identify species' and workers' needs can have long-term negative effects on projects and goals of an institution. As such, animal care staff were included in the deliberations of the design, layout, and animal-related equipment selection of the facility. They were designated as stakeholders and sub-consultants to give specialized guidance to the engineers and contractors. In the planning stage of the facility, the animal care staff gave their evaluation of different space allocations of rooms, schematic animal rack layouts, large equipment layouts, workflow charts, surface materials, and storage requirements. In the designing phase of the facility, the animal care staff gave their assessment of environmental monitoring technology, air-exchange exhaust schematics, and workstation locations and their features (electric outlets, sinks, etc.). In the equipment fitting phase of the facility, the animal care staff gave their sample appraisal of different individually-ventilated caging systems and their components,



biosafety cabinets, shelves, PPE, material containers, and environmental enrichment as it was related to the specialized immunodeficient animals. After comparing the original design of the facility to what was later commissioned and built, we concluded that the facility was greatly improved in the alternative design solutions offered by the animal care staff for the SCID animals. In addition, their contributions assisted in the comprehensive evaluation of constraints applied to the animal care workload efficiency by means of identifying and communicating potential issues to the engineers and contractors. Our conclusion thoroughly recommends an integrated team environment when pursuing the expansion or the designing of a new facility to include animal care staff as stakeholders in the enterprise.

### **P127 Phenotype and Husbandry Information System for Rooms Harboring Multiple Strains of Genetically Engineered Rodents**

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Vital to efficient operation of a rodent research facility is the ability to readily access accurate phenotypic information and specific husbandry needs of animals. This is especially true in facilities such as ours, that house multiple genetically engineered strains of mice and rats. Successful communication is complicated when several investigators, veterinarians, and husbandry staff are involved. It is vital for husbandry staff to recognize health issues and determine appropriate action. Sometimes veterinary attention is required, but sometimes animals may exhibit phenotypical or physical behavior that may not require veterinary attention, but could instead be monitored by colony animal care staff. A line-specific informational system in the animal rooms was needed to make these assessments and provide guidelines for less conversant personnel to perform daily colony management duties. The system developed included a colony management line specific instructions handbook that records strain nomenclature, research applications, phenotypical behavior/appearances, and basic husbandry procedures. Each animal rack contains its line-specific information from the handbook. Each cage card lists the strain name and is color-coded to match the handbook information. This handbook became an important reference tool and makes it easy for any team member to enter the animal room, observe an animal, and compare its behavior and appearance to the line-specific information on the animal rack. This will allow one to determine if the behavior/appearance is normal or if it needs veterinary attention. The flow of communication and information among colony management, animal care staff, and veterinarians make it easier for someone less familiar with the room to step in to help when needed. The system has resulted in better colony management and has become a key factor for efficient use of resources. It has also proven very valuable for training of new personnel, veterinary students, and incoming laboratory animal medicine residents.

### **P128 Bacterial Reduction in Rodent Water Bottles Using Smaller Sized Pinhole Sipper Tubes**

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The purpose of this experiment was to determine if decreasing the size of the holes in the rodent water bottle sipper tubes would decrease workload in the facility without compromising water hygiene. We proposed that a smaller hole would reduce the amount of water wasted from cage movement. With more water remaining in the bottles, water bottle changes could be extended from 7 to 14 d. Water bottle sipper tubes were customized with a 1 in long, pinhole (1/32 diameter) commercially available sipper tube. In addition to maintaining higher water levels, we needed to ensure that the cleanliness of the water would not be adversely affected by the additional days between water bottle changes. Division of Veterinarian Resources (DVR) Bacteriology provided the procedure and

microbiological testing to assess bacteria levels in the water bottles. Per National Institutes of Arthritis and Skin Disease (NIAMS) facility standard operating procedures (SOP), all rodent drinking water is autoclaved. On day 0, 3 mL of water was collected from each water bottle. As expected, all samples were negative for bacteria. Following water collection, the bottles were added to the rodent cage. At day 7, water samples were taken from the standard sipper tubes. Standard sipper tube size is (5/16 diameter). This gave us a baseline to compare with new customized sipper tubes. Group A (control sample) with the standard sipper tubes that are used for 7 d water bottle change. Group B (test sample) with the customized sipper tubes for the proposed 14 d water bottle change. The next step was to compare control and test samples. We found that in 7 d of use from both groups A and B, water samples from group A had more bacterial growth than group B. What we didn't expect was after 14 d, 80% of group B samples showed less bacterial growth than the samples taken from group A after one 7 d of use. The results prove that the smaller sipper tubes provide cleaner, longer-lasting drinking water for rodents. In addition, the workload was reduced due to less frequent water bottle prepping and changing.

### **P129 The Dirty Truth: An Electronic Device Sanitation Study**

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With increased technology, our facility issued electronic tablets to all animal care staff. These tablets could possibly serve as a fomite and a source of cross-contamination. While there is literature on the efficacy of various cleaning products on smartphones, we did not find any literature on the use of an accelerated hydrogen peroxide (AHP) based products for the sanitation of electronic devices. We used adenosine triphosphate testing (ATP) to measure the effectiveness of various AHP products. We tested the premoistened wipes (0.5% hydrogen peroxide), the ready to use AHP spray (0.5% hydrogen peroxide), and the AHP concentrate (4.25% hydrogen peroxide) diluted to 2 oz per gallon spray at a 1-, 3-, and 5-min contact time. We determined that the premoistened wipes had the most significant decrease in relative light units with ATP testing overall.

### **P130 Chlorine Dioxide or Hydrogen Peroxide: Can We Switch? Do We Want To? Did It Work?**

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Maintaining a high level of sanitation is essential for animal research facilities. Researcher and technician compliance, including appropriate application, is a common problem. Historically, our standard protocol included using a chlorine dioxide disinfectant followed by a wipe down with a less corrosive cleaner. However, the chlorine dioxide disinfectant required a 5-min contact time, was corrosive to many surfaces, including stainless steel, and required a follow-up cleaner. We identified a new hydrogen peroxide-based disinfectant that is both a disinfectant and a cleaner. It requires only a 1-min contact time, claims to be non-corrosive to surfaces, and comes in a convenient pre-soaked wipe format. Before deciding which product would best suit our needs we conducted side by side testing, for effectiveness. Using agar plates, we compared the level of contamination present on surfaces before and after use, following the manufacturers' recommended procedures. After 3 rounds of testing, we determined that both chemicals were equally effective. We then considered many other factors: expense (wipes in particular), shorter contact time (improved compliance), less corrosion of our stainless equipment (long-term cost savings), safety of the product (no aerosol/wipes), longer shelf life (up to 2 y), the need for only 1 chemical (no cleaner required), ease of use (wipes), and user preference. Our final determination was that while the hydrogen peroxide-based disinfectant was more expensive, especially the wipes, it was an effective agent and offered many advantages justifying the increase in cost. We continue to see the positive effects of our switch based on quarterly health monitoring, yearly agar testing, and

equipment longevity. In combination with a comprehensive training program, our change to a hydrogen peroxide-based disinfectant has ensured a high level of sanitation in our facility.

### **P131 How's Your First Impression? Effective Onboarding of New Employees**

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The first week on the job comes with a barrage of reading materials and required paperwork. We were getting the job done, but were we doing it well? Our institution recently recognized a need for improved staff engagement and challenged departments to create a more thorough and standardized onboarding program. Onboarding is a prime opportunity for employers to win the hearts and minds of new employees; this first impression is a crucial step in setting an employee up for success. Onboarding does not stop when all of the paperwork is completed; effective onboarding promotes employee engagement, builds trust, enhances the quality of work, and increases retention. Some of the recent improvements we made include a welcome letter that provides links to campus maps, parking and an overview of university policies and benefits for new employees to review prior to their first day; individualized information such as items to bring, dress code, date, time and place to report; an agenda for the first day to include a meeting with the operations manager to give the new employee an overview of the expectations, responsibilities and job duties; a welcome lunch with the administrative staff; standardized training program with a dedicated departmental trainer; and a custom, online SOP husbandry training video series. Fostering an appreciation for our impacts on research, we also invite researchers to all-staff meetings to present their projects and connect the value of our support to their studies. These are important to help animal care staff understand their role in the mission of the organization and promotes engagement. Some lessons learned: use checklists to ensure important items are covered for each area; assign an onboarding buddy, someone other than the supervisor to whom the new hire can go with questions, we found that this simple assignment promoted inclusion to the team. Peter Martel, senior talent development consultant at Harvard Business School, reports that employee engagement is equally important to compensation for employee retention and it starts with the first impression of an organization. How is your first impression?

### **P132 A Comparison of Dirty Bedding versus Exhaust Air Dust Sentinel Program during a 12-Month Routine Health Screening in an IVC Mouse Facility**

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Health monitoring in rodent facilities with IVC cages is a challenging duty. Most facilities perform quarterly testing with specific screening panels. However, several different monitoring methods exist. Most frequently soiled bedding sentinels are used, but with a large variance in the number of cages to be controlled via 1 sentinel cage. Recently, exhaust air dust control (EAD) emerged as a new monitoring method by using a sensitive and specific real-time PCR assay with promising results. We performed a year-round testing of the EAD monitoring in parallel to the existing regular bedding sentinel program on a quarterly interval in all of our rodent units: quarantine, 3 experimental units with different health status level, SOPF nucleus breeding, and embryo transfer (all units fitted with IVC). Additionally, we tested 4 different providers of EAD analyses as we equipped all our IVC filter towers with double testing material for EAD analyses. During the year-round routine health controls, monitoring by EAD was able to detect a range of pathogens like *Helicobacter spp.*, *Pasteurella pneumotropica*, *Spironucleus muris*, astrovirus, norovirus, mouse minute virus, and pinworms. Some of these agents were not detected with the routine bedding sentinel program including pinworm. In one case, only 1 cage in an experimental unit was contaminated with *Syphacia obvelata* due to

an import. This 1 positive cage out of 280 cages led to a positive EAD result whereas the regular bedding sentinel setting (1 sentinel cage for 140 cages) was not able to detect this pinworm infestation. In order to exclude false positive results during this routine control, additional testing was undertaken. Testing included PCR analyses of fecal pellets (pooled per row of the IVC racks in question) and direct microscopy after fecal flotation and confirmed infestation of only 1 cage with imported mice. The mouse minute virus infection was revealed by EAD as well as by the routine bedding sentinel program. The implementation and inclusion of EAD in a routine health monitoring program first need investment. EAD delivers reliable results. Close communication with the providers of the different analyses and monitoring methods is crucial for decision making, especially in case of unexpected findings.

### **P133 Water Pouch Water Sterility: A 30-Mo Study**

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The in-house produced, commercially available water pouch system provides ultra-filtered, chlorinated sterile water to our rodent population. We performed testing to determine the possible shelf life of these water pouches. Pouches were produced in October 2015 using a dilute chlorine solution (8 oz of bleach in 5 g of water; 5-10 ppm at the time of production) added to ultrafiltered water. Pouches were saved in standard storage containers in a typical storage room environment. We used a commercially available ATP detection system to assess for sterility. We swabbed the inside surface of the pouch at 1 d; 1 and 2 wk; and 1, 2, 4, 6, 12, 18, 24, and 30 mo. The data demonstrate that in-house produced water pouches remain sterile for at least 30 mo.

### **P134 From Biocontainment to Bioexclusion: Successful Maintenance of Germ-free Mice on a Biocontainment Unit Rack under Negative Pressure Ventilation**

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Gnotobiotic animal studies are gaining renewed interest coincident with the growing body of research evaluating the relationship between the microbiome in health and disease. Many gnotobiotic programs operate using Trexler-style flexible film isolator housing, which has been historically successful. However, isolator maintained research study limitations include time-consuming aspects of chemical sterilization of isolator housing, difficulty of in isolator manipulations while working with bulky gloves, and the space requirement of isolators, which can only house 1 microbiome at a time. Alternative housing strategies for studies of multiple stable microbiomes with 16-wk duration or less were of interest. In this study, we describe a negative pressure rack system with hermetically sealed cages used to house mice germ-free for a minimum of 16 wk. Procedures developed for animal care required the use of minimal chemical sterilant and minimal training for skilled technicians. Mice were supplied with autoclaved food, bedding, nesting materials, cage components, and sterile water. Fecal samples for culture were collected from a minimum of 1 mouse per cage at cage change. Animals remained sterile across 8 cage changes occurring at 14 d intervals. Sixty-nine mice were maintained germ-free throughout the study housed in groups of 2 or 3 same gender mice per cage. Six out of 6 mice sent to a commercial laboratory for testing at week 17 were found to be axenic in all testing criterion (serology, axenic bacteriology screening, PCR assessment, endo and ectoparasite screen, and histology). All cages tested negative in weekly in-house aerobic and anaerobic culture tests for a total of 24 wk. This study validated the repurpose of underutilized biocontainment unit (BCU) equipment at our institution for germ-free housing. This housing system allows for both germ-free and gnotobiotic mouse experiments at the cage level, including those requiring biocontainment. In conclusion, the study found that a BCU

rack successfully housed germ-free mice under negative pressure within an individually ventilated cage system for 24 wk.

### **P135 Just Let It Flow: A Closer Look at the Quality and Sterility of Standard NSG Mouse Drinking Water**

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Everyone knows the saying, “water is essential to life.” However, does 1 type of water excel over the rest? Husbandry practices for immunodeficient strains such as NOD.Cg-PrkdcscidIl2rgtm1Wjl/SzJ (NSG) mice are at a higher standard to ensure animal welfare and consistent research results. In addition to getting weekly cage changes, NSG mice commonly receive acidified or autoclaved acidified water bottles. Because NSG mice are to be handled and cared for with delicacy in order to protect their health and the research results, we wanted to compare pH levels and microbial growth over the course of 1 wk in the 2 most common types of water delivered to immunodeficient mice in our facility. The purpose of this study was to discover whether or not the pH level was affected by autoclaving and/or time, and if microbial growth differed over time between the 2 water types. The pH of acidified bottles (n=4) and autoclaved acidified bottles (n=4) was recorded before and after autoclaving, and on day 7. For sterility testing, samples from day 0 and day 7 were sent to our veterinary pathology program and cultured on blood agar plates. There was little to no change in the pH after autoclaving and over time, and there was no bacterial growth for any sample on day 0 or day 7. To take a step further, day 0 (n=8) and day 7 (n=7) samples were then sent to a laboratory for full bioburden testing. There was no microbial growth from any sample undergoing bioburden testing as well. These results indicate that there is no need to autoclave acidified water in our facility. However, it is important to note that our facility distributes reverse osmosis water to all animals, and these results might not be consistent for facilities using tap water or other water types instead.

### **P136 Comparison of Spot Change Frequency and Associated Husbandry Parameters for Individually Ventilated Mouse Cages Bedded with Corn Cob and a Paper-based Material**

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Recommendations in the *Guide* stipulate a maximum sanitation interval of 2 wk for rodent cage bottoms, yet in many instances, cages need to be changed earlier due to excessive contamination of the microenvironment. We sought to understand the actual early changing rate of individually ventilated mouse cages (IVC) across several facilities within our institution. In addition, we hypothesized that a switch from corn cob bedding to a paper-based material would significantly reduce the spot changing frequency. We evaluated the frequency that cages were changed prior to the 2-wk interval for mice housed in IVCs, in over 3,700 cages from 23 different housing rooms, during a 2-3 mo timeframe (4-6, 2-wk intervals). During this timeframe, staff reported decreased levels of environmental dust and odor in the housing rooms, as well as a reduction in cage flooding. On average, 18.2% (0.8-58.9%) of corn cob bedded mouse cages were changed before the 2-wk interval. In contrast, cages bedded with a paper-based material had an average spot change frequency of 1.76% (0-6.3%) over the measured timeframe. We also observed that cages with paper-based bedding had a statistically significant decrease in intracage ammonia levels compared to matched corn cob controls for cages containing male mice ( $P < 0.0001$ ), and paired breeding mice with litters ( $P < 0.05$ ). We found a statistically significant increase in the incorporation of bedding material into the base of the nest for mice housed on paper bedding ( $P < 0.0001$ ). This study shows that paper-based bedding provides a potential reduction in the spot change frequency of IVC mouse cages, which allows for improvement of animal welfare, husbandry efficiency, and environment for personnel working with the animals.

### **P137 Flexible Space for the Housing of Sheep: Assessment of Feasibility**

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In many research facilities, hoofed stocks are often housed in runs with raised flooring. Likewise, our facility solely used this set up for large animals species such as sheep (*Ovis aries*), goats (*Capra hircus*), and pigs (*Sus scrofa*). In the past year, the facility’s sheep population drastically increased requiring additional space. As such, we devised a unique way of housing these animals. When the animal facility was built, several rooms were constructed and were coined as flex rooms as they were flexible in potentially housing rodents, aquatics, or large animals, depending on the needs of the facility. To transition the flex room for housing sheep, lixit lines were installed, as well as partition walls to enclose the sheep, and drain covers to keep out debris. The decision was made to house sheep on the floor on bedding. We discovered that we can house more sheep per room and waste less hay since the sheep can graze on the hay that is on the ground. It takes about half the time for husbandry technicians to clean this room. The flex room also encourages more natural herd and grazing behaviors which are important for the enrichment of sheep. Since this has been successful, our facility’s future goals are to improve our current flex room housing and to transition from modular runs to housing on the floor in our remaining rooms. We will use this opportunity to compare the quality of various bedding by monitoring indoor air quality.

### **P138 Vole Husbandry: Managing Chaos**

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There are many variables to consider when housing new rodent species in an animal facility, especially if they are USDA regulated. Important considerations include space, equipment, pathogen screening, and properly trained personnel. In 2015, we had the opportunity to work with prairie and meadow voles. Since there is not much published on this unique animal model, animal care technicians assembled informational packets that included vole behavior, diet, handling, breeding, caging and enrichment, temperature and humidity ranges, and light cycles. Prior to arrival, the housing room was organized, sentinels established, and harem breeding cages were made using aspen chip bedding, alfalfa cubes, veggies chips, cotton pieces, a PVC tube, an igloo, and sunflower seeds. Voles also received a laboratory rabbit diet and a water bottle. Since voles cannot be picked up by their tails, a small plastic cup was used to scoop them during cage changing. Gardening bite-resistant gloves were also worn. As we worked with the voles we found several unexpected differences compared to mice and rats. Cage changing took longer than expected due to their messy behavior and special handling techniques. Other such differences included higher water consumption and enhanced enrichment requirements. When the program switched from bottles to the water pouch system, the voles were included. Being notorious chewers, the voles destroyed the water pouch valve causing floods or dehydration. They were successfully moved back to bottles with stainless caps. Because of these differences, time in motion analysis was used to establish accurate per diem rates. Using both rats and mice, we used our established sentinel program to test for pathogens and the only pathogen detected was *Mycoplasma volis*. The voles were prolific breeders and the colony expanded, quickly leading to space constraints within the housing room. Overall, we found working with voles to be a rewarding challenge and a great learning experience. Technicians and voles adapted quickly to new handling practices and overcame the quirks of caring for chaotic voles.

**P139 Movin' On Up: Assessment of Temperature in a Mouse Rack during Relocation Conditions**

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As campuses grow or expand their vivarium, it becomes necessary to relocate large numbers of mice for short distances. Transferring large numbers of mice into traditional transport containers for relocation is time and labor intensive, creates a large amount of waste, and has the potential for identification errors in the multiple transfers into and out of the transport boxes. Our university recently opened a neuroscience institute necessitating the relocation of over 5,000 cages of mice 40 blocks (2 miles) from their original campus. In the interest of reducing labor, waste, and errors, we developed a system to move the mice in their home cages. The day before relocation, mice were transferred into clean cages containing bedding, feed and a gel water source. During relocation, the rack was wrapped with moving plastic to prevent cages from shifting and secured for transport on a temperature-controlled vehicle. To assure that cages would not overheat when wrapped in plastic, we tracked the temperature in 20% of the cages in the wrapped full rack (all cages containing 5 adult mice) compared with the same rack of cages unwrapped. The temperature in the wrapped cages increased by an average of 2 degrees Fahrenheit within the first half hour, but stabilized and did not increase further over the remaining 8 h. To date, over 5,000 cages of mice have been successfully moved by this method.

**P140 Environmental and Psychological Enrichment for Nonhuman Primates Reimagined and Repurposed**

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Our institution has a large array of housed animals that had required expensive devices. We struggled with a lack of manpower to carry out daily enrichment activities. Environmental and psychological enrichment must be provided to nonhuman primates. The focus of these devices must be on facilitating the expression of species-typical behaviors by use of physical exercise, novel manipulation of devices, and cognitive adventures. The department of veterinary resources began rethinking our enrichment program by focusing on species-specific needs. We then reimagined and repurposed our existing equipment, thus creating the perfect toys. High caliber research consumes much of our skilled veterinary staff, so husbandry has owned the enrichment component and incorporates it into daily rounds with a focus on creativity and documentation of results. Husbandry spends the greatest amount of time with these animals and has made enrichment part of its critical duties. Providing an engaging foraging toy is carried out along with checking temperature, water lines, and cleaning enclosures. We have been successful creating an endless source of budget-conscious enrichment devices while minimizing waste. The institution has created ownership of the powerful enrichment component where designated animal care technicians, called Happiness Crews, directly impact the animals' wellbeing. The end goal is a happy nonhuman primate exhibiting species-specific behaviors and representing wellness has been met at our facility.

**P141 The Use of Immediate Feedback Adenosine Triphosphate (ATP) Testing to Improve Macroenvironment Sanitization in the Vivarium**

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Surface sanitization in a vivarium is important for reducing microbial contaminants which can negatively impact colony health and research

results. Since microorganisms cannot be detected visually, vivarium managers rely on cleaning efficacy. Historically, sanitization has been verified through traditional microbiological culture methods. These methods are time consuming and lack immediate feedback. Adenosine triphosphate (ATP) is found in cells and its existence indicates cellular presence. ATP can be quantified by measuring the reaction of a surface swab's contents with luciferase using a luminometer and reporting as relative light units (RLUs). This process is called ATP testing. Our institution's comparative medicine facility uses a commercially available luminometer with commercial surface swabs. RLU thresholds were set according to the facility's standards as clean walls (<10RLU), clean floors (<40RLU), dirty walls (≥30RLU), and dirty floors (≥75RLU). Results between those numbers are the warning range. Initial post-sanitization ATP testing of 6 technicians' work over 11 animal rooms elicited dirty results. Sanitization procedures were investigated and proper methods reviewed. Testing results improved but were still within the dirty range. It was not until immediate feedback was provided that testing results were low enough to indicate sanitization. To perform immediate-feedback ATP testing, floors and walls were swabbed after sanitization in inconspicuous areas. Within seconds of testing the staff member who performed the cleaning was contacted. A clean result was applauded. Staff was alerted to warning range results. Dirty results required resanitization of the area after which ATP testing was repeated. All results were in the clean range when retested. Results continued to improve in subsequent months for all technicians and RLU readings for all rooms have been in the clean range for the last 4 mo. Investigations have shown a brief disinfectant wet-contact time and weak dilutions to be the main barriers to substandard cleaning in this facility. When faced with measurable numbers, the vivarium staff followed sanitization procedures more closely resulting in a cleaner macroenvironment.

**P142 Prevalence of *Staphylococcus xylosus* as Determined by Exhaust Air Debris Testing**J Bailey<sup>1</sup>, G Nault<sup>1</sup>, RS Livingston<sup>2</sup>, DL Fong<sup>1</sup>, CM Mitchell<sup>1</sup>, LM Habenicht<sup>1</sup>, JK Leszczynski<sup>1</sup>, CA Manuel<sup>\*1</sup><sup>1</sup>University of Colorado Denver, Aurora, CO; <sup>2</sup>Molecular Diagnostics, IDEXX Bioresearch, Columbia, MO

*Staphylococcus xylosus* is a gram-positive bacterium that is presumed to be a commensal on the skin of a variety of animals including rodents. In mice, *S. xylosus* has also been reported to cause opportunistic infections and is a primary pathogen of strains deficient in phagocyte superoxide production and immunodeficient strains with impaired innate immune cell recruitment. The goal of this study was to determine the prevalence of *S. xylosus* throughout rodent housing facilities at an academic institution. Sterile, flocked swabs were used to sample the air exhaust debris of 369 IVC racks. Racks were equally distributed between 2 research vivaria that are managed using identical procedures. Each swab submitted represented a maximum of 140 cages by sampling 1 or 2 single-sided racks or 1 double-sided rack. All racks sampled were in service for approximately 1-6 mo. Swabs were submitted to a diagnostic laboratory for qPCR analysis and relative copy number determination. In total, 88% (227/257) of swabs were positive for *S. xylosus*. When a single swab was used to sample a single-sided rack, 88% (59/67) were positive. Sample copy number had a positive correlation with the number of cages on the racks ( $r = 0.177$ ,  $P = 0.008$ ), but did not correlate with the duration that the racks were in service ( $r = 0.037$ ,  $P > 0.05$ ). All rat racks were negative for *S. xylosus* ( $n = 15$ ). The 5% of samples with the highest copy number were traced back to their original racks for additional investigation into general population trends. None of these racks had a recent history or increased incidence of skin pathology, but were all associated with breeding colonies. Our results demonstrate that *S. xylosus* can be readily detected within the exhaust plenums of IVC racks by PCR and is very prevalent among laboratory mice, but not rats. While a statistically significant, positive correlation was identified between the relative copy number and the number of cages on racks, the quantity of *S. xylosus* DNA detected still appears multifactorial.

### P143 Animal Recovery and Enrichment Bed

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The use of equipment that serves multiple purposes can be an effective strategy for saving space and reducing costs. We developed a bed that can be used for both anesthesia recovery and environmental enrichment. For use as a recovery bed, the goal was to provide a soft surface for comfort and thermal support. For use as an enrichment bed, the goal was to provide a retreat from the hard floor and/or to encourage natural behaviors. The bed is durable and relatively easy to clean. The frame is constructed from 3.4 in PVC with metal conduit to provide support. The bed fabric is ballistic nylon and has a pouch beneath that allows for insertion of thermal support devices. Optional blankets made from French terry cloth attach via heavy duty velcro and provide a soft blanket for recovery, or provide enrichment by encouraging digging, burrowing, and rooting. Through the use of surveys, we solicited feedback from users. Based on the responses, several modifications were made, including reducing the size of the corner cut-outs, making the blankets smaller and detachable, and modifying the bed size to fit snugly in a recovery cage. The beds have been tried for enrichment and/or anesthetic recovery in ferrets, rabbits, turkeys, dogs, and pigs. For use during recoveries, we found that the bed is most useful for small or medium-sized animals such as rabbits, turkeys, and beagles. For use as an enrichment bed, swine and ferrets appear to interact with it the most. Eighty percent of survey respondents indicated they plan to use the bed again, and future improvements being considered include offering a variety of sizes (shorter version for weanling pigs; smaller version for ferrets) and multiple options for blankets (soft fabric for recoveries; durable fabric for enrichment).

### P144 Use and Sanitation Frequency of Enrichment Shelters in Mouse Breeding Colonies

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At our institution, research staff frequently place enrichment shelters in mouse breeding cages, which are then sanitized every 2 wk coincident with cage change, resulting in significant labor costs. The *Guide* permits flexibility in sanitation intervals, recommending these be based on performance criteria. Recent studies have validated increased sanitation intervals for other rodent cage components. Our hypothesis is 2-fold: 1) the shelter is not used for nesting, and 2) the sanitation interval can be extended to 6 wk. This study used mice from 2 genetic backgrounds (n=22 cages), and assessed sanitation intervals for red bottle-shaped mouse enrichment shelters based on shelter weight as a measure of accumulated debris and ATP luminometry. Nude mice used shelters more frequently than B6 mice (68.7% and 42.5% of observations, respectively). The shelters were used most often by adults and older pups while neonates were kept in a separate nest, suggesting a potential benefit to providing shelters to breeding cages as a nesting site for older mice when neonates are present. At 4 and 6 wk, neither hut weights nor ATP levels were significantly higher than at the standard 2-wk change interval. There were no significant differences in hut weights or ATP levels between the 2 genetic backgrounds. However, there was a strong positive correlation between ATP levels and the number of mice over 10 d old, with 2 cages housing very large litters having heavily soiled shelters that were statistical outliers in ATP levels. Based on these findings, the sanitation interval for mouse enrichment shelters may be extended up to 6 wk without compromising animal welfare, provided that heavily soiled shelters are spot-changed as needed.

### P145 Modified Flexible Film Isolators Proved Safe and Effective for BSL-3 Aerosolized Studies Verified by Computational Fluid Dynamics

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Computational fluid dynamics (CFD) was used to evaluate and verify the integrity of modified flexible film isolators for containment of aerosolized BSL-3 select agents. Three isolators were located inside an animal biosafety level-3 (ABL-3) room to provide an extra tier of protection and to permit different infectious studies within the same room. Multiple case studies of failure scenarios were investigated including isolator breaches through plastic membrane seam separation, different rip sizes, and exhaust fan failure. To breach the containment, it required the rare event of a plastic membrane rip plus the improbable malfunction of the dual back up exhaust fans. Each isolator has a blower motor plus a backup blower motor including battery power in case of electrical failure. Even with this rare double event, the ABL-3 room air exhaust system easily contained the few agents released. The modified flexible film isolators with negative airflow proved safe and effective for aerosol studies using BSL-3 select agents.

### P146 Toxigenic Profile of *Clostridium perfringens* Isolated from Natural Ingredient Laboratory Animal Diets

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*Clostridium perfringens* is a gram-positive, anaerobic, spore-forming bacterium considered ubiquitous in the environment and commonly isolated from the gut of numerous mammalian species. *C. perfringens* is a frequent enteropathogen in humans and animal species; however, the trigger for pathogenesis is uncertain and likely the sequelae to dysbiosis and Clostridia overgrowth. The Clostridia genus produces more toxins than any other known bacterial genera. *Clostridium perfringens* is primarily categorized into 5 toxinotypes (A, B, C, D, and E) based on the production of 4 major toxins, alpha (a), beta (b), epsilon (e), and iota (i). Some *C. perfringens* strains produce other toxins, including the Clostridium perfringens enterotoxin (CPE) which is the third leading cause of food poisoning in humans. We normally screen our animal feeds for aerobic, enteric pathogens such as *Salmonella* spp. or *E. coli*, but recently started anaerobic screening as well. To date, we have isolated several *Clostridium* spp. including *C. perfringens*, from all batches of our standard rodent diet (NIH-31). We also tested multiple natural ingredient rodent diets and one swine diet (NIH-2004) from other institutions and isolated *C. perfringens* and 3 additional *Clostridium* spp. from almost all batches. While the isolation of *C. perfringens* from unsterilized, natural ingredient diets is not surprising, there have been no published reports of its isolation from laboratory animal feeds. As such, we wanted to perform a toxigenic profile analysis of the various isolates. Our findings demonstrate that toxin-producing strains of *C. perfringens* are present in these diets; however, there are no reports of disease due to *C. perfringens*. As studies progress with immunocompromised strains of laboratory animals or microbiome-related research using gnotobiotics or animals with perturbations in gut flora, the presence of *C. perfringens* could result in disease and is an important reason that these diets be sterilized prior to feeding.

**P147 Effects of Cage Color on Reproduction and Behavior in ICR and SJL Mice**

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Tinted caging has been suggested as a refinement to decrease rodent stress. Because rats and mice cannot perceive red and yellow wavelengths, red- and yellow-tinted cages allow the rats and mice to feel as though they are in a darkened shelter without compromising the ability of staff to perform daily health checks. The purpose of this study was to examine the effects of tinted cage color on the breeding success in mice. Eighteen breeder pairs of ICR (good breeders) and SJL (poor breeders) mice were established. The breeder pairs were randomly allocated to 3 different treatment groups: (1) clear, (2) red, or (3) dark gray (opaque). The breeder pairs were followed for 140 d. The breeder production index, or BPI, was used to assess breeding success. Two male and 2 female mice from each litter were maintained for anxiety assessment at approximately 95 d of age using the elevated plus maze. For the ICR mice, there were no significant differences between groups. For the SJL mice, there were no significant differences in BPI between groups, but there appeared to be a trend of improvement in the opaque and red conditions compared to the clear. There were no significant differences time to first litter for either strain. There were no significant differences between the groups for the proportion of time spent on the open arms for the ICR mice, but the SJL mice that had been reared in the red caging environment showed a significant decrease in the proportion of time spent on the open arms. Although the tinted caging did not appear to confer any significant benefit for the mice on this study, the study suggests that housing in red tinted caging may increase the distress of mice that are already predisposed toward anxious behavior.

**P148 Interdepartmental Teamwork Approach to a New Rack Sanitation Program**

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The introduction of a new ventilated rack and cage (IVC) system required an interdepartmental teamwork approach to update the rack cleaning and sanitation SOP. Review of operations manuals and discussion with manufacturers before racks arrived provided insufficient detail to update sanitation practices in advance. The quality assurance laboratory, facility trainer, husbandry and cage wash team leaders, worked together to define new procedures. Areas of specific focus included water valves and air plenums. New racks utilized quick disconnect water valves. Concerns regarding the potential for cross-contamination were addressed. Active exhaust systems resulted in the potential for increased debris accumulation within the rack plenum. Different cleaning methods and materials were evaluated for the disinfection of water valves during cage wash and cage change out over an 18-mo time period. Racks (n=160) transported for sanitation in cage wash were visually inspected for debris, dust, and rust. Hoses and exhaust ducts were inspected for accumulated debris and different methods for cleaning (high-pressure wash or rounded brushes) were compared. Colored zip ties were used as a method of communication about the progress of testing. Samples were taken for testing with a combination of ATP monitoring system, ATP luminometer and sterile swabs for bacterial culture. Studies completed with husbandry and training staff allowed for input from personnel working directly with the new equipment that could be incorporated into the new SOP. Increased involvement of staff within separate groups allowed for a smooth transition and increased confidence concerning new sanitation practices.

**P149 Assessment of Available Chemical Cleaning Agents for Facility Antimicrobial and Electrostatic Contamination Control Mats Using Real-time ATP Testing**

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A routine ATP test of facility electrostatic contamination control mats called in to question the efficacy of current chemical cleaning agents and launched a search for a more rigorous cleaning solution compatible with the mats' manufacturer's guidelines. Three cleaning agents were chosen from the manufacturer's recommended chemicals list to determine the best cleaning agent (as determined by ATP test of <10 Relative Light Units), we chose a 1-step disinfectant cleaner (n-alkyl dimethyl benzyl ammonium chloride (8.19%), didecyl dimethyl ammonium chloride (8.7%)), a 70% ethanol solution, and a neutral cleaner (alcohol ethoxylates (20-30%), sodium xylene sulfonate (1-3%), fatty acids, C8-18 and C18 unsaturated (1-3%) and terpene solvent (.1 - <1%)). Twice daily each mat was cleaned with water, left to dry and the chosen solution applied, after which the compounds evaporated and a real-time ATP swab test was used to determine effectiveness. Ideal levels per the ATP test kit manufacturer's guide recommend less than 10 RLU to pass. Three high traffic areas were used to assess how each chemical would perform in the worst case scenario and because of this an average score of 11-29 RLU (caution zone per manufacturer) was used to determine overall effectiveness. On average all 3 chemicals failed on 2 or more surfaces even after removal of outliers with 70% ethanol failing to reach the requisite minimum of 29 RLU disqualifying it from further testing. Of the 2 remaining chemicals, the neutral cleaner was found to score in the 11-29 RLU range consistently and was the preferred cleaner recommended for use by custodial staff. Based on these results the neutral cleaner is proposed as a replacement to current cleaners with applications no less than twice daily to achieve acceptable microbial levels.

**P150 When to Wean? A Visual Guide to Mouse Pup Growth P14 to P28**E Brogan<sup>\*1,2</sup>, SM Kirchain<sup>1</sup><sup>1</sup>Center for Comparative Medicine, Brigham and Women's Hospital, Boston, MA; <sup>2</sup>SoBran Inc, Danville, OH

Laboratory mouse pups are commonly weaned between postnatal day 21-28. While utilization of an exact day post-partum is a concrete metric for management in laboratory animal facility operations, this metric does not account for variation such as developmental differences among genetically modified strains, differences among research and animal care personnel in assessing pup developmental stage, different breeding schemes which can lead to pups weaned too early due to overcrowding, and lack of precision estimating actual birthdate for nontimed pregnancies. Clinical and operational sequelae of weaning pups too young may include failure to thrive, unsuitability for study assignment, undue distress on the pups, and extensive time and resource commitment to provide additional monitoring and supportive care for pups weaned too early. Point-of-use visual representation is a valuable tool to help vivarium personnel and researchers identify mouse pup age and developmental stage. However, resources currently available are either too cumbersome at the point-of-use (written descriptions) or incomplete (posters and photos only up to 14 d of age). Here we present the creation of a photographic poster depicting mouse pups (C57BL/6J) from age P14 to P28. Photographs were taken of normal pups with a ruler to show size and gross appearance. Visual aids have shown to have a positive impact on learning among the research and animal care staff at our institution. With both the animal care and research staff more experienced in the normal progression of weaning growth, they will be more equipped to make better assessments when determining the appropriate day to wean mice without the need for veterinary guidance. Positive outcomes from the poster include lower morbidity,

fewer delays to study start, less need for veterinary interventions, and less time spent by personnel placing wet food and water on the cage floor. The poster is now included in the vivarium orientation and training program at our institution.

#### **P151 Performing a Disinfectant Price Analysis**

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Recently our institution's program underwent a change of disinfectant from a chlorine dioxide compound that was made up weekly with a 7-d shelf life, to a ready-to-use hydrogen peroxide solution that has a shelf life lasting 2 y from the factory mill date. Before committing to this transition, we did a price analysis of the 2 products. The hydrogen peroxide solution appeared to be over 7x more expensive than the chlorine dioxide solution based on a volume per volume comparison. This was done by comparing the cost that would be associated with each bottle (21 oz) of the disinfectants. Recognizing the already large price difference between the 2 products, we decided to investigate further by considering several of the characteristics that would contribute to cost. One factor was the amount of wasted product associated with the chlorine dioxide that resulted from switching bottles every 7 d. The hydrogen peroxide product was filled only when the solution ran out, thereby eliminating waste. We looked at each room and predicted the amount of use per year based on the known weekly consumption. We then used this predicted volume to account for how many actual bottles of the hydrogen dioxide disinfectant would be used. By looking at the amount of total volume from the chlorine dioxide compared to the less wasteful hydrogen peroxide solution the total price for a year of the showed the chlorine dioxide was now only double the cost. We calculated the difference associated with the labor cost. The chlorine dioxide product was more laborious in that it involved the weekly collection and redispersing of the bottles, whereas the hydrogen peroxide product was only refilled as needed. With the high labor cost associated with the chlorine dioxide, the hydrogen peroxide solution was now 1.2 times more expensive. By taking the new costs for comparison into consideration, we looked at factors that did not have monetary value as easily captured, including efficacy, the effects on equipment and personnel, and convenience of use. We switched to the hydrogen peroxide product as we would be recapturing the money from the initial loss of going with the more expensive chemical and would be selecting the disinfectant that was the best fit for our program hydrogen peroxide solution.

#### **P152 Development of an Improved Technique for Serial Survival Cerebrospinal Fluid Collection in Mice**

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Multiple sclerosis (MS) is an immune neurodegenerative disease characterized by intrathecal inflammation. Progression of the disease is difficult to determine until severe symptoms arise. Biomarkers for neurodegenerative diseases like MS are not easily found in the blood, making it imperative to obtain cerebrospinal fluid (CSF). CSF is in direct contact with the extracellular space of the spinal cord, the epicenter for the disease, and can show more precise changes in the spinal cord and disease progression. Normally, to monitor the disease in mice, the spinal cord is collected using a serial sacrifice method and the tissue is used for immunological analyses. However, spinal cord collection requires a high number of mice to obtain sufficient data and spinal cord biopsy is not available in human patients. CSF is more readily accessible in patients, making CSF collection in mice optimal for translating findings in mice to human patients. We have set up a method for serial CSF collection in the Theiler's Murine Encephalomyelitis Virus-induced Demyelinating Disease (TMEV-IDD) mouse model of progressive disability in MS. To enable serial collection of CSF from the cisterna magna, aseptic surgical techniques, including instrument sterilization between each mouse, is required.

Instead of cutting muscle like in necropsy CSF collection, separating the muscle with forceps and making a smaller entry incision aids healing. Anesthesia is monitored and pre-warming the recovery cages also eases recovery following surgery. Serial CSF collection is performed approximately every 2 mo with a per entry volume of 2-10µl of CSF normally obtained. Amounts vary depending on size, hydration, and health of the mouse. From such a small amount of CSF, we are able to measure several different biomarkers of intrathecal inflammation and neurodegeneration. These all assist in monitoring disease progression in TMEV-IDD, which can ultimately be translated into human research. The serial CSF collection method also decreases the need for serial sacrifice, resulting in the use of a diminutive number of mice, therefore supporting the 3Rs of animal research.

#### **P153 Novel Creep Feed Cage Modifications Support Reintroduction of Mother and Infant Pigtailed Macaques at 1 Month of Age**

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Removal of infants from dams at birth is sometimes necessary for research or clinical reasons. To improve animal welfare and reduce personnel time required to care for infants, reintroductions are attempted whenever nursery-rearing is not required by research protocols. A successful reintroduction requires exhibition of appropriate behaviors by both dam and infant including affiliation, proper suckling positioning, adequate lactation, and sufficient nursing. Previous experience has found introduction of *Macaca nemestrina* infants to their mothers shortly after delivery likely to succeed. Here we report a failed mother-infant reintroduction rendered successful at 1 mo of age through the development of a creep feeder caging arrangement. An infant separated at birth was first reintroduced to his dam's home cage 16 d and 17 d postpartum. The multiparous dam acted appropriately, but the infant, who had been housed in the nursery and bottle fed, would not remain on the dam, did not nurse, and attempts to offer formula bottles drew dam interference. To increase the probability of a successful reintroduction we created a separate infant feeding area without major equipment alterations by linking an adult cage to 2 adjacent infant cages and modifying the infant cage divider locking mechanism to reduce the passageway to accommodate only the infant and placing formula bottles in the infant-only area. At 29 d side-gates between maternal and infant cages were opened. Upon reintroduction, behaviors were consistent with the initial reintroductions, but the pair was able to remain united since the infant had access to supplemental formula. This study indicates that novel caging arrangements can succeed in reintroduction of infants to dams or foster dams at later ages and may also apply to situations where behaviors are intact but the dam is unable to nutritionally support her infant.

#### **P154 LED Light Conversion in a Vivarium: Yes, No, Maybe?**

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Energy reduction targets in research animal facilities are variable, but 1 common denominator and regulatory requirement is the provision of light. Vivarium lighting has traditionally been supplied using broadband fluorescent tube lighting. With typical light cycles of 12:12 and a bulb life average of 10,000 h, 1 quality fluorescent tube in a 4-light ballast needs to be replaced ~ 6 mo. Most animal holding rooms hold multiple ballasts and light tubes which leads to multiple outages, inconsistent light levels, noncompliance, and constant work order submission for bulb replacement. In order to provide consistent, compliant, and energy-efficient lighting, we considered facility conversion to white light-emitting diode (LED) lighting. We initiated

the project in November 2015, replacing fluorescent light tube ballasts with LED drivers in 2 empty animal holding rooms. Floors were tape demarcated in multiple spots to determine light level compliance and what we termed workability—the ability to conduct normal husbandry tasks within the room. Test rooms were maintained for 3 mo and researchers were consulted throughout the process. Once the appropriate drivers and ballasts were determined, full facility retrofitting was completed in March 2016. Approximately 8 mo following installation, animal care staff noted increased algae growth in the *Xenopus laevis* holding tanks. Additional sanitization strategies were implemented, yet unsuccessful. Algal sampling revealed non-pathogenic strains, growing exponentially. No other species or rooms have had untoward effects. The spectrum of the LED lighting was measured and compared to the spectrum of fluorescent lighting. LED lighting output is stronger at blue wavelengths (430–470 nm) and this is likely the cause of the algae growth. We have experienced increased cost savings and energy efficiency (no light bulbs have been changed to date), but tank sanitization and cageside observation times have increased significantly due to algal overgrowth. Light filters and ballast modulators are being investigated as ways to reduce the blue light emission from the LEDs reaching the tanks. The use of filters may allow for the continued use of the LED lighting without resulting in increased algae growth.

#### **P155 Protect Your Germ-free/Gnotobiotic Mice: Have You Checked Your Isolator Gloves Lately?**

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Isolators containing germ-free or gnotobiotic animals require an environment that is free from all bacteria, viruses, and parasites. General maintenance of the isolator is required to ensure that the isolator unit operates efficiently while still maintaining the sterility. The frequency in which general isolator maintenance is performed may vary based on the durability of the isolator parts. In this particular case, we are focusing on the isolator gloves, which are one of the major components of the isolator. Pinholes can be a source of entry for unwanted contaminants within the germ-free isolator so it is important to inspect the gloves for breaks. In previous years, we set the frequency of changing the isolator gloves at intervals of every 6 mo. However, we noticed that during glove inspections, small pinholes were frequently found around the cuff, before the next scheduled glove maintenance change. After evaluating the frequency of changing the gloves and methods of checking the gloves for pinholes, along with reassessing numerous types of gloves, we were still unsuccessful in preventing the pinholes and had switched the frequency of changing the isolator gloves at intervals of every 3 mo. Recently, we thought of a new method of installing the gloves onto the isolator by using the plastic rings as a protector for the cuffs of the gloves. Now the plastic rings are on the inside of the isolator and on top of the cuff of the gloves. Thirty-nine isolators were used to monitor the daily inspection of both the left-hand and right-hand of the gloves using this new method for approximately 7 mo. In conclusion, the new method to protect the cuff of the gloves has proved to be successful. As a result, we have changed the frequency of changing the isolator gloves back to intervals of 6 mo. We plan to continue to evaluate the integrity of the gloves and the new method of installing the gloves to increase the time between changing gloves that in turn will save labor and expense.

#### **P156 Effects of Cage Density and Sanitation Frequency on Animal Welfare**

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The *Guide* recommends the minimum amount of space required for group-housed rodents, as well as cage sanitation frequency.

Adjustments to these guidelines may be allowed if reviewed and approved by the IACUC and any changes should be based on performance indices of animal wellbeing and research quality. Our institution has developed cage density and cage sanitation guidelines based on evidence from published literature that differ from the *Guide's* recommendations. We evaluated the effects of cage density and sanitation on animal growth, welfare, and airway lesions that could be associated with ammonia concentration in rodent cages. The *Guide* recommendations were compared to the standards adopted in our production barriers. For sanitation, the *Guide* recommends a complete cage change weekly. The comparison used in this study was a monthly complete cage change with only a bedding change weekly. Sprague Dawley rat breeding cages were housed according to our internal (1 x 1; 120 in<sup>2</sup> per animal) or *Guide* standards (1 female with litter; 124 in<sup>2</sup> per animal). The number of pups born and weaned was recorded, as well as pup and parental morbidity, mortality, ammonia, clinical conditions, animal behavior, and cage cleanliness. After weaning, the pups were housed with 15–40 in<sup>2</sup> per animal from <100 - >400g. The *Guide* recommends 17–70 in<sup>2</sup> per animal from <100 - >500g. Body weights of the pups born were recorded from weaning until 12 wk of age. At the end of the study, 5 rats per group were randomly selected from different cages and were examined for pathology of the nasal cavity and lungs. The data showed no statistically significant differences between the groups in body weight, morbidity, mortality, cage cleanliness, or behavior. There were also no statistically significant differences in average litter size or the percentage of pups weaned (pup survival from birth to weaning). Cages that were housed in accordance with the *Guide* standard had lower ammonia levels in the last 2 to 3 wk of study, however, the values were below 25 ppm. The pathology findings indicated that there were no lesions in the nasal cavity or lung that would be associated with high levels of cage pollutants. There were no detrimental effects of using the higher cage density and lower sanitation frequency that is recommended by the *Guide*.

#### **P157 Using Business Operations Software for Inventory and Supply Control in a Complex Animal Program**

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The comparative medicine department at our institution manages 9 vivaria in a medical center. The department has a dedicated shipping receiving dock on the main campus with a 5-person crew. Managing supplies and inventory for a large animal program presents many challenges. Our cumbersome tracking system made it impossible to accurately track on-hand inventory. Facility managers routinely over ordered consumables which exacerbated restricted storage capacity and impacted an already tight budget. We decided to work with our purchasing group, to add our list of consumables and vendors to the main inventory/purchasing process. The list was then loaded into the business operations software. Supply levels were determined based on historical use data to establish our min/max points. Once these limits are reached in the system, a purchase order (PO) is automatically generated and sent to the vendor. The institution carries the cost of the asset until the asset is distributed. Every week, each facility submits a weekly consumables/feed/bedding order. This is done using a spreadsheet created from our inventory system. The order is submitted to our dock staff, which creates a pull sheet used to fill the order at the warehouse. The products are then pulled and palletized for delivery to each facility. Once delivered, a goods issued entry is made in the software program. This deducts the items from the electronic inventory, charges the facility's cost center and if necessary, automatically generates a PO. Deliveries are received through our dock, a goods receipt entry is made, and these items are added to our electronic inventory. The vendor delivery is divided between our dedicated dock area (feed/bedding/enrichment) and our main warehouse. Using the power of the institutional purchasing and inventory management system through the software has allowed us to control and track on-hand inventory, decrease costs and storage needs



while maintaining adequate supplies for our operation. Using the power of our purchasing department has freed our managers to manage many other things besides supplies.

#### **P158 Evaluation of Optimal Strategies for Floor Contamination Control in Rodent Individually Ventilated Caging Facilities**

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Strategies for controlling floor contamination in rodent individually ventilated caging (IVC) facilities have traditionally consisted of disposable shoe covers, but alternative technologies, such as contamination control flooring, have been developed to minimize introduction of organic debris and pathogens. We evaluated the relative reduction in organic load between rodent IVC facility entrances and housing areas for 4 different floor contamination control conditions: 1) shoe covers alone, 2) contamination control flooring alone, 3) both shoe covers and contamination control flooring, and 4) neither shoe covers nor contamination control flooring. We hypothesized that facilities employing a contamination control method would show a significant reduction in organic load, with the greatest reduction seen in the facility employing both shoe covers and contamination control flooring (condition 3). Floor swabs were collected from facilities twice weekly for 6 wk, and organic load (ATP) was measured via a handheld luminometer. Intrafacility organic load was significantly reduced compared to animal facility entrances in all facilities, including the facility not employing a floor contamination control strategy (condition 4). Biweekly examination of organic load in the same facility while changing the floor contamination control strategy every 4wk showed that using both shoe covers and contamination control flooring resulted in the greatest reduction in ATP levels. To examine whether floor contamination control strategies affected the environmental presence of both excluded (pinworms, mouse hepatitis virus, mouse parvovirus) and nonexcluded (mouse norovirus, *Corynebacterium bovis*, *Helicobacter* spp., *Staphylococcus xylosum*) murine pathogens, swabs were taken at facility entrances and within animal rooms weekly for 6 wk and submitted for PCR testing. Excluded murine pathogens were not detected in any facility, but non-excluded pathogens were detected sporadically in animal rooms, regardless of floor contamination control strategy. These findings show that floor contamination control strategies help reduce organic load in rodent IVC facilities but do not enhance protection from environmental contamination of murine pathogens.

#### **P159 On Your Mark, Get Set, Manage!**

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Oftentimes, technicians are promoted to management positions simply because it seems like the next logical step. Many lack the soft skills and other necessary skills to make them successful in the role. My department has implemented a Leadership Training Program (LTP) to help give technicians interested in leadership roles the tools and skills necessary to be successful. The LTP is a yearlong course designed to give each candidate skills required for leadership responsibilities. A few of the resources include human resource classes, seminars, case studies, and tours of other facilities. To date, we have had 3 research support technicians (RST) that have completed the program go on to become supervisors within our division. The success of our leadership program is measured in multiple ways. One way that success is measured is by conducting surveys each year. We want to hear from our program participants about what went well, and in what ways we can improve. The managers of the participants also give feedback to assess short and long-term goals. We also measure success by the number of people that end up in leadership roles, whether at our organization or somewhere else. We have had graduates of our program promoted to leadership roles. We have had several of our graduates give testimonials regarding how the program has given a

deeper understanding of management and helped with self-awareness in making personal improvements. Many of our program participants are RSTs, which is a team leader role. While our RSTs are not involved in handling disciplinary issues, they are expected to perform many leadership duties such as ordering, communicating effectively with staff, and helping with scheduling, among other tasks. We are able to determine that the technicians can use these leadership skills after the training by observing them in their role. Also, leadership skills require development, which is a continuous process.

#### **P160 A Plastic Water Pouch Delivery System for Rodents Is not Associated with Estrogen Activity or Bisphenols in Their Drinking Water**

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Estrogen is a naturally occurring steroid hormone most notable for the development and regulation of the female reproductive system. Synthetic compounds known as xenoestrogens mimic estrogen and can disrupt normal reproductive physiology in mammals. Bisphenol A (BPA) and Bisphenol S (BPS) are xenoestrogens used as the base material for the synthesis of thermoplastics including polycarbonate and polyethylene. Laboratory animal water bottles are commonly made from these thermoplastics due to their ability to withstand repeated exposure to extremely elevated temperatures, experienced during steam sterilization, without significant degradation. Recent publications show that some plastic water bottles found in rodent facilities have estrogen activity (EA) and leach BPA into the drinking water. To increase accuracy and reproducibility in scientific studies, it is important to eliminate xenoestrogens from the drinking water and reduce their exposure to laboratory animals. This study aimed at testing if there were detectable amounts of EA, BPA, and BPS leached into the water from patented polyethylene plastic water pouches. Reverse osmosis (R/O) water was compared to city water for a duration of 2 weeks to 8 mo to provide for both acute and chronic leaching of these compounds. Two distinct water samples within glass bottles and 6 distinct water samples in plastic pouches were sent to independent laboratories for evaluation. Known positive controls (a polycarbonate thermoplastic) and negative controls (polyester and glass bottles) were used. Human ovarian cancer cell line, BG1Luc4E2, cells were seeded onto plates along with an estrogen receptor-response Firefly luciferase reporter gene plasmid which measured the amount of EA leached into the water samples. The levels of BPA and BPS were assessed using pressurized fluid extraction (PFE) and by high-performance liquid chromatography (HPLC). The findings reveal that no estrogen activity or bisphenols were detected in either water supply when kept in the pouches or the glass bottles. It is recommended that facilities test their water delivery systems to determine EA and xenoestrogens within the water supplied to their animals.

#### **P161 Development of a Biocontainment Procedure for the Transport of Zika Virus-infected Macaques between Research Facilities for Imaging Purposes**

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NIAID, NIH, Dickerson, MD

Zika virus (ZIKV) is a flavivirus that is spread mostly by mosquitoes. While supporting a ZIKV study our research team was tasked with transporting ZIKV(+) infected macaques from a satellite facility to the main facility for imaging purposes, which was 30 miles away. Due to the experiment's imaging schedule being time sensitive, our research animals had to be transported during the summer months, which was also peak mosquito season. While animals infected with biohazardous agents are routinely transported within the confines of their facility walls for numerous reasons without incident; how does one approach safely transporting ZIKV(+) animals outside of the animal facility?

Since it was necessary to transport ZIKV(+) animals during this critical time we needed to develop procedures which ensured the animal was appropriately protected from mosquitos during its brief periods outside, such as loading and unloading from the transport vehicle onto the loading lock and prior to entry into the facility. A biocontainment isolator transport cage was fabricated consisting of 2 parts; the primary interior cage and the secondary exterior cage. The interior cage housed the macaque while the exterior cage completely enclosed the interior cage. The exterior portion included 3 air vents lined with mosquito netting to allow for adequate airflow during the transport while at the same time prevented any mosquitos from entering and accessing the ZIKV(+) macaque. Other biocontainment procedures included mosquito traps at the facility entrance, air curtains installed at each of the facilities loading dock entrances, and additional mosquito netting installed into the back of the transport vehicle. The additional netting prevented any mosquitos from entering the back of the truck while the door was opened to load/ unload the bio-transport cage. Our biocontainment procedures were approved by our biosafety committee (IBC) and we were able to transport our ZIKV(+) animals safely without incident.

#### **P162 Urine Collection from Göttingen Minipig within a Laboratory Environment**

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Urinalysis is a commonly used method of evaluating urinary tract or kidney function and to screen for progression of chronic conditions. To perform a urinalysis, urine must first be collected. The ideal urine sample would be free from contamination, but methods to collect sterile urine can be invasive. The ideal urine collection technique would be easy, pain-free, and provide a good quality sample with minimal contamination. We needed to collect urine from Göttingen minipigs on multiple occasions over the course of various studies. Multiple methods of urine collection were considered, but all had drawbacks. A creative, low-cost alternative method was developed using the modular run housing unit that was already being used to house the minipigs. Standard sized household screen doors were placed under the runs where the cage pans were typically placed. On the floor below the screen, 2 rabbit pans were placed. This allowed the urine to pass through the flooring and into the rabbit pans, being filtered by the screen to prevent any fecal material from contaminating the urine. This method allows for an easy, nonstressful, pain-free method of urine collection that results in a good quality sample with minimal contamination. Although the typically group-housed animals must be separated during periods of urine collection, they are still housed in their standard caging with tactile, visual, and olfactory access to their cage mates.

#### **P163 Assessment of Mechanical Washer Disinfection as a Means of Decontaminating Biohazardous Materials**

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Autoclaving is one of the most common methods of decontaminating biohazardous materials for purposes of biosafety, such as ABSL-2 housing and of biosecurity and the exclusion of murine pathogens. While effective, its disadvantages include high cost, limited availability, and incompatibility with certain materials. A literature search indicates there are several other acceptable decontamination methods. Here, we investigated 3 methods as potential alternatives to autoclaving for decontaminating cages of the murine pinworm *Syphacia obvelata*, a relatively hardy agent in the environment, or of *Citrobacter rodentium*, a representative gram-negative bacterium. A total of 43 cages were tested: Group 1) quaternary ammonia detergent

pretreatment for a 10-min contact time followed by 82.2°C (180°F) water and non-phosphoric acid cleaner for a 27-min cycle (n=11); group 2) 82.2°C (180°F) water and nonphosphoric acid cleaner for a 27-min cycle (n=9); Group 3) 82.2°C (180°F) water only for a 10-min cycle (n=12); and Group 4) autoclave for a 1 h and 12-min cycle (n=11). Dirty cages were emptied under a biological safety cabinet. Efficacy of decontamination was determined via PCR of pre- and posttreatment cage swabs. *S. obvelata* DNA was not detected on 16/22 PCR samples of clean cages: Group 1) 4/5 samples, 80% efficacy; Group 2) 5/6 samples, 83% efficacy; Group 3) 5/6 samples, 83% efficacy; Group 4) 2/5 samples, 40% efficacy. *C. rodentium* DNA was not detected on 18/21 PCR samples of clean cages: Group 1) 6/6 samples, 100% efficacy; Group 2) 3/3 samples, 100% efficacy; Group 3) 6/6 samples, 100% efficacy; Group 4) 3/6 samples, 50% efficacy. We conclude that autoclaving, although an effective decontamination method based on our autoclave monitoring reports, did not eliminate DNA on the cages, and that the use of 82.2°C (180°F) water alone or with the addition of a chemical is an effective method for eliminating *S. obvelata* and *C. rodentium* DNA from contaminated supplies and equipment.

#### **P164 Can Year Old Mice Be Effective and Reliable Sentinels?**

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Rodent sentinel screening for adventitious pathogens is an integral part of all biomedical research institutes and universities that use rodents in research. Typical screening programs involving live sentinel animals purchase new, young sentinel animals which are sampled and replaced quarterly. In efforts to reduce the numbers of live animals used in our sentinel program, we wanted to investigate the possibility of keeping sentinel animals in-house for periods of 12 versus 3 mo at a time. We exposed mice (38-40 wk of age) to murine norovirus (MNV) to test whether they could reliably produce detectable levels of antibodies (similar to younger mice) to a typical murine adventitious pathogen. Mice first exposed at 38-40 wk of age were able to seroconvert to MNV after direct exposure (via gavage) and after indirect exposure (from soiled bedding transfer) as well as or better than mice first exposed at 8-12 wk of age. These findings support the possibility of reducing sentinel animal numbers used by extending sentinel residence from 3 to 12 mo. This practice, in combination with other non-animal testing modalities (eg., exhaust duct sampling) can be combined to increase sensitivity and specificity of rodent surveillance programs and minimize the use of live animals.

#### **P165 Husbandry of African Pygmy Hedgehogs**

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Our conventional facility will house African pygmy hedgehogs (*Atlerix albiventris*) for an investigator. We needed to write a husbandry SOP and design a cage that was portable, durable, affordable, and met the hedgehog's environmental needs. Our staff was not familiar with hedgehog handling. We started our housing review by conducting a literature search and submitting an online query. We consulted an author of an upcoming book on hedgehogs. Much of the published information was specific to private ownership, and not fully applicable to a vivarium setting. We purchased 33 x 20 x 14 in (84 x 51 x 36 cm) plastic bins with lids that could be cleaned in the cage washer, as well as running wheels, huts, bowls, and water bottles. We modified the bins by drilling holes in the side for the sipper tubes and bottle hanger attachment, and in the lids to allow for airflow, while keeping animals contained. The enclosures were housed 2 per shelf on commercial steel shelves. We purchased rabbit cage paper pan liners, crinkle paper, and

a commercial hedgehog food. We tried various types of gardening gloves worn over nitrile gloves, as well as mouse boxes or other small containers for moving or housing in between cage cleaning. Hedgehog diet is measured by the teaspoon. The PI and our trainers held training sessions for the staff. The hedgehogs have been housed for 8 wk in the current setup. The running wheels were the most likely to become soiled, followed by the pan papers. We proposed twice weekly cleaning of the wheels and twice weekly changing of the pan paper liners. Crinkle paper is changed weekly. Temperature is kept between 75-80°F (24-27°C), and humidity, <40%. Sanitation of the bottles and bowls is done weekly, and the cages are sent through the cage washer, with all components fully sanitized every 2 wk. We found that 1 hedgehog was unable to understand the use of a sipper tube, and required a water bowl. We recommend placing both types of water sources until it is clear which one the animals are using. This protocol is subject to change as the hedgehog colony continues to stay with us. The hedgehogs have spaces for hiding as well as exercise, and the investigator can easily monitor food and water intake.

#### **P166 Refinement of a Zebrafish (*Taeniopygia guttata*) Enrichment Program Decreases Fighting and Improves Welfare**

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A large, breeding Zebrafish (*Taeniopygia guttata*) colony was newly established at our institution in 2014. At that time, standard housing density, cage set-up, and enrichment program were established based on the current literature and consultation with peers within the field. As the colony continued to grow, it was observed that there was increased aggression despite the established programmatic parameters in place. As space was limited, decreasing cage density was unfortunately not an option. A new enrichment program was devised to try and mitigate the aggression within the colony. The new enrichment program included increasing the frequency and variety of fresh food offerings as well as encouraging foraging via novel toys and/or devices on an established rotational schedule. There was an almost immediate decrease in the number of health reports of fight wounds and/or decreased feather loss in the cages by approximately 62%. The decrease in health issues has continued since implementation, and further refinement of the program is in place to continue to elicit species-specific behaviors and minimize aggressive dominance behaviors.

#### **P167 Veterinary Care Supply Storage Solutions for a Vertical Building**

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Optimizing medical supply procurement and storage organization for veterinary use in a satellite facility can be a daunting task when the primary drug distribution center to obtain treatment supplies are several miles away and where transportation services are limited. There are also additional challenges with efficient measures to supply a vertical facility comprised of animal housing on many floors. To preserve time and prevent frequent trips to the main campus supply facility, and as well to minimize the number of trips between floors within our satellite facility, a system based on lean production was implemented. Lean is a set of operating principles based on waste minimization. In our application of lean, our goals consisted of improving productivity and eliminating non-essential steps to gain efficiency. At the end of our assessment of the problems facing drug supply and storage at our vertical satellite facility, we were able to implement several strategies to help maintain an appropriate inventory as well as to make the inventory accessible and understandable to the varied veterinary care staff that would need to access and use these supplies. Some of the tasks we completed to help us with these goals included developing a reusable master inventory list and in using preprinted labels for aliquots of supplies and to

denote where items should be placed in storage areas through labeling. Through the implementation of this organizational system, we were able to substantially reduce trips to our main supply facility, and improve the overall supply chain organizational system within our vertical satellite facility.

#### **P168 Microenvironmental Findings and Undetectable Changes in Inflammatory Cytokines Support Extended Cage-change Frequency for Rats**

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Currently, the *Guide* recommends solid-bottom caging be changed weekly, but allows for the flexibility to establish facility- and equipment-specific standards. Within our facility, a large percentage of our rat population is singly housed due to scientific need. We therefore sought to establish a performance standard for extended cage change frequency of singleton rats in individually ventilated caging. We hypothesized that microenvironmental conditions would not change significantly over a 1-mo period even without change-out of cage bottom, accessories, or contact bedding. We measured intracage temperature, humidity, carbon dioxide, ammonia levels, and microbiological load (ATP) in addition to performing health assessments and fecal scores (0 to 5 scale that accounted for amount of feces, gross appearance of the cage and amount of moist bedding) for 2 different weight classes of singleton Sprague Dawley rats (n= 5 per group, heavyweight 518 g and lightweight 374 g on average). Intracage temperature and humidity did not differ significantly and there was limited accumulation of CO<sub>2</sub>. Intracage ammonia was not detected until 7 d in the heavyweight group and 14 d in the lightweight group. At end of study (30 d), the heavyweight group averaged 23.0 ppm ammonia and the lightweight group 7.4 ppm ammonia whereas ATP was 248.8 RLU and 157.0 RLU for these groups. End of study average fecal scores did not exceed 4 in either group (heavyweight average 3.6 and lightweight average 3.2) which was consistent with a grossly clean cage with moderate dry fecal accumulation having < ¼ of the cage bedding noticeably moist. Inflammatory cytokine levels were measured in these rats after 30 d without cage change and were all below the limit of detection. Taken together the data support that extending cage change to once monthly for singleton rats, regardless of size, does not compromise their microenvironment or wellbeing as suggested by lack of detectable inflammatory cytokines and observable health issues.

#### **P169 Trash Birds Receive Trash: Enrichment for Wild-Caught Corvids on a Limited Budget**

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Corvids are a species of bird that has been proven to be extremely smart and require a great deal of mental stimulation while in captivity. Although our colony has twice as much space as required per bird, staff have noticed incidences of poor welfare such as feather loss, panting, and other stress indicators. The current budget for the corvid colony is limited, which has driven staff to become more creative which requires great communication. Given these challenges, we hypothesize that we could repurpose existing enrichment items from other species, take donations of household items, and use tracking sheets to manage the required daily edible enrichment items to foster employee communication. Video data taken of the crows showed that they return to a positive state of welfare with reduced vocalization and increased preening within 10 min of staff leaving the room. They readily interact with novel enrichment items within 15 min after cleaning. Due to the difficulty of telling the crows apart, a broad time budget was established for resting, preening, interacting with enrichment, or moving/flying and utilizing a scan sampling analysis at 5 min intervals, we found that indicators of positive welfare (resting,

preening, and interacting with enrichment) were greatly increased after altering the enrichment protocols, as compared to before they were implemented. Use of recycled materials with improved documentation methods for enrichment of corvids is an easily adoptable approach that increases positive welfare in birds.

#### P170 The Rabbit Condo: An Original Solution

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Ethical treatment of laboratory rabbits implies providing them with physical comfort and psychological wellbeing. While the reproduction of their natural conditions of life would contribute to these goals, it is difficult to achieve such a thing in a laboratory setting. In order to find solutions to this challenge, our institution's environmental enrichment committee was mandated to develop a special kind of cage. It should stimulate exercise, normal body postures and foraging, while providing a complex and safe environment. Nonetheless, the solution should not cause major interference in daily room routine and should respect some resource constraints. The committee proposed the rabbit condo, an enrichment system developed from recycled outdated equipment, which presents numerous advantages. The rabbit condo was made in-house and the device allowed animals to exercise, forage and assume normal body postures in a novel and safe space. This new approach was retained as part of the environmental enrichment program in both laboratories in North America. The participation of the animal staff in the search for these solutions gave them a sense of achievement and valued their creativity in improving the rabbits' wellbeing.

#### P171 Conservation of Water Quality Following an Aquatic System Disruption

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When keeping aquatic frogs in a laboratory research setting, many details need to be taken into consideration. Every day, the quality of the water must be tested by measuring components such as the pH of the water, the nitrate levels, and the oxidation-reduction potential (ORP). Testing of the water quality is primarily required for the life support of the frogs, and secondarily for experimental consistency. Therefore, when the entire sump of the circulating frog system in our laboratory was discovered drained, our team jumped into action to avoid a major disruption to the quality of the water. Through the course of a daily husbandry check, a technician discovered large pools of water covering the housing space floor. They then noticed that the sump was nearly empty and that the housing cages were excessively soiled with debris. Upon further review, the technician discovered that an empty cage had been incorrectly docked, causing an excess of water to flood the cage and leak out of the system. The sump was refilled to capacity and a standard dose of marine salt was added. Subsequently, veterinary assistance was sought as the team began to come up with a solution to preserve the water quality and avoid frog casualty. After further review of the issue at hand, a plan was devised: turn off the system, change all mechanical filters, and use a hands-on filtration method to filter the debris out, while retaining as much of the conditioned water as possible. One half of the frog population received a water change using the retained filtered water. The water from the changed soiled frog tanks was coarse filtered and added back to the sump. The other half of the frog population remained in soiled tanks. Tanks housing all frogs were maintained overnight in static conditions, while the system was turned on and the water supply to the empty cages was opened fully to allow residual debris to filter out. The next morning, the system water appeared clear and the mechanical filters were changed an additional time. The frogs were placed back on the

circulating water system and the water quality was checked twice daily for the next week to ensure that it was maintained and the health of the frogs conserved.

#### P172 Effect of High-dose Irradiation on a Commercial High-fat Diet for the Provision of Germ-free Mice

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Germ-free or axenic mice are bred and maintained in environments that contain no detectable microbial life including bacteria, fungi, parasites, and viruses. For sterilization of materials in germ-free production, steam, gamma irradiation, or liquid and gas chemical sterilization processes are utilized. Gamma irradiation is considered a convenient and reliable method of sterilization of medical supplies as well as irregularly shaped particles such as pelleted chow, and is the only acceptable method for sterilization of high-fat diets that would not withstand the heat of autoclaving. FDA regulations allow feed manufacturers to irradiate feed between 10-50 kGy, with an average of approximately 25 kGy. For gnotobiotic and germ-free mice, the FDA has approved exemptions for the use of higher levels of irradiation (35 kGy minimum) in feed for certain research facilities. Inbred C57BL/6Ncr1 (B6N) germ-free mice were fed a high-dose irradiated (minimum 35 kGy) high fat western diet (40% kcal from fat). Within 2-3 wk of feeding the high-dose irradiated diet, animals displayed neurological signs which manifested as rolling in the longitudinal axis when held by the tail and severe ataxia. While necropsy revealed no major findings, further analysis showed major losses of vitamin A (56.12%±5.11 loss) and thiamin (91.41%±2.28 loss) in the diet due to the high level of gamma irradiation that were not present during commercial manufacturing. Clinical signs of animals switched onto a non-high-dose irradiated diet resolved within 24 h. Reformulation and fortification of the high fat diet prevented clinical signs in subsequent feed trials. Appropriate levels of irradiation have not been identified for germ-free work and most irradiator sources can only guarantee a minimum exposure level and not a maximum level. Furthermore, due to the nature of irradiation, careful consideration on size and density of packaging should be considered to mitigate uneven gamma penetrance and exposure that can occur during processing. Careful consideration of possible nutritional changes secondary to autoclaving or high-level gamma irradiation of diet is required when designing germ-free studies to reduce the risk of affecting the health and data collected from animals enrolled in such studies.

#### P173 Systematic Evaluation of Sterilization Procedures for Gnotobiotic Rodent Flexible-film Isolators

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The use of germfree animals such as mice and fish has grown exponentially over the past decade. Standards for the maintenance and care of such sterile environments were outlined in the 1950s, however, there is little evidence-based information on optimal care and operational procedures. One key aspect of gnotobiotic mouse studies is initial sterilization of the environmental enclosure in which animals are kept. Flexible-film isolators are most commonly used for this purpose. The objective of this study was to evaluate specific criteria in choosing the safest, most economical, and efficacious sterilization protocol for flexible-film isolators. To determine which microbes were the most relevant to use for testing, we collected fecal samples from 4 isolators housing gnotobiotic animals that were inadvertently contaminated from unknown sources. Using 16S rRNA PCR and sequencing we identified several different contaminating pathogens, including *Paenibacillus macerans*, *Paenibacillus thermophilus*, *Bacillus licheniformis* and *Micrococcus luteus*. We then tested 6 various products

commonly used to sterilize hospital rooms, kitchen surfaces, and veterinary facilities for activity against the isolated contaminating bacteria by inoculating various sterilant concentrations into bacterial suspension assays. We then performed surface testing inside the isolator by fogging various concentrations of sterilants onto inoculated Luria broth [LB] and minimal media agar plates over various time intervals. The results showed chlorine-based sterilants had the widest margin of safety in respect to the bactericidal and sporicidal activity in vitro, were the most rapid-acting. Based on these studies, we conclude that the chlorine-based sterilant, has a good combination of high sterilization activity, speed, and least corrosive action, but is also the least cost-effective. By comparison, hydrogen peroxide-based sterilants can be effective alternatives, as are generally more cost effective but more corrosive to acrylic surfaces.

#### **P174 Transportation and Monitoring of Anesthetized Sheep to and from a Remotely Located Magnetic Resonance Imaging Facility**

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Magnetic resonance imaging (MRI) is a valuable imaging technique used for research and diagnostic purposes. MRI on large laboratory animals like sheep can present challenges when the MRI facility is located within a hospital setting remote from the animal facility. Safe and effective anesthesia and physiologic monitoring during transport and conduct of the MRI is necessary. Important logistical factors, including the provision of MRI compatible equipment, multi-department collaboration, contingencies for emergencies, communication between veterinary and hospital staff, sensitivity issues traversing hospital corridors, and even mapping the most efficient path to and from the MRI facility was considered. Veterinary staff training on MRI safety and MRI staff training on working with sheep was conducted. Mock test runs with all involved staff using no animals, followed by live test runs using control sheep identified areas for process improvement. Staff gained comfort and confidence with the process during test runs. Over time we identified several technical and veterinary considerations for safe anesthesia and transportation of sheep for MRI procedures. Some examples included: renovating existing animal housing rooms in close proximity to the MRI facility to accommodate sheep and minimize the distance between the two areas; IV propofol anesthesia administration by continuous rate infusion with a syringe infusion pump, and using a compact respiratory ventilator to provide portable ventilation throughout transport and the procedure.

#### **P175 Measuring Ammonia in 2 IVC Systems**

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Many facilities base their cage-change protocols on ammonia levels. The *Guide* recommends changing static open topped cages every week and individually ventilated cages (IVCs) every 2 wk, with considerations granted for the use of wire bottom or perforated-bottom caging. As there are not definitive limits in the *Guide* for ammonia levels at cage change, each facility must determine their own institutional performance standards for intracage ammonia limits. Breeding scenarios are typically high-density scenarios and special considerations are required to develop cage change policies in this area. This study evaluates ammonia levels in breeding cages of C57Bl/6J mice in vendor A's IVC and vendor B's IVC. The vendor A system operates on 60 air changes per hour (ACH) and a 14-d cage change interval. The vendor B system places bedding on a perforated false bottom and introduces the airflow from below the bedding. The vendor B design operates at 30 ACH and a 21-d cage change interval, as permitted via the use of a perforated bottom cage. It was hypothesized that the vendor B design would support a low ammonia

environment throughout an extended cage change in a breeding scenario. Ammonia levels were measured by an electrochemical sensor and by wireless metal-oxide-semiconductor type sensors. The sensors were tuned to record an ammonia value once every 3 h over the course of a 6-mo long study. Cages (n=16) were monitored individually and fresh cages were provided the day after pups were observed in the cage. Average ammonia level on day 14 in a vendor A cage was 47 ppm (n=27) versus 25 ppm in the vendor B cage (n=33). Ammonia on day 21 in the vendor A system was 49 ppm, but comparison was hard to make because these cages had been changed 7 d prior. In the vendor B system on day 21 the ammonia was 49 ppm. Ammonia trends via persistent capture with the wireless system will be compared in each system showing different cage population densities. Overall, the vendor B system was found to be a generally low ammonia environment suitable for mouse breeding on a 21-d cage change schedule.

#### **P176 Pole and Collar Training of *Chlorocebus* Monkeys for Ophthalmic Study Purposes**

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We use pole and collar training for macaques to facilitate handling, especially in combination with chair restraint for dosing or procedures. Animals undergo a period of training to acclimate them to having a pole introduced into their cage and having it clipped to their collar. Animals are then trained to exit the cage for study purposes such as sitting in a restraint chair. Pole and collar training allows technicians to safely handle macaques that are too large for hand-catch restraint and can minimize the use of chemical restraint for handling. This voluntary participation with positive reinforcement may lead to overall stress reduction for the animals. We were faced with the challenge of conducting an ocular study using adult *Chlorocebus* monkeys. These animals were not accustomed to being hand-caught and manually restrained, and many of them were too large to be safely restrained manually. In order to minimize animal stress and the need for chemical restraint, we decided to pole and collar train the *Chlorocebus* monkeys so that we could use restraint chairs for routine study procedures. The pole and collar training process for *Chlorocebus* monkeys was very different than our normal pole and collar training procedure in macaques. Major differences included a longer training period needed for *Chlorocebus* to be considered trained (approximately 30-40% longer versus cynomolgus macaques) and the stoic nature of the *Chlorocebus* compared to macaques. Our trainers noted that that *Chlorocebus* monkeys tended to show little to no facial, vocal, or body cues during the training and responded better in a quiet environment with few distractions during the training. It was also found that the *Chlorocebus* tended to become accustomed to their original trainer, and consistently worked better for that trainer than for other technicians, which is not something we see frequently in cynomolgus macaques. We were ultimately able to successfully use pole and collar handling for these animals on study, which facilitated day to day study activities, as well as veterinary assessment and care. Our experience shows that this technique is applicable to *Chlorocebus* monkeys, but requires the training of personnel to adapt the training procedure and be flexible to the needs of the animals.

#### **P177 Gerbil Enrichment in Laboratory Environments**

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The Mongolian gerbil (*Meriones unguiculatus*), while relatively uncommon as a laboratory animal model, represents an important niche in the overall body of scientific literature including neuroscience and infectious disease research. There is currently a dearth of established standardized methods for administering enrichment in a laboratory environment. Current enrichment standards for other laboratory rodents may be inappropriate for the social, physical, and

mental wellbeing of gerbils in relation to their physiological and psychological health. Current understanding of captive Mongolian gerbil behavior suggests frequent enrichment is beneficial to the welfare of the animal. To determine if there is a correlation between positive behaviors and enrichment, we are evaluating behavior patterns within 3 different enrichment strategies. Gerbils are divided into 3 study groups as follows: 1) baseline enrichment (given once during weekly cage change, consisting of 1 cardboard box or 1 cardboard tube, and 1 dust bath); 2) enhanced enrichment A (given once during weekly cage change, consisting of baseline enrichment plus 1 toilet paper tube stuffed with treats, 1 bundle of nesting material, and 1 wooden block); and 3) enhanced enrichment B (baseline enrichment plus daily addition of either 1 toilet paper tube, treats, 1 bundle of nesting material, or 1 wooden block). Daily behavioral observations are then taken on the 3 different enrichment types using an ethogram of positive, negative, and neutral behaviors. Five behavioral observations of each cage over 15–20 min are used to record the frequency of individual gerbil behaviors. Initial analysis of data strongly suggests that providing enhanced enrichment, particularly with daily application, produces a higher occurrence of positive behaviors. In summary, enrichment amount and frequency may be directly correlated with positive behaviors in captive Mongolian gerbils and should be considered an essential element in shaping future husbandry practices for this species.

#### P178 Sterilization of Nonhuman Primate Handling Gloves

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Leather gloves worn during the handling of nonhuman primates provide a protective barrier against physical trauma and can also prevent the spread of zoonotic disease to the handler. In addition, these gloves can harbor bacteria that can be harmful to the nonhuman primate. In order to prevent the spread of organisms between groups of nonhuman primates, gloves are often assigned to individual rooms or hallways at our facility. Once nonhuman primates are finished with a study or are removed from quarantine, these gloves may be disposed of in order to prevent exposure of organisms to nonhuman primates in other rooms. It has not been established if nonhuman primate handling gloves can be sanitized and remain viable after the decontamination process. To determine if these gloves can be disinfected, ATP (measured in RLU or relative light units) and colony-forming units (CFU) were measured before and after sterilization. Gloves were then sanitized by one of the following methods: chlorine dioxide dip with a water rinse, accelerated hydrogen peroxide dip with a water rinse, chlorine dioxide spray with a water rinse, accelerated hydrogen peroxide spray with no rinse, ethylene oxide, dry heat sterilization, detergent dip with dry heat sterilization, hydrogen peroxide fogger, and ultraviolet light. Testing of CFUs after sterilization revealed that all methods, except chlorine dioxide spray, lead to levels that were considered passing. Passing levels of CFUs include those that were 100 CFUs per mL/100m<sup>2</sup> or less. ATP levels were not acceptable after sterilization with ethylene oxide and hydrogen peroxide fogger decontamination. Passing ATP levels include those that were 100 RLU or less. Any sterilization method that soaked the glove in a liquid left it no longer usable. In conclusion, dry heat sterilization and UV light irradiation gave passing ATP and CFU levels as well as leaving the gloves in working condition.

#### P179 Practical Study of Demand Controlled Ventilation in a Rodents' and Primates' Facility with 50% HVAC Energy Saving

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Since the 8th edition of the *Guide* published, some variable air volume (VAV) systems had been installed in newly built animal facilities. Because the *Guide* recommends VAV systems as, «VAV systems allow

ventilation rates to be set in accordance with heat load and other variables. These systems offer considerable advantages with respect to flexibility and energy conservation.» In our previous study, a multiplexed IAQ sensing device had been used as input device for VAV control. In this study, in order to make installing IAQ sensing device easier, an alternative system which used. Simple CO<sub>2</sub> sensors and an advanced controller was installed. CO<sub>2</sub> sensors which were installed on exhaust ducts of each room and a common supply duct measured CO<sub>2</sub> concentration continuously. As the first step, these CO<sub>2</sub> concentrations were being measured throughout 2 wk under the constant 12 ACH for references which were traditional ACH in this facility. VAV control in accordance with CO<sub>2</sub> differential measurements between supply and room exhaust air was practiced at rodents' and primates' rooms under the real breeding conditions. Three modes correspond to set points (SP) of the VAV control were practiced. In the SP low mode (SP =  $\mu + \sigma$ . Here,  $\mu$  is an average and  $\sigma$  is a standard deviation of the CO<sub>2</sub> differential measurements at the foregoing 12 ACH operation), ventilation rates varied synchronized with animal biorhythm (circadian rhythm) and saved integrated ventilation air by 32.0–38.4%. On the other hand, in the SP high mode (SP =  $\mu + 3\sigma$ ), ventilation rates almost only increased during in-room activity (such as cage changing or room cleaning) and saved integrated ventilation air by 47.3–47.7%. In addition, in order to cancel out potential effects of sensor drift, ventilation rates were increased as scheduled and the parameter which adjusted differential measurements between supply and exhaust CO<sub>2</sub> was revised simultaneously. It confirmed that there were no significant differences in food and water consumption, weight increase, and breeding performance. Results of standard microbiological monitoring were also negative. From the point of view of animal health, both the SP low and SP high modes seemed to be acceptable.

#### P180 Improvement of Equipment Production Processes

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Maintaining adequate equipment is an integral part of any research facility. Within our vivarium, we encountered cage supply issues that resulted in overproduction of unnecessary equipment and inadequate production of necessary equipment. This was observed during a period of time when the facility was transitioning from older to newer caging units. We had 2 different types of caging equipment in the facility paired with an extensive cage change schedule that required our cage wash staff to produce 6–8 different types of caging. Without a defined and simple structure or schedule, the production was not consistent with the demands of the facility. In order to streamline this process, we produced an equipment schedule for our entire facility. We used animal housing room numbers, census, and our bi-weekly cage change rotation to develop a detailed schedule that was based on a 4-wk cycle. From this we were able to determine how much of each type of caging was required and went a step forward in determining how much of each type of bulk truck would need to be produced on a weekly basis. Our animal care and cage wash staff now communicate effectively and work efficiently while avoiding many disparities that were previously hindering the progress of our technicians. With cage wash now having the ability to produce the precise number of bulk trucks required, our technicians have adequate equipment to perform their tasks. Although cage wash equipment failure occurs occasionally, implementation of our schedule has improved and stabilized equipment production.

#### P181 Ergonomic Considerations while Working with Gnotobiotic Flexible Film Isolators

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Working with gnotobiotic mice in flexible film isolators poses some unique challenges to routine husbandry and care for the animals.

Space for husbandry supplies and procedures is significantly more limited and less flexible than what an employee typically experiences in a standard rodent housing room. There are limited positions an employee can work from while wearing the gloves in an isolator, and this can cause additional strain from repetitive movements and lack of posture adjustability while working. Multiple layers of protective gloves worn during mouse handling and restraint can lead to hand fatigue. We have found that many small changes and additions to procedures have led to increased employee comfort and morale. We use platforms and equipment of different sizes and heights within the isolator to elevate cages for cage changing and mouse manipulation. We have identified specific ear notchers and styles of mouse restraint that are easier to use in the context of the isolators. Supply allocation and waste removal are planned well in advance, to ensure that the limited space is used efficiently and to decrease use of the single port of entry and exit in each isolator, further decreasing ergonomic strain. Another important system that we use is the buddy system. Two technicians work together to help remind one another of proper procedures when they are starting to adopt poor posture or technique and thus avoid repetitive motion injuries. To this date, employees have fortunately not sustained any occupational injuries while working with mice in our flexible film isolators for over 10 y.

#### **P182 Improving Efficiency in Handling of Rabbits (*Oryctolagus cuniculus*) by Visibly Posting Individual Animal Temperament**

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As part of our institution's comparative medicine center's regular veterinary care program, animal care staff trims rabbits' nails once per month. The standard method of calming a rabbit during trimming is to cover its eyes. However, staff repeatedly observed that some rabbits reacted to this perceived calming step by fidgeting and pulling away when their eyes are covered. Staff often have to stop to change their restraint, making the nail trimming process less efficient. Therefore a rabbit temperament chart was devised, listing each rabbit in the room, whether it is comfortable with its eyes covered, and its sociability score (from 5, indicating negative behaviors, to 1, indicating positive behaviors). We hypothesize that if staff know in advance how a rabbit is comfortable being handled, the nail trimming procedure will be less stressful and more efficient. A scheduled monthly nail trim was performed on 29 rabbits without the rabbit temperament chart posted. The number of times the staff changed restraint due to animal movement was counted, and the overall procedure time was recorded. At the next monthly nail trimming, the rabbit temperament chart was posted on the wall of the animal room and 23 rabbits had their nails trimmed following standard operating procedures. Handling time and number of restraint changes were recorded again. With the chart posted, the number of times the staff changed restraint dropped from 8 to 0, and the average handling time per rabbit was reduced by an average of 66.92 sec per rabbit ( $P = 0.0009$  by 2-tailed T-test). In conclusion, the animal care staff found that routine handling is faster and less stressful with the temperament chart posted.

#### **P183 Novel Enrichment for Ferrets (*Mustela putorius furo*) Used in Biocontainment Research**

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Ferrets (*Mustela putorius furo*) are a commonly used animal in biomedical research, particularly infectious diseases. The ferret shares many anatomical, metabolic, and physiologic features with humans that make it a valuable animal model. Ferrets are regularly used in respiratory pathogen research studies that utilize the unique biocontainment features at our institution. A challenge of utilizing any animal in infectious disease research is the cleaning, sanitization, and longevity of enrichment devices. Traditional enrichment used for

ferrets include nesting boxes, bowls, pipes or tubes, hammocks, and chew toys. These devices accommodate the ferret's instinct to burrow and nest and are generally reusable. Due to the mischievous nature of the ferret, commercial hammocks are not a fiscal or safe option because they are frequently destroyed and difficult to decontaminate. From these challenges, we have developed a novel enrichment device that encourages the ferret's normal behavior and is fiscally reasonable for frequent replacement and decontamination. The "ferret futon" utilizes items that are easily found in most animal facilities: a standard mouse cage, stockinette material, and zip ties. This enrichment is designed to encourage the ferret to nest, burrow, and hide within the nesting box.

#### **P184 Rederivation of the Domestic Ferret (*Mustela putorius furo*): A Method to Create an SPF Health Status**

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Ferrets are an incredibly valuable model for studying influenza transmission and pathogenesis, as well as new vaccines and treatments. Therefore, there is a need for ferrets free of any influenza exposure. We developed a procedure for surgical rederivation and introduction of ferret litters into a complete barrier environment. The procedure is necessary to introduce ferrets with a desired genetic background into the barrier and ensure they have the required health status. Ferrets with the desired genetic background were time-mated, and pregnant jills were clipped and transported to the procedure space within 24 h of when they were estimated to whelp. The procedure was divided into 4 stations that were designed to decrease the risk of any pathogen transmission during the delivery process. Delivery was performed via hysterectomy and cross-fostering of kits to time-matched jills inside the barrier. Once inside the barrier, the kits, and foster jills, were monitored to ensure acceptance of the new litter and the health of the kits. The specific pathogen-free status of the kits has been monitored via the collection of random blood samples to screen for several pathogens, including screening for the presence of titers for the currently circulating influenza strains. Ferrets have successfully fostered to the new jills, and the kits have remained negative for influenza titers as well as other pathogens. Therefore, this procedure has proven to be a successful method for introducing ferrets into a barrier environment to maintain an SPF health status for studying infectious disease, such as influenza.

#### **P185 Care of Laboratory Ferrets: Special Requirements for Pregnant and Lactating Jills as Well as Neonatal Kits**

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Marshall BioResources, North Rose, NY

Ferrets are a valuable model in many areas of biomedical research, most notably influenza research and neurobiology. Because ferrets are born rather prematurely compared to other species, there is a certain amount of brain development that occurs postnatally, and neonatal ferrets offer a unique ability to study brain development processes that occur in humans and other species while still in utero. This creates a demand for pregnant ferrets as well as ferrets that are only a few days old upon arrival. Pregnant or lactating jills, as well as neonatal kits, require specific care during transport and upon arrival. They must be provided with the appropriate materials to build a nest; and they must be protected from extreme temperatures, loud noises, and distractions. Ferrets are very sensitive to the photoperiod, and a longer photoperiod must be maintained throughout pregnancy, parturition, and lactation. A proper diet is also very important for maintaining the health of the jill and the kits. This poster will review these unique requirements for the care of pregnant and lactating jills, as well as neonatal ferret kits, and will provide some recommendations to maintain the health of these animals within the laboratory.

**P186 Search for Definitive Senescence Biomarkers in Naturally Aged Mice**

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Our facility has kept many naturally aged animals (mice and rats) used for gerontology and geriatric researches. When a scientist conducts research using these animals, it is important to consider the effects of various genetic and environmental factors on them. However, a clear criterion for defining aged (old) mice has not been established. We report various age-related characteristics observed in our mice. Male and female C57BL/6NCrSlc mice (4 wk) were purchased from a vendor every 3 mo and kept over their lifetime. Physiological (measurement of body weight, survival rates, and urinary corticosterone), behavioral (the rotarod tests and the grip strength tests), and morphological (autopsy, MRI, and histological examination) analyses and blood test (WBC, RBC, HGB, HCT) were performed. Body weight peaks at 18-20 mo (approximately male 46.0g, female 35.0g) and only male mice show rapid decrease at around 25 mo. Survival rates start to decline at approximately 21 mo and are somewhat lower in female aged mice. Urinary corticosterone levels are relatively higher in female mice and tend to increase with age. Rotarod performance peaks at 3 mo in male (208±61.4 sec) and at 6 mo in female (235.3±75.1 sec) mice and then continues to decline. Rotarod performance and grip strength of aged animals show relatively low score. Enlarged seminal vesicles or splenic tumors were often found at autopsy. Blood test shows that the total WBC count starts to decline at around 18 mo and the composition of WBC tends to change with aging. Various age-related changes (such as body weight, motor performance, the total WBC count, and its composition) found in our aged B6N mice can be candidate senescence indicators at an individual level. We will investigate these parameters in detail and continue to search for novel biomarkers (such as fecal IgA levels).

**P187 An Approach to Internal Sustainability by Means of Recycling and Reuse in a Laboratory Animal Care Facility**

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Research animal resources currently reduce significant amounts of waste from landfills through efforts to compost soiled animal bedding, recycle cardboard wastes, and process carcasses via the institution's tissue digester. However, efforts to reduce recyclable waste were lacking within animal rooms, where all paper, cardboard, and plastic wastes were being discarded in the trash instead of being recycled. A project was conducted by several animal husbandry staff to decrease laboratory wastes and implement recycling within animal rooms. In order to remain cost-effective, unused standard mouse caging (7.75" w x 12" d x 6.5" h) were gathered, labeled, and distributed to SPF animal rooms for use as recycling bins. Additionally, commonly used flagging cards for mouse husbandry were laminated and could, therefore, be marked with wet-erase markers and reused instead of thrown away after completion. A trial run for the recycle bins was conducted in 2 animal rooms before expansion into all animal rooms. At first, recycle bins were seldom full within a 2-wk period. When research and veterinary staff were notified of the recycling bins and what could be recycled, bins quickly became full within a 1-wk period. Similarly, laminated cards were implemented in a single animal room for a trial period of 1 mo. Research staff were notified of the change and instructed on how to properly use and care for the laminated cards. Follow up discussions with both research and animal care staff yielded positive opinions or preference to the new laminated cards. After observing a success in reducing waste in the trial rooms, recycle bins

were expanded to all SPF animal rooms, and laminated cards were expanded to 5 animal rooms. Across 20 animal rooms, over 70% of recycle bins are full weekly. This project successfully reduced waste within animal rooms in a simple, cost-effective manner which could be easily implemented within other facilities.

**P188 Effect of Sanitation Frequency on Microbial Burden of Cage Lids and Feeders in Static versus Ventilated Mouse Cages**

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The frequency of sanitation of cage components (such as feeders, cage lids, and watering devices) has a direct impact on animals as well as workload of husbandry technicians. The *Guide* recommends that enclosures and accessories such as tops and feeders be sanitized at least once every 2 wk, but recognizes that decreased frequency may be justified as long as microenvironmental conditions are not compromised. The objective of this study was to determine if sanitation frequency of cage lids and feeders for both static and ventilated microisolator cages could be extended beyond the 2-wk recommendation without adversely impacting animal welfare or the microenvironment. A total of 10 cages (5 mice per cage) were used; 5 cages were housed on a ventilated rack and the other 5 in static cages. Cages were monitored weekly for 6 wk, using ATP luminescence and environmental monitoring plates to quantify organic material and microbial burden, respectively. The study was then repeated by switching the mice into the alternate housing type in the same rooms; final results were combined for analysis. Mice were weighed at the beginning and at 7 wk and monitored during cage changes for general wellbeing. After week 4, due to the level of growth on environmental monitoring plates and high relative light units (RLU) by ATP measurement on static cage components, testing on these cages was discontinued. In contrast, RLU and microbial growth on environmental monitoring plates for ventilated cage components remained within acceptable limits for 6 wk. Average colony counts for ventilated cages were  $\leq 4$  for lids and  $\leq 8$  for feeders at all time points. RLU values varied unexpectedly from week to week; higher values 1 wk did not predict similarly high values the next. All mice gained weight (mean = 27%) and no health problems were observed. This study demonstrated a significant difference between static and ventilated cages, despite all cage lids and feeders looking visibly clean. We concluded that static cage feeders should be changed every 2 wk, while lids on static cages and both feeders and lids on ventilated cages can be changed once every 4-6 wk without adverse impact on animal health and with minimal increased microbial counts.

**P189 Staff Attitudes on Mouse Welfare and Ease of Use of a Simple Restraint Tool for Murine Nail Trims**

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Ulcerative dermatitis is a common, frustrating disease to treat in mice, but trimming the rear toenails has been found to be an effective treatment. Many ulcerative dermatitis lesions are located on the nape of the neck, making it difficult to scruff the mouse for restraint without affecting lesioned tissue. We hypothesized that the use of a 50 mL conical tube with a breathing hole as a restrainer will increase comfort for both mouse and operator. Tube restrainers for trimming mouse toenails have been described, but not the staff attitudes regarding their use, which is critical to effective implementation. We also developed a novel method to guide mice into the restraint tube. Twenty-one participants (2 students, 5 husbandry staff, 8 technicians, 6 veterinarians) were asked to trim rear nails on healthy, adult, C57Bl/6 background mice, using both scruffing and tube restraint methods in a randomized order. Participants then filled out an anonymous survey addressing ease of use and perceived mouse welfare for each method



using visual analog scales. Restraint using the tube method was not more difficult than scruffing, and nail trims were significantly easier with the tube method. Participants felt that the tube restrainer was significantly more comfortable for the mouse, especially in the presence of ulcerated lesions. Additionally, participants' stress levels were significantly decreased by approximately half. Importantly, all participants reported they would use this method again, while half indicated they would only use the scruffing method if they had no choice. Due to the positive response at our institution, we have implemented the tube restrainers in our rodent facilities. Using a 50 mL conical as a mouse restrainer for nail trims is practical, cost-effective, and easy to construct. This method is easy for staff to perform and appears to improve welfare for mice with ulcerative dermatitis.

#### **P190 Simplified Cesarean Rederivation Procedure to Generate Germ-free Mice Using Isolation Cages in Lieu of Isolators**

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The use of gnotobiotic animals in biomedical research has had a major resurgence over the past decade as the impact of the microbiome on many aspects of human health has become increasingly apparent. Much of the work is being done in commonly used inbred strains, such as the C57BL/6 mouse, which is commercially available in the germ-free state from multiple vendors. However, to address questions concerning the interactions of the microbiome with a specific genotype, gnotobiotic genetically modified mouse models are needed. Due to the large number and demand for these models, we needed to establish a rapid, efficient, and cost-effective method to rederive mice into the germ-free state. Traditionally research facilities have used Cesarean rederivation in which the gravid uterus is removed and passed into an isolator housing a germ-free foster dam. However, isolators are costly to maintain and the procedure is cumbersome and requires multiple staff. Rederivation isolators need to be maintained for several weeks until the microbiological status of the rederived mice has been verified, limiting throughput. Moreover, if contamination occurs, the entire isolator must be rebuilt resulting in additional cost and loss of time. Our modified rederivation procedure is done entirely inside a horizontal laminar flow hood. It is fast, straightforward, and can be performed in its entirety by a single operator. The progeny is then housed with their foster dam in an individually ventilated isolation cage. We have shown that this procedure can be performed successfully without contamination and yields germ-free mice, which after microbiological testing, can be transferred to an isolator for colony expansion. An additional advantage is that in the case of contamination during the rederivation only a single cage is affected.

#### **P191 Caging Survey: Meeting the Needs of Nonhuman Primates and Laboratory Professionals**

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Nonhuman primate caging continues to evolve with the times, and leading cage manufacturers now offer a wide variety of caging options. Balancing the physical, behavioral, and social needs of the animals with the safety, ergonomic, and accessibility needs of animal care and research staff can be a tall order. In addition, the ease and expense of maintenance and repair needs to be taken into account. At our research center, we created a survey to evaluate our current caging in an effort to better understand the various needs of both the primates and the employees. Forty-one employees from across 5 facilities participated, including colony management, veterinary, husbandry, facilities, behavioral management, nursery, research support, and research staff. We evaluated 4 cage sizes (groups 3, 4, 5, 6) across 4 caging types (A to

D). Pros and cons per cage type were solicited and each respondent ranked the types in order of preference. With its larger doors, fully-opening cage front, and versatile connectivity options, cage type B ranked highest (41%), particularly among researchers (45%), research support (50%) as well as other staff (nursery, facilities; 67%). Cage type A, the only 4-pack design, ranked last overall (44%), particularly amongst husbandry (58%), due to its larger size and weight, fewer social contact options, and smaller, awkwardly placed door. Though type D received negative feedback for being outdated, when rated on a scale of 1 (easy) to 3 (difficult), it rated highest in maneuverability and ease of repair. In addition, 33% of husbandry and behavioral management staff ranked type D as preferred, partially because it offers the ability to socialize adult male and female pairs without the risk of pregnancy. These survey results are useful for cage manufacturers and for facilities looking to optimize their future caging purchases.

#### **P192 Successful Transition to Researcher DEA Registration for Scheduled Drugs at a Large Academic Institution**

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Our institution recently underwent a change in the method scheduled drugs are obtained by animal researchers for approved IACUC studies. In the past, scheduled drugs for analgesia and anesthesia were provided to researchers through the animal program. In order to better serve the growing needs of the institution, while at the same time meeting regulatory compliance requirements, it was determined animal researchers would acquire and maintain independent federal Drug Enforcement Administration (DEA) registrations specific to their research needs. A team comprised of representatives of the animal program, state and federal drug enforcement officials, campus Environmental Health and Safety (EHS), and institutional leadership were brought together to outline a plan for the change and begin implementation. Goals for the transition included minimizing disruption to researchers, establishing working timelines, providing support and training resources, effectively communicating the steps to be taken to impacted groups, and following up with researchers to assure completion. The planning and preparation stages took approximately 8 mo and included the initial messaging to researchers about upcoming changes. While not all researchers applied for DEA registration, 91 out of 126 previously active in animal controlled substance administration did. The collective period to complete the process for new DEA applicants from the time of application was about 12 mo and included the time to carry out in-person training on ordering, record-keeping, and disposal. Given the complexity for researchers and their staff, the challenges from having a large campus with many sites to inspect, and the need to carry out multiple types of training, we felt this timeframe worked well. Though researcher support will be ongoing, we met the goals we set out to achieve. By using a team approach and involving major stakeholders, we were able to prevent disruptions to ongoing animal research while transitioning to a model based on individual DEA researcher registration.

#### **P193 Evaluation of Options for Rodent Health Surveillance in Ventilated Cages and Conventional Housing**

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Health surveillance in rodent facilities typically uses live sentinel animals euthanized at periodic intervals for serology, parasitology, and necropsy evaluation. Recent literature has suggested the number of animals needed could be reduced by PCR testing filters or fecal and fur swabs from colony animals instead of sacrificing sentinels. To evaluate this in our facilities, we compared PCR testing of filter tops from

sentinel cages (SPF), room exhaust filters (conventional), and random fecal and fur swabs from colony animals to results obtained from sentinel sacrifice. Our hypothesis was that the alternative testing would provide equivalent results to sentinels. Two mouse rooms and 1 rat room with commercially available positive pressure IVC cages with rack filtered incoming air and exhaust through the cage top filter to the plenum were evaluated. Six rack sides were evaluated in each room. The sentinel cage filter top was marked and not changed for 8 wk. After 8 wk a 1 x 1-in piece of each filter was submitted for PCR testing. Fecal and fur swabs were also taken from colony animals by sampling animals in 1 cage/row. Samples from each rack side were pooled 10 samples/test. In conventional rooms, sections of room exhaust filters were also submitted for PCR. Serology and PCR were sent to a commercial laboratory, with in-house necropsy and parasitology for sentinels. SPF mice historically were positive for mouse norovirus and *Helicobacter*. Conventional mice were positive for these plus mouse hepatitis virus, mouse parvovirus, epizootic diarrhea of infant mice, pinworms, and fur mites. SPF rats were positive for rat theilovirus (RTV) and *Helicobacter*. Our results showed that filters consistently picked up *Helicobacter*, but not mouse norovirus (MNV) or RTV. However, the results of direct colony sampling for mice matched the sentinel results and did not pick up any additional agents. For rats, the direct samples were negative for agents found by sentinels. We thus concluded that by holding mouse sentinels for longer intervals and adding direct random colony sampling we could reduce animal numbers while not compromising our ability to detect rodent pathogens. Further evaluation may be needed for rats.

#### P194 An Approach to Occupational Incident Analysis

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The occupational health and safety program (OHSP) at an institution is an essential component of its animal care and use program. To maintain an effective OHSP, a thorough evaluation of occupational incidents is critical to identify issues, guide appropriate interventions, and measure the corresponding response. Herein we describe a system developed to quantifiably evaluate occupational health-related injuries and illnesses. This system compiles reports submitted by personnel, which are then investigated and categorized according to the relevant occupational health concern: allergy, ergonomic injury, other physical injury, sharp injury, injury by animal, burn/chemical injury, and other. Data are further subdivided into laboratory personnel and animal care staff and reported as a proportion of total incidents. Trends are assessed through comparison of incidents over the course of the year as well as in relation to the previous year. Using this evaluation system, the top 3 categories comprising 66% of total incidents reported at our institution were other physical injuries, injury by animal, and ergonomic injury. Animal care staff injuries were primarily physical in nature, while injuries to laboratory personnel were mainly allergies or injuries by the animals. There were no substantial trends observed over the course of the year. These results highlight the different occupational health concerns that can be identified when taking an in-depth approach to incident analysis, which can then be utilized to justify the appropriate allocation of resources to implement a robust OHSP.

#### P195 Comparing Efficacy of Antimicrobial Hand Wipes and Registered Disinfectants in the Surgical Suite

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Surgical suites in laboratory animal science facilities are used for both

survival and nonsurvival surgery. Various animal species may be presented during a day. Fast, efficient table disinfection is required for a suite being recycled between procedures. Using ATP technology to evaluate the effectiveness of disinfectants, the authors compared prepared concentrations of potassium peroxydisulfate, chlorine dioxide bleach, quaternary ammonium, a commercially prepared sanitation wipe, and a commercially available antimicrobial surface wipe. Each trial consisted of starting with a clean table, exposing each section to the soil, and then measuring ATP. Each section of the table was then cleaned with the corresponding disinfectant and ATP measured again. This was repeated up to 3 times or when an ATP measurement of under 5000 RLU (relative light units) was reached (ATP measurements of 0 ideal, indicating complete disinfection). The unit for measurement was a commercially available luminometer with a commercially available swab. Results indicated that disinfecting soiled surgical tables required more cleaning before adequate disinfection could be performed. While there are many things to consider when it comes to choosing the right disinfectant for a facility, such as contact time, corrosivity, and solution preparation, the best performers in our study were bleach and 2 commercially available products.

#### P196 The Pigsicle: An Adaptable, Swine Enrichment Device for High Biocontainment Facilities

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Providing enrichment to research animals enhances their mental and physical health and leads to better scientific results. Many types of enrichment require careful attention to decontamination processes, which create difficulties in a high biocontainment facility. Creative enrichment items that encourage species-specific behaviors of swine (foraging and rooting) are limited. Toys, foraging balls, and hiding treats in substrates (such as hay) can be used, but do not promote a long interaction time and complicate waste removal processes from animal rooms. Our facility houses large swine that devour edible enrichment and damage conventional toys quickly. Pigs interact with our layered, frozen device comprised of various treats and a flavored oral rehydrator, the pigsicle, by pushing, carrying, gnawing, chasing (as it rolls away), and licking the melting trail. Depending on the size of the pig and group, it lasts between 1 and 4 h. The swine become visibly and audibly excited when we introduce our new pigsicle to their environment. This device has decreased competition within a group in comparison to other enrichment, because it disperses edible enrichment over a large area as it is manipulated. This enrichment device creates no waste. The pigsicle is highly customizable, in regards to shape, size, and incorporation of flavors and types of treats. It promotes species-specific behaviors, increases interaction time, produces no waste, and allows for variation when offered repeatedly. The pigsicle is a layered, frozen device comprised of various treats and a flavored oral rehydrator.

#### P197 Comparison of 1/4 and 1/8 Inch Corn Cob Bedding: Effects of Ammonia, Behavior, and Respiratory Pathology in Mice and Rats

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Rodents housed on automated water racks are at risk of flooding from the water valve. The 1/4 in corncob bedding has been associated with a reduced incidence of flooding compared to the 1/8 in both at our and other institutions. In this study, we compared the effects of these 2 corncob bedding sizes on cage ammonia levels, behavior, and respiratory pathology in mice and rats to better understand the potential implications of using the 1/4 in instead of 1/8 in bedding throughout our facility. We hypothesized that the beddings would not significantly differ in their effects on these parameters. Two strains of

male mice (C57BL/6 and 129S1/SvIm; 5 mice per cage) and two stocks of male rats (Sprague-Dawley and Long Evans; 2 rats per cage) were housed in static, filter top cages with 1 of the 2 bedding types for the duration of the study (12 wk). Intracage ammonia was measured during 1 wk of the study on days 0, 3, 5, and 7. Behavior was evaluated with circadian rhythm, Morris water maze, and open field tests. Animals were euthanized with injectable euthanasia solution and respiratory and ocular tissues were collected for histopathological lesion scoring, with animals euthanized immediately upon arrival from the vendor serving as negative controls. Behavior and ammonia levels did not differ significantly between bedding sizes for either rats or mice. Average intracage ammonia levels (d 7) for 1/4 in bedding were 575ppm in rat cages and 525ppm in mouse cages. For 1/8 in bedding, average ammonia levels were 580ppm in rat cages and 533ppm in mouse cages. The two bedding sizes also did not result in significantly different lesion scores in the eyes, nose, trachea, and lungs for mice. The 1/4 in bedding was associated with a significant increase in the severity and frequency of lesions within the nose of rats, but not in the eyes, trachea or lungs. In summary, the 1/4 in bedding has comparable effects as 1/8 in. on ammonia levels and behavior for rats and mice. Although the 1/4 in bedding does not increase the risk of respiratory pathology in mice, the larger bedding might be associated with greater damage to the nasal mucosa in rats and should be used with more caution in that species.

#### **P198 Developing an RFID Rack Tracking System at a Large Academic Institution**

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Keeping track of individually ventilated rack (IVC) locations in rodent facilities can be challenging. Movement of IVC racks occurs for change out during biannual sanitation, for consolidation or expansion of housing capacity, for equipment maintenance, or for animal health reasons associated with surveillance testing and restricted pathogens. Historically, tracking of IVC racks and live sentinel cages was done using a database log, visual checking of rack location on a weekly basis, and relying on animal husbandry staff for updates. Our sentinel process had focused on the use of live animals previously, however, our new racks allowed for the use of plenum testing, and a decision was made to implement this process. As a result, tracking of the IVC plenum filter and rack became critical to the success of our sentinel testing. Our institution has been using an RFID census system for over 6 y, however, the vendor did not have an inventory control component to the system. Our team therefore developed a novel solution for tracking our rodent IVC racks. An RFID cage card-holder was attached to each rack and was used to correspond with the rack serial number. A sham protocol was entered into the system to allow for submission of a sham animal order to generate barcode numbers. The barcode is used by the system to identify individual census units in that database. The RFID is associated with the barcode for each IVC rack, and the information automatically uploaded into the database. The database is accessible from any workstation, and provides a room location for each rack at the time of the weekly census activity. The database is checked weekly by the quality assurance (QA) staff and compared to the prior week to identify any racks that may have been moved without notification of the QA staff. The implementation of the RFID rack tracking system has made it easier to verify the location of racks in our facilities and know when racks have been relocated, thereby making the IVC plenum sentinel testing more effective.

#### **P199 Validating the Effectiveness of Contamination Control Floor Mats for Use in Rodent Vivaria**

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Many methods are used to protect facility floors from outside debris. A possible new approach is that of contamination control polymer floor mats. Through silver impregnation on the surface of the mats, particles are locked in place and bacterial growth is inhibited, preventing unwanted entry and spread from footwear. We sought to test these as a potential improvement over traditional PPE and spraying of casters. We installed mats in 2 different rodent facilities for assessment of multiple data points. There was heavy foot traffic in our larger facility versus the smaller facility (which also had carpeted halls leading to the vivarium). The most effective placement of the mat was near the entryway to the vivarium directly over existing floors, preferably near the PPE gowning area. Recommended mat sizing required 3 steps to allow sufficient contact with the mat to strip off contaminants. Cleaning consisted of mopping with a disinfectant, then removal by a squeegee. To properly test the efficiency of the mat, we tested for adenosine tri-phosphates (ATP) using a luminometer. The mats were highly efficient at capturing organic debris but required a balanced cleaning frequency to keep them effective. An 11-fold higher level of organic debris (404 RLU) was measured on floors before the mats, while a significant reduction in ATP (38 RLU) was seen on floors directly after walking across the mats. The larger facility mat required cleaning every day, however, the smaller facility mat was functional with cleaning 3 times/week. The overall effectiveness and maintenance of these polymer mats depended on several variables including traffic patterns, climate, and placement within the facility. Contamination control floor mats proved to be an effective innovation to help manage floor cleanliness. The contamination control mats were easily installed within a couple hours, have a durability of 3-5 y, and can immediately replace the need for shoe covers. We were able to maintain floor cleanliness while avoiding cross-contamination and potential slip and fall concerns associated with shoe covers, improved investigator compliance, minimized the environmental waste impact, and saved over \$5,600 annually, providing an ROI of about 4.5 y.

#### **P200 Daily Rodent Cage Monitoring: The Effects of Flashlight Choice on Animal Awareness and Operational Success**

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The comparative medicine center at our institution operates using a lean management approach where all staff applies the concept of continuous improvement to their daily work. Opportunity cards are filled out when staff finds problems or setbacks in their daily work flow that they feel can be improved upon. We identified that our rodent cage check flashlights were operationally deficient—making daily cage checks more difficult and increasing the amount of time required of technicians to perform quality checks. In response, an improved, lightweight flashlight was purchased at half the cost of the current flashlight. The new flashlight was selected with veterinary guidance and then tested on mice representing 4 different strains. Four cages of each strain were evaluated over a 4-d period. Data was compiled documenting: 1) animal awareness (AA) or how reactive the animals are to the flashlight type, 2) cage check time, and 3) number of health concerns found. Despite being brighter than the original flashlight, the new flashlight failed to produce a significant statistical change to AA. When cage check time was analyzed, the time it took husbandry staff to check one cage went from 3.06 to 2.25 s, a reduction of 0.81 s. With a facility that holds ~29,000 cages, daily checks using the new procedure could project savings of 6.5 h/d. In addition to the reduction in cage check time and cost as well as the minimal increase in AA, animal health alert records data show the new flashlight allowed technicians to find 11% more health concerns—supporting our mission to reduce pain and distress in laboratory animals and provide more reliable and responsive laboratory animal care using a lean approach. The results showed a significant reduction in waste without sacrificing cage check quality.

**P201 Visual Task Completion Records Using a Magnetic Schedule Board, Laminated Log Sheets, and Photos For Electronic Archives**

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In an ongoing effort to eliminate the use and storage of paper records, we are implementing visual task completion records. We started with more common areas that require frequent oversight to ensure that each task is completed per SOP. Daily, weekly, quarterly, and bi-annual schedule lists are posted. These color-coded lists allow staff members to have a clear knowledge of the tasks and the expected completion schedule. Husbandry tasks are tracked on a scheduling board and laminated logs are used for cage wash. The scheduling board is a 1wk magnetic and dry erase grid. Each staff member has a line for noting completion of assignments and use color coordinated magnets to indicate completion of tasks throughout the day. Directly next to this board is a time and date clock. This at-a-glance layout allows supervisors to quickly make adjustments to the schedule. For cage wash, each area has a large, laminated monthly log sheet. The staff member uses a dry erase marker to initial each task on the day the specific task was completed. For record keeping, photos are taken at the end of the week for the schedule board and the end of the month for the cage wash logs. The photos are electronically filed in a common use folder. The archived photos remove the need for keeping paper records and allow access to all members of the department for review. The visual nature of these records is providing staff members a self-accountability while ensuring that all tasks are completed as assigned.

**P202 Nutritional Management of Mature Guinea Pigs: What Are You Feeding?**

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Nutritional requirements vary between adult, mature guinea pigs and growing, pregnant, or lactating animals. Adults are prone to diet-associated clinical abnormalities including urolithiasis and ectopic tissue mineralization. Currently available pelleted laboratory diets are alfalfa-based, which is higher in calcium and protein compared to commercial diets for pet guinea pigs, which are Timothy hay-based. Alfalfa is associated with urolithogenesis in adults due to a high macromineral profile. This pilot study sought to determine whether the removal of the alfalfa-based diet in mature animals was able to improve body condition and decrease blood and urine macromineral levels. Three 4-year-old experimentally naive female Hartley guinea pigs, which had been previously maintained on an ad-libitum alfalfa-based pelleted diet supplemented with Timothy hay, were provided a diet consisting solely of Timothy hay and a daily vitamin C supplement. Prior to the diet change, weights, serum and urine chemistries, as well as full body radiographs and gross photographs were collected. Animals were weighed on a weekly basis with urine chemistries collected at 8 wk and 12 wk. Serum chemistries, radiographs, and gross photographs were again collected at the end of the 12-wk study period. All 3 animals demonstrated an initial weight reduction and a significant improvement in body condition that was maintained over the course of the diet trial. Blood and urine macromineral levels including calcium:phosphorus ratios as well as urine protein:creatinine ratios were reduced in all animals over the duration of the study period. These results suggest that mature guinea pigs may benefit from a lower macromineral and less energy-dense diet made available from standard research diet manufacturers. Additional larger-scale studies are required to fully characterize the clinical significance of diets with reduced macromineral and protein content.

**P203 Optimizing Colony Production in CNTNAP2 Mice by Reducing Microenvironment Disturbances**

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Cntnap2 knockout mice exhibit hyperactivity, social and communication behavioral impairment, spontaneous seizures, abnormal cortical neuron migration, and abnormal neural synchrony. They may be useful in studies related to cortical dysplasia-focal epilepsy syndrome and autism spectrum disorders. Colony management services provide a comprehensive set of specialized services related to breeding and line maintenance for research purposes. When presented with a nonproductive breeding colony of CNTNAP2 knockout mice, a troubleshooting checklist was implemented to achieve a strategic plan for colony maintenance and revitalization. This analysis included reproductive criteria (litter size at birth, litter size at weaning, pup morbidity) as well as cage location. These results were collected from 14 CNTNAP2 breeder cages each containing a monogamous pair. They were determined to be in good health and of the appropriate age of sexual maturity. Crinkle paper was introduced into each breeder cage to redirect displaced energy to discourage cannibalization. This increased the rate of production but had little effect on the dam's nurturing behavior postpartum to maintain pup viability. The breeder cages were then moved from the top row of the housing rack to the bottom row. Complete cessation of production was observed. The housing rack was located at front of the room, exposed to microenvironment disturbances such as foot traffic, noise, and light variances. After 2 failed breeding cycles, the breeder cages were relocated to the top row of a different housing rack located on the opposite side of the room the entrance. This location provided fewer disturbances. The change of the housing rack and position resulted in a decreased mating to parturition time from 32-44 d to 21-28 d. Litter sizes increased from 3-5 pups to 6-13 pups. Overall viability increased from 0-2 to 6-11 pups at weaning age. The results suggest that a more secluded cage location appeals to the behavioral impairments of this strain. When placed on a rack away from foot traffic and noise pollution, the colony thrived resulting in efficient breeding and pup viability. This concludes that the cage and rack location within the animal housing room has a direct impact on the breeding success of a CNTNAP2 knockout colony.

**P204 Chemical Control Measures for Husbandry Chemicals at an Animal Research Facility**T Fountain<sup>1</sup>, R Fest<sup>2</sup>, M Thompson<sup>1</sup><sup>1</sup>Environmental Health and Safety, Yerkes National Primate Research Center, Atlanta, GA; <sup>2</sup>Animal Care, Yerkes National Primate Research Center, Atlanta, GA

Animal research facilities use a multitude of chemical detergents that are necessary for daily husbandry activities. To reduce costs, these detergents are generally maintained in bulk quantities with individual container sizes ranging from 55 gal drums to 275 gal totes. Many environmental health and safety challenges were encountered at our center with these large chemical containers. Due to indoor space limitations, the bulk chemical containers were primarily stored outdoors on secondary containment berms in multiple locations. While the majority of containers were partially protected from weather elements, inadequate equipment design led to incidental drips, occasional spills, and rainwater that filled the containment berms over time. In 1 particular location, this culminated in the detergent saturating the asphalt and bubbling into a nearby storm drain to the local creek during a heavy rainstorm. Immediate changes were addressed; however, it is ultimately important to address the root cause of the problem. In order to accomplish this, stakeholders were identified and an interdepartmental team was formed to collaboratively address and improve control measures for chemical storage and usage. The team was represented by management from facilities, animal care, and the center's environmental health and safety

office. The work process for use of these chemicals was evaluated and possible solutions were devised. The team completed a risk assessment and cost analysis to present the current and proposed changes to the center's administration in a request for additional funding. The request was approved. While the changes are still being implemented, there have been significantly less drips and spills. Our success is possible through the commitment of all the involved stakeholders addressing the root cause as a team, involving daily husbandry technicians, and presenting a comprehensive proposal that ultimately benefits the entire center.

#### **P205 Guide Recommended Space Provides Inadequate Intracage Environment for Guinea Pigs Housed on 3 Different Bedding Types**

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The *Guide* recommends a minimum cage space of 101 in<sup>2</sup> for guinea pigs weighing  $\geq 350$ g. Little additional regulatory guidance is available to define ideal housing parameters for guinea pigs. To determine ideal housing conditions, we assessed 3 different bedding types for pair-housed guinea pigs in 212in<sup>2</sup> static micro-isolator cages. The bedding assessed included paper bedding, cellulose bedding, and corncob bedding. We hypothesized that cellulose bedding would be ideal for guinea pigs because it is soft and highly absorbent. Twelve pair-housed animals were housed 2 per cage for a total of 6 cages. Two cages were provided each type of bedding for a period of 1 wk. The bedding type was rotated at the end of each week so that all animals were housed on each type of bedding. Once per week, each guinea pig was weighed and a brief physical examination performed. Intracage environmental parameters including ammonia (NH<sub>3</sub>) and carbon dioxide (CO<sub>2</sub>) were measured 5 d per week and intracage temperature and humidity were measured daily. Cage wetness was assessed daily and cages were changed if more than 1/4 of the bedding was wet. Cages were unable to reach a standard 1 wk change interval with any bedding tested due to rapid increases in NH<sub>3</sub> and wetness. For all bedding types, acceptable NH<sub>3</sub> or wetness thresholds were exceeded by 2–5 d. Some animals showed transient plantar redness indicative of foot irritation, though animals on paper bedding were the least affected. In conclusion, 101in<sup>2</sup> per animal may not be enough space to adequately meet performance standards in static micro-isolator cages for pair-housed guinea pigs, regardless of bedding type. Additional studies are necessary to determine the ideal bedding type in other cage setups.

#### **P206 The Quatranasia: A Multi-Chamber Euthanasia Unit**

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In vivo rodent biomedical studies often rely on data collected post-mortem. Euthanasia may be part of the experimental design or may be a necessary step to relieve an animal's distress, requiring humane euthanasia practices. Here at our institution, we developed an apparatus, the Quatranasia, a multichamber tabletop euthanasia unit to allow for a maximum of 20 mice to be euthanized simultaneously through a gas overdose. The ability to euthanize mice from separate cages at the same time without combining them into 1 chamber, which is discouraged, or one-by-one as with a standard euthanasia chamber, prevents unnecessary distress to the animals and decreases personnel time spent performing the euthanasia. The reduced euthanasia time minimizes emotional stress for personnel and is useful for time-sensitive studies. A lightweight, clear plastic design allows the Quatranasia to be placed on any flat surface and stored if not in use. It can easily be used in areas where larger, more expensive, high-volume euthanasia systems are not warranted. The Quatranasia connects via hose to a tank or wall unit of CO<sub>2</sub> and uses a flow rate of 4 L/min to fill all 4 chambers simultaneously while providing a displacement rate of 10% to 30% of the chamber volume per minute. A hinged lid left

open between cycles permits gas to dissipate from the chambers and cleaning excrement and odors from the chambers after a cycle expedites gas dispersal. The simple design and lack of programming make the Quatranasia accessible and easy to use. The Quatranasia provides a means of increasing the efficiency of low volume euthanasia in laboratories and vivaria without deviating from the *AVMA Guidelines for the Euthanasia of Animals*.

#### **P207 Use of a Centralized Transgenic Mouse Breeding Colony to Maximize Breeding Productivity and Operational Efficiency**

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Large-scale research programs dependent upon the use of transgenic animals, especially those necessitating advanced breeding strategies, must maximize breeding output to meet scientific goals while adhering to the ethical and financial mandate of minimizing waste. One successful approach to balance these potentially opposing aims is the use of a dedicated breeding colony, with expert colony managers who utilize current best practices and technologies. The responsibility for managing >400 active transgenic lines, containing targeted genetic modifications at up to four independent loci, lies with the transgenic colony management (TCM) team. This team effectively interfaces with the other vivarium teams, including the husbandry staff and veterinary technicians, as well as teams outside the vivarium including the molecular biology team (for genotype analysis) and the researchers (end users), including the transgenic technology team. The TCM team uses up-to-date principles of colony management and electronic (software) tools to process breeding requests, devise breeding strategies, schedule tasks (such as paw tattooing, tail biopsies, ear notching, and weaning), log genotype information, track breeder productivity, and notify end users when young adult animals are available for use. The use of approved work instructions ensures consistency across the team. Two tools have been effective at minimizing the number of unused mice, defined as healthy mice of the correct genotype that are not used for breeding or experiments. The first is implementing a system where the colony managers determine the breeding strategy (or at least inform the end user on the best approach), based on the number of experimental mice needed per month. The second is the periodic issuance of a colony census report by the TCM team so that end users can compare the cage count for each line to their budgeted allocation. Following full implementation of these refinements in mid-2017, the number of unused mice decreased by 55% in Sep-Nov, compared to Jan-May. We propose the use of a dedicated breeding colony with expert, vigilant colony managers to reduce waste and improve overall operational efficiency in the transgenic mouse vivarium.

#### **P208 The Litter Sizes of Embryo Transfer Mice Are Uncorrelated with Gene-modified Modes of the Donor**

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Embryo transfer, as a basic assisted reproductive technology, was extensively applied in the construction and cryopreservation of gene-modified mice and rederivation of infected rodent strains. Our study was undertaken to examine whether the litter sizes of mouse by embryo transfer was related with modes of gene-modified of donors. We transferred 2-cell embryos of transgenic mice to 15 recipients, and 2-cell embryos of knockout mice to 9 recipients. The numbers of embryos, which were transferred to the unilateral oviduct of ICR recipients, ranged from 10 to 15, and the embryos were all conceived by IVF. The background strain of all embryos was C57BL/6. The strains of transgenic donors were Podo-C3 transgenic mice (1 recipient), TRAMP transgenic mice (3 recipients), OT-I transgenic mice (7 recipients), SM22-GOT1 transgenic mice (1 recipient),  $\alpha$ -MHC-GOT2 transgenic mice (1 recipient), Tek-mGOT1 transgenic mice (2

recipients), and the strains of knockout donors were Nipa2 knockout mice (2 recipients), Lyg1 knockout mice (1 recipient), F2r11 knockout mice (2 recipients), CDO knockout mice (1 recipient), mGot1-flox knockout mice (1 recipient), TRVP3 knockout mice (1 recipient), Col4a3 knockout mice (1 recipient). The average pups born by every recipient were 3.50 and 3.67 for transgenic and knockout mice ( $P = 0.86$ ). In conclusion, under our experiment condition, the pup numbers born by embryo transfer mice were not correlated with gene-modified modes, this effect could be excluded when we seek the factors that affect the litter sizes of embryo transfer recipients.

### P209 The Necroptosis Machinery in the Pathogenesis of Neonatal Necrotizing Enterocolitis

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Necrotizing enterocolitis (NEC) is a devastating disease of premature infants that is characterized by excessive inflammation and cell death in the intestine, often leading to infant death. We hypothesize that necroptosis, a recently identified highly inflammatory form of programmed cell death, plays a role in the pathogenesis of NEC. Here we used in vitro culture of intestinal epithelial cell (IEC) crypts, termed enteroids, to test the effects of 2 variables, namely hypoxia and exposure to bacterial endotoxin, on intestinal epithelial cell inflammatory cytokine production and cell death. Additionally, we employed a well-characterized experimental mouse model of NEC on pups ( $n = 0$  C57BL/6,  $n = 14$  MLKL<sup>-/-</sup>) from postnatal day 7 through 10, and harvested ileal tissue for RT-PCR, Western blot, and immunohistological analysis of tissue inflammation and cell death. This model was carried out in the presence or absence of inhibition of the necroptosis pathway. In vitro results showed both IEC differentiation and exposure to endotoxin caused an upregulation of necroptosis gene expression (e.g. receptor-interacting protein kinase 1 and 3 (RIPK) and mixed lineage kinase domain-like protein (MLKL)), while hypoxia caused a decrease in expression. In vivo work showed the necroptosis machinery to be transcriptionally and translationally upregulated in the neonatal mouse ileum at a time when the pups are susceptible to experimental NEC. Induction of NEC in these animals further upregulated the necroptosis machinery. Importantly, both pharmacological and genetic inhibition of necroptosis during NEC decreased the upregulation of inflammatory gene transcription and resulted in decreased tissue inflammation as measured by 3-nitrotyrosine staining, a marker of free radical-mediated damage. Overall, upregulation of necroptosis genes in the immature intestine suggests that these genes may play a role in normal intestinal development, but also that the neonatal intestine may be primed for death by necroptosis. Since necroptosis is upregulated in NEC and inhibition of this pathway results in decreased intestinal inflammation, pharmacological inhibition of this signaling pathway may provide therapeutic options for decreasing the severity of neonatal NEC.

### P210 Minipig Model of Atopic Dermatitis: Assessment of In Vivo and In Vitro Activity of Recombinant Porcine Interleukin-4 and Interleukin-13

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Atopic dermatitis (AD) is a common skin condition that clinically presents as erythematous, dry, pruritic skin. Minipigs are used frequently for toxicity/safety of dermally applied products, and thus a model of AD in minipigs would be beneficial for preclinical efficacy tests of such medications. This study assessed sensitivity of Hanford minipigs to recombinant porcine (rp) IL-4 and IL-13, 2 major cytokines involved in human atopy. Peripheral Blood Mononuclear Cells (PBMC) isolated from 3 female Hanford minipigs demonstrated

approximately a 4-fold increase in STAT6 phosphorylation when challenged with rpIL-4, but not rpIL-13. When 3 female Hanford's received a single intradermal dose of rpIL-4 or rpIL-13, erythema and edema were not different from vehicle control dose sites. However, repeat intradermal injections for a period of 5 d did elicit increased erythema and edema in rpIL-4 dose sites relative to vehicle control, but not rpIL-13 dose sites. The peak irritation was observed approximately 5 min after dose administration, similar to histamine injections in minipigs. Interestingly, perivascular or dermal lymphocytes were observed in ~25-38% of rpIL-4 and rpIL-13 dose sites, but were not present in the vehicle control sites. Perivascular eosinophils were observed in ~25% of the rpIL-4 dose sites, while not observed in vehicle or rpIL-13 dose sites. This suggests that intradermal injection of rpIL-4 and rpIL-13 may recruit lymphocytes to dermal tissues. These findings that rpIL-4 and rpIL-13 appear to have biological activity in Hanford minipigs, and are good candidates for further exploration in developing a porcine model of AD.

### P211 Comparison of the Gingival Vein and Cranial Vena Cava as Blood Collection Techniques in Guinea Pigs

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When compared to other rodents, blood collection methods in guinea pigs are limited due to their short necks and limbs and lack of a tail. Gingival venipuncture is a novel blood sampling technique that uses the gingival vein located within the gingiva just below the pair of mandibular incisors. The technique has previously been shown to be minimally traumatic with no significant alterations in hematological parameters when multiple blood samples are collected over time. The purpose of this study was to determine if gingival venipuncture could be used as an alternative blood collection technique in guinea pigs, such that: (1) no contaminants from the oral cavity would be introduced into the sample and (2) hematological parameters would be consistent with samples collected from the cranial vena cava. We hypothesized that there would be no bacterial contamination present with either collection method and no detectable differences in hematological parameters between sample sites. An aseptic skin preparation was performed using chlorhexidine and alcohol at the cranial vena cava sampling site. The gingiva was flushed with sterile saline to prevent irritation to the mucous membranes with an antiseptic. A 28G insulin syringe was inserted caudally approximately 3-5 mm deep into the gingiva immediately below the lower incisors. Blood samples were successfully obtained from the gingival vein in 5 of 7 anesthetized Dunkin Hartley guinea pigs and from the cranial vena cava in 7 of 7 guinea pigs, with a minimum of 200  $\mu$ L submitted for blood culture. Blood samples were again successfully collected from the gingival vein in 4 of 7 anesthetized guinea pigs and from the cranial vena cava in 7 of 7 guinea pigs, with at least 250  $\mu$ L submitted for complete blood counts. All guinea pigs showed bacterial growth at the gingival sample site, but not at the cranial vena cava site. There were no significant differences in complete blood count values when comparing the gingival vein blood samples to those from the cranial vena cava. No adverse effects were detected at either site. These results provide evidence that gingival venipuncture may be used as an alternative blood collection method for guinea pigs for analyses such as complete blood counts, but may not be suitable for blood culture. A chlorhexidine oral rinse should be considered as an alternative to saline in future studies, given the positive blood cultures.

### P212 Determining the Specificity and Efficacy of MSLN BiTE for T Cell Engagement in an Model of Pancreatic Adenocarcinoma

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Pancreatic ductal adenocarcinomas (PDAs), malignant neoplasms affecting the exocrine portion of the pancreas, are the third leading cause of cancer related deaths in the United States. There are several mechanisms impeding the effective treatment of PDAs, requiring novel treatment modalities to be discovered. Mesothelin (MSLN), a protein normally expressed on mesothelial cells, has been shown to be over-expressed within PDAs and, when targeted, yields a robust immunologic response. Bispecific T cell engager (BiTE) antibody constructs, a biologic agent capable of targeting tumor self-antigens, binds to T cells (CD3) and a tumor-associated molecule, like MSLN. Human and mouse MSLN BiTE molecules were used in 2 independent studies to verify their specificity to traffic T cells within PDAs and to determine their effect on tumor size over time. In 1 study, 12 NSG mice were orthotopically implanted with a 2mm x 2mm tumor composed of a luciferase-expressing human-derived PDA cell line (AsPC-1), and subsequently 2 injections of  $6.0 \times 10^6$  activated human T cells, 100U of human recombinant IL-2 (rIL-2), and either a high (1 mg/kg) or low dose (0.1 mg/kg) of a human MSLN BiTE were administered intraperitoneally. Control NSG mice (n=5) underwent the described tumor implantation and injections of human T cells and rIL-2 at the same doses; no MSLN BiTE was administered to control animals. To compare the efficacy of the varying doses, tumor size, as indicated by radiance (photons/sec/cm<sup>2</sup>/sr), was measured longitudinally using an in vivo imaging system. The control cohort demonstrated a mean radiance of  $50 \times 10^8$  at day 21 post-transplantation; tumor size was reduced in both treatment cohorts with the low dose cohort mean radiance at  $14 \times 10^8$  and the high dose cohort mean radiance significantly reduced to  $10 \times 10^8$  (KW  $P = 0.0071$ ) at this time point. The human MSLN BiTE is effective, over a range of doses, for limiting the growth of PDAs in this mouse model. Future directions include monitoring tumor growth within existing AsPC-1 orthotopic tumor groups and determining the survival curves of each cohort.

### P213 The Effect of Aseptic Technique on Bacterially Colonized Cranial Chambers in Macaques

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Cranial recording chambers are frequently used in neuroscience to obtain electrophysiology data from the brain. These chambers require frequent maintenance to prevent infection. While bacterial colonization of chambers is common, the chambers often contain exudate suggestive of localized infection. The Association of Primate Veterinarians recommends aseptic technique when opening cranial chambers; however, chamber maintenance protocols vary by laboratory and no studies have evaluated whether the use of strict aseptic technique mitigates levels of colonization within chambers. A small pilot study was performed using a crossover design, in which existing/longstanding (1-3.5 y) cranial recording chambers of 3 male *Macaca radiata* and 1 male *Macaca mulatta* were cleaned 3 times weekly using either strict aseptic technique or the routine technique of the participating laboratory. The process included cleaning with alternating rounds of iodopovidone, hydrogen peroxide, and sterile saline. After 3 wk, the cleaning technique was switched for each group. The qualitative appearance of fluid within each chamber was rated throughout the study, and fluid cytology, microbial cultures, and bacterial quantification via colony forming unit counts were performed at 3-wk intervals. Culture results were variable and suggest a fluctuating bacterial population within the chambers regardless of

cleaning technique. *Corynebacterium ulcerans* and *Staphylococcus aureus* were the most common species identified on culture, affecting 100% and 80% of the chambers at various time points throughout the study, respectively. There were no significant differences between treatments in gross fluid characteristics or bacterial burden. There was also no apparent correlation between gross fluid quality, inflammation based on cytology, and bacterial burden ( $P = 0.25$ ). Although study power was low due to small sample size, the use of strict aseptic technique for maintenance of existing cranial recording chambers did not significantly reduce colonization or improve the qualitative appearance within cranial recording chambers.

### P214 Functional Impairment and Proliferative Changes in the Gut Following Partial-body Irradiation in Göttingen Minipig Model of Gastrointestinal Acute Radiation Syndrome

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The Göttingen minipig (GMP) displays classic gastrointestinal acute radiation syndrome (GI-ARS) following total body irradiation (TBI) at GI doses which is 100% lethal by 10-14 d. Here, we developed a partial body irradiation (PBI) model by exposing only the abdomen and lower extremities to study clinical signs and digestive system impairment out to at least 30 d. Twenty-four 5-6-mo-old, male GMP were exposed to either 12 or 16 Gy PBI employing 40-50% bone marrow sparing. Natural history data was obtained as CBCs, clinical observations, gross, and histopathology (H&E, proliferation markers- Ki67 and BrdU and apoptosis-TUNEL). In addition, assessment of digestive system processes, namely food tolerance, digestion, absorption, and mucosal function (citrulline) were evaluated over time (pre-PBI and 4 post-PBI phases). PBI at 16 Gy yielded higher lethality than 12 Gy. Unlike TBI, PBI did not cause severe pancytopenia or external hemorrhage. Compromised animals showed inactivity, anorexia, vomiting, weight loss, and changes in stool consistency. Histology revealed that in 12 Gy animals, lesions occurred only in the early phase post PBI but in 16 Gy animals lesions were more pronounced and were seen in both early and intermediate phases (phases 1 and 2). BrdU and Ki67 labelling demonstrated dose-dependent loss of crypts and subsequent mucosal ulceration which recovered over time. TUNEL analyses revealed apoptosis only to a minimal extent at both doses. Reductions in food tolerance, amino acid absorption, and citrulline production were dose-dependent. Digestion capacity was lost earlier at 16 Gy compared to 12 Gy. Loss of citrulline reached a nadir between 6-12 d and then recovered partially. In conclusion, PBI GMP models allow for 30-d survival at otherwise lethal GI doses. Classical signs of GI-ARS such as vomiting, diarrhea, constipation, and weight loss were seen. H&E and proliferative markers indicated dose-dependent structural damage to the intestine with a subsequent recovery seen in the lower dose. Food tolerance, amino acid absorption, and citrulline production were impaired in both a dose and/or time-dependent manner resulting in quantitative decreased overall gut functionality.

### P215 Assessment of General Activity on Open-field Test of Mice Postvasectomy Using Tramadol and Meloxicam for Pain Relief

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An adequate pain management for laboratory mice under invasive procedures allows animal experimentation refinement. Behavioral parameters can be assessed to evaluate pain protocol relief, such as

general activity, body posture, aggressiveness and lack of grooming. The aimed of this study was to evaluate general activity and self-grooming of non-operated and vasectomized male mice after analgesic administration. C57BL/6J male mice, aged 8-12 weeks, were divided into groups (n=8 each): CTR- non-operated control; CT- non-operated treated with tramadol; CM- non-operated treated with meloxicam; CTM- non-operated treated with tramadol + meloxicam; VT- vasectomized treated with tramadol, VM- vasectomized treated with meloxicam; VTM- vasectomized treated with tramadol + meloxicam; and VTML- vasectomized treated with tramadol + meloxicam + lidocaine. Animals received tramadol 20 mg.kg<sup>-1</sup> and meloxicam 10-20 mg.kg<sup>-1</sup>, by intraperitoneal injection 90 minutes before behavioral tests, except for CTR group. Surgery was conducted in vasectomized-treated mice groups 30 minutes after analgesics administration under general inhalation anesthesia, and behavioral tests were performed within 60 minutes after anesthesia recovery. Lidocaine 10 mg.kg<sup>-1</sup> was administered by subcutaneous via at the incision site (scrotal sac) in the VTML group. Open-field tests were recorded from 5 to 10 minutes and analyzed with OpenFLD.Ink software. Comparisons of locomotion time, frequency of rearing and grooming, time spent in central and peripheral zones were analyzed among groups using one-way ANOVA followed by a Bonferroni post hoc test ( $P < 0.05$ ). Non-operated treated and vasectomized-treated mice showed spontaneous locomotor activity similar to what was observed in the CTR group and they spent most part of the time in the periphery of the open field, with the exception of CM (20 mg.kg<sup>-1</sup>). This group demonstrated significantly lower ambulation activity and the mean time spent in the central area was longer than in the periphery. Frequency of rearing was reduced in CM, CTM, VT, VM, VTM and VTML groups when compared to CTR and CT groups. Grooming behavior, such as licking of the paws and genitals, was significantly lower in CM (20 mg.kg<sup>-1</sup>) group in comparison with other groups. We conclude that tramadol 20 mg.kg<sup>-1</sup>, meloxicam 10 mg.kg<sup>-1</sup> and lidocaine association did not interfere in general activity of these animals and can be used for pain relief after vasectomy.

#### **P216 Utilizing a Toponome Imaging System to Study Epstein-Barr Virus Infections in Immunocompromised New Zealand White Rabbits (*Oryctolagus cuniculus*)**

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Epstein-Barr virus (EBV) is a gamma-herpesvirus that infects over 90% of the adult human population, is the main etiologic agent of infectious mononucleosis, and is associated with cancers such as Hodgkin's lymphoma and Burkitt lymphoma. EBV has been studied in lab animals using EBV-like viruses, but an adequate animal model has not yet been established. This project seeks to utilize the rabbit model to study EBV in conjunction with systemic immunosuppression and to demonstrate significant amplification of the viral program in immune tissues. Ten rabbits (female; n=4, male; n=6) were infected with EBV via marginal ear vein injection to create a rapid, systemic dispersal of the virus. Systemic immunosuppression was induced using cyclosporine A (CsA) injected subcutaneously either five weeks after virus infection (modeling reactivation from latency; n=6) or concurrent to viral infection (modeling primary infection; n=4). CsA treatments were administered at 15 mg/kg daily for five days, then at 20 mg/kg twice weekly for two weeks, so that the immune suppression was maintained over a three week period. Peripheral blood lymphocytes (PBL) and body temperatures were monitored weekly. Rabbits rapidly lost weight and, at humane endpoints, demonstrated significant EBV activity in the spleen, PBL, and immune cells of the liver. The use of a Toponome Imaging System (TIS), a novel image cyler microscopy, demonstrated incidence of several viral proteins in rabbit spleen cells in situ. Various viral proteins including EBNA2, EBNA3C, LMP1,

VCA, BZLF1, BMRF1, EBNA1P were detected histologically, and EBV EBNA RNA by in situ hybridization. The TIS results showed viral proteins present in rabbit B-cells but not in rabbit T-cells. The results also showed that viral genome amplification in PBL was more extensive in the rabbits receiving concurrent EBV and CsA treatments when compared to the delayed CsA treatments. The rabbit EBV model requires further characterization, but is a promising new preclinical model to test anti-viral medications and prophylactic vaccines for EBV in patient populations.

#### **P217 Skin Ulceration after Subcutaneous Administration of 20 mg/kg Meloxicam in C57BL/6 Mice**

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Studies using the Mouse Grimace Scale have shown that doses of nonsteroidal anti-inflammatory drugs (NSAID) of at least 20 mg/kg may be necessary for adequate peri- and postoperative analgesia in the mouse. However, it is not known whether such NSAID doses exceed the threshold for gastrointestinal ulceration or induce other relevant pathology. We administered equal volumes (0.1 ml) of saline or injectable meloxicam at a dose of 20 mg/kg subcutaneously to 8 male young adult C57BL/6 mice daily for 6 d and performed necropsies on all mice on the seventh day. No gross pathological lesions in the stomach, kidneys, or liver were noted, and complete blood counts and serum chemistries were within normal limits. However, skin ulceration (ranging from 1-6mm in diameter) was noted at the injection site in 3 of the 4 mice that received meloxicam. The fourth mouse had intact skin but displayed hemorrhage in the subcutaneous fat around the injection site. On histopathology, the injection sites of the first 3 mice that received meloxicam had full-thickness skin necrosis, and all mice had necrosis of the underlying fat and muscle. The mice that received saline subcutaneously displayed either no lesions or mild foreign body responses at the injection site. These results demonstrate that undiluted meloxicam (5 mg/ml) injected subcutaneously at a dose of 20 mg/kg likely causes skin pathology. Further research will examine the effects of similar doses of meloxicam at lower concentrations.

#### **P218 A Simple and Effective Method for Eliminating Pathogens in Imported Mouse Strains**

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The increasing number of genetically modified mice available makes collaborations between researchers from all around the world more and more frequent. We import close to 100 cohorts of mice each year. Imported mice destined for our barrier facilities are either tested to confirm the absence of infectious agents or rederived to eliminate pathogens excluded from the barrier. Traditionally, rederivation is performed by embryo transfer. Although this method is excellent at eliminating undesired pathogens, it requires technical expertise, surgical skills, specialized equipment, and often uses wild-type animals which can delay the production of animals of the desired genotype. We have explored several methods for rederivation as an alternative to embryo transfer: cesarean section, cross-fostering, and artificial insemination. We propose a cross-fostering procedure that requires minimal manipulation of the fostered pups. The limited handling is combined with fostering the litter within 18-24 h of birth, using a foster dam of the cleanest available commercial source, and administering antibiotics to the donor dam during gestation. We have shown this procedure to be effective at eliminating murine norovirus, *Helicobacter* spp. and *Pasteurella pneumotropica* as confirmed by PCR testing of fostered litters, with pup survival rates similar or superior to those of wild-type strains. The specific pathogen-free (SPF) status of the rederived strains has been maintained for over 2 y. Rederivation by cross-fostering requires few manipulations, limited technical expertise, and can use as little as 1 male and female mouse to



produce litters of SPF animals. Furthermore, we can mate animals strategically to produce pups of the desired genotype. Cross-fostering allows us to offer a simple, cost-effective, and efficient method for our researchers to obtain animals of a health status comparable to that of animals purchased directly from commercial vendors without generating significant delays to research projects.

### **P219 Cardiomyocyte-specific Transgenic Expression of TRPV4 as a Tool to Investigate Calcium Homeostasis and Cardiac Remodeling in the Murine Heart**

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Transient receptor potential vanilloid channel subtype 4 (TRPV4) is a calcium-permeable nonselective cation channel found in many cell types throughout the body, including cardiomyocytes. Abnormal calcium handling by cardiomyocytes is associated with several cardiomyopathies. However, there is limited data on the functional role of TRPV4 on calcium homeostasis and remodeling in the heart. The goal of this project was to develop a transgenic mouse model with tamoxifen-inducible overexpression of TRPV4 in cardiomyocytes. A mouse model harboring an  $\alpha$ -MHC promoter driven (loxP)mCherry-STOP(loxP)-TRPV4 transgene was bred to the MerCreMer inducible cardiac-specific Cre recombinase driver mouse. In this double-transgenic mouse model, cardiomyocytes exhibited expression of the mCherry fluorescent reporter protein prior to tamoxifen treatment (50/50 cells sampled from 4 mice; n=5 to 15 cells sampled per mouse). After feeding tamoxifen (500 mg/kg diet for 2 wk, followed by 4 wk normal diet), mCherry-reporter positive cells were no longer observed (0/29 cells from 3 mice; n=6 to 15 cells sampled per mouse), and Western blot analysis of isolated cardiomyocyte homogenates revealed a ~3 fold increase in TRPV4 protein expression. Future studies will use this novel animal model to determine if TRPV4 contributes to calcium-dependent signaling and cardiac remodeling following defined physiological and pathological stimuli.

### **P220 Sampling of Neural Tissues in Preclinical Toxicity Studies**

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There has been a recent increase in compounds in preclinical toxicity studies that are intended to treat neurological disorders or those suspected to cause neural toxicity. Therefore, specialized processes and techniques are needed to facilitate the sampling and processing to get optimum quality neural tissue sections along with other tissues collected for standard histopathology from the same set of animals. Twelve rats were used to compare whole-body perfusion fixation to 2 different immersion fixation methods to determine the procedures that produced the highest-quality tissue sections in the most efficient manner. To evaluate immersion fixation, 4 rats were collected with all neural tissue remaining intact on the carcass and exposed post-fixation. Four additional rats were collected with all neural tissue intact on the carcass and exposed at necropsy via laminectomies of the vertebral column to reveal the spinal cords and dorsal root ganglia, as well as the removal of applicable muscles on the limbs to expose distal peripheral nerves. Four additional rats were fixed via whole-body perfusion, with all associated neural tissues collected and exposed post-perfusion. From all animals, spinal cords with intact dorsal roots and ganglia were collected in situ with the peripheral nerves from the hind limbs and forelimbs intact with the corresponding muscles. Additionally, the superior cervical and cervicothoracic (stellate) ganglia from the autonomic nervous system were collected post-fixation from all 12 animals. Tissues were evaluated microscopically by a board-certified anatomic pathologist and no notable differences were found between the different fixation methods. Since immersion

fixation is less expensive, more efficient, and flexible enough to allow for sample collection for multiple analyses in the same set of animals, this method is recommended for most general safety assessment studies with compounds intended to treat neurological disorders or those with neural tissue as a suspected toxicity target.

### **P221 Pharmacokinetics of Sustained-release Meloxicam Compared to Oral and Subcutaneous Meloxicam over 72 h in Male Beagle Dogs**

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Meloxicam is a commonly used and FDA approved COX2-preferential nonsteroidal antiinflammatory drug in dogs administered orally or subcutaneously every 24 h. A sustained-release formulation of meloxicam (SR-meloxicam) has been shown to last up to 72 h in other species, maintaining therapeutic levels and decreasing dosing interval. This crossover study compares the pharmacokinetics of a single subcutaneous (SQ) dose (0.6 mg/kg) of SR-meloxicam (10 mg/ml) to oral and SQ meloxicam formulations dosed for 3 consecutive d in 7 adult male dogs. Plasma concentrations were compared at 0 (baseline), 1, 4, 8, 12, 24, 48, and 72 h post initial administration. SR-meloxicam peaked at 1-h time point (2180  $\pm$  359 ng/mL) while oral and SQ meloxicam peaked at the 4-h time point (295  $\pm$  55 ng/mL and 551  $\pm$  112 ng/mL, respectively). SR-meloxicam yielded significantly higher plasma concentrations than the oral and SQ formulations until the 48 and 72-h time points, respectively. SQ meloxicam formulation was significantly higher than oral formulation at 4, 8, 12, 24, 48 and 72-h time points. No lesions were noted at SR-meloxicam injection sites. The results show that SR-meloxicam can provide adequate plasma concentrations for at least 72 h in male dogs. The oral and SQ meloxicam formulations provide adequate plasma concentrations for 12 to 24 h.

### **P222 IL-1 Release Is Dependent on NLRP3 Signaling and Appears to Be Protective in a Murine Model of *Escherichia coli*-Induced Meningitis**

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*Escherichia coli*-induced meningitis is the leading cause of bacterial meningitis in premature infants but is rarely seen in adults. Amongst other differences, the neonatal immune system has decreased inflammasome signaling compared to adults. However, it is unknown what role inflammasome activation plays in *E. coli* meningitis. We set out to determine whether neonatal meningitis associated *E. coli* (NMEC) strains activate inflammasome pathways, and how this activation contributes to the outcome of NMEC infection. First, we performed in vitro infections of mouse peritoneal macrophages with 2 well-characterized clinical isolates: NMEC58 and NMEC RS218. We found that stimulation of inflammasomes was dependent on the strain of NMEC, as only the NMEC RS218 strain elicited IL-1 $\beta$  secretion, and that this difference between strains is in IL-1 $\beta$  processing, not transcription. We also found that this response is dependent on NLRP3 and Caspase 1/11. This effect was recapitulated in infections of microglial cell lines. In follow-up in vitro experiments, we determined that extracellular bacteria alone were sufficient for IL-1 $\beta$  production, and that while NMEC RS218 induced cell death, this death was not dependent on inflammasome activation or necroptosis. Finally, we performed in vivo infections to determine the contribution of IL-1 to the outcome of *E. coli* meningitis. Wild-type and IL-1R<sup>-/-</sup> mice were given intracranial injections of 500 bacteria in 1 $\mu$ l PBS, and then monitored frequently to develop survival curves (n=10/group). Mice were assessed based on a pain and distress scale developed for the model which included semi-quantitative scoring metrics for activity level, general appearance and posture, and neurologic signs. When

mice reached the predetermined cut off score, they were euthanized, and considered a mortality. Survival data was analyzed via Kaplan-Meier survival log rank. While overall mortality was not significantly different between groups (8/10 WT, vs 9/10 IL-1R<sup>-/-</sup>), the mice lacking the IL-1 receptor did succumb to the infection sooner, leading to a statistically significant difference overall ( $P = 0.018$ ). This suggests that NLRP3-dependent IL-1 is playing a protective role in NMEC infection.

#### **P223 Assessing the Efficacy of Heterozygous CD-1 Nude (nu/+) Mice as Soiled Bedding Sentinels**

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Heterozygous (HE, nu/+) nude CD-1 mice have been used as soiled bedding sentinels (SBS) for rodent health surveillance for decades. It has been postulated that HE nude mice should not be used as sentinels as they possess immune system differences from wild-type mice. We compared HE nude and wild-type CD-1 mice as soiled bedding sentinels (SBS) to assess possible differences in antibody response and agent transmission between the 2 strains. For each strain, 2 cages of female-specific opportunistic pathogen free (SOPF) mice were housed in static microisolator caging. Baseline testing by PCR using feces, body swabs, and oral swabs confirmed the absence of rodent pathogens. Pet shop animals infected/infested with multiple agents including viruses, bacteria, protozoa, and parasites were used to supply the source of rodent pathogens. SBS cages received 25% soiled bedding at biweekly cage changes. Twenty-five percent soiled bedding was produced by combining 50 parts soiled bedding pet shop mice, 50 parts soiled bedding from an SOPF mouse isolator, and 100 parts clean bedding. One SBS mouse from each cage was submitted monthly for testing by PCR and traditional methods for 3 mo. MPV, MNV, and Theilovirus were detected by PCR and serology for both the HE nude CD-1 and CD-1 wild-type SBS. MFIA scores and estimated PCR copy numbers were similar between the 2 strains for these viral agents. MHV was detected only in the HE nude CD-1 SBS by both PCR and serology. Adenovirus, K virus, MCMV, and rotavirus, present in the pet shop mice, were not transmitted to any of the SBS. Astrovirus was detected by PCR in both mouse strains, although the copy number detected was higher in the CD-1 wild-type SBS. *Helicobacter* and pinworms were detected by PCR in both mouse strains, and the estimated copy number was similar. Fur mites and entamoeba were detected by PCR only in the HE nude CD-1 SBS. The data from this study support that HE nude CD-1 mice are appropriate for use as soiled bedding sentinels and subtle perturbations to their immune system status does not appear to impact agent transmission as overall suitability as sentinels.

#### **P224 Temporal Microbiome Evaluation in Wistar Han Rats after Shipping from a Vendor to a Preclinical Testing Facility**

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It has been reported that the mouse gut microbiome (GMB) will stabilize in 5 d when transported within a research facility, but that transport from a vendor still shows substantial changes even at 5 d and may take longer to stabilize. Rats are commonly used in toxicology research and are typically acclimated for 3 d prior to study enrollment. The outcome of therapeutic treatment success in humans has been linked to the GMB. Similarly, using rats before their GMB has stabilized could impact study results. We hypothesized that the rat GMB would continue to change beyond the typical 3-d acclimation period. In this study, we evaluated the GMB of 6-9-wk-old, male and female Wistar Han rats (CrI:WI(Han)) before and after transit from production barrier rooms to the preclinical research vivarium. At both locations, rats were housed in individually ventilated caging (IVC) systems with water and food ad libitum within a standard barrier

room. Fecal pellets were collected at the vendor on the day of shipment and again upon arrival (Day 0) and on day 1, day 3, day 7, day 14, and day 21 at the research facility. Fecal metagenomic DNA was extracted and a 16S rRNA sequencing library was prepared according to the 16S metagenomics sequencing library preparation protocol targeting the V3 and V4 hypervariable regions of the 16S rRNA gene. We evaluated the GMB alpha and beta diversity using a Miseq 16S rRNA sequencing protocol. There were no substantial variations in rat GMB in the barrier room control samples over time. However, among animals shipped to the research vivarium, alpha and beta diversity analysis observed continuous changes in the GMB from arrival through 21 d. The greatest increases among genera at 21 include Parabacteroides (666%), Coprococcus (617%), Sutterella (188%), Lachnospira (150%), and Coprobacillus (113%). The greatest decreases among genera include Sarcina (95%), Turicibacter (87%), Caloramator (79%), Clostridium (79%), Bifidobacterium (78%), and Lactobacillus (67%). In support of our hypothesis, the rat GMB did not stabilize after 3 d and continued to change over the time points evaluated in this study. Additional time points beyond 21 d may be necessary to determine the time required to stabilize the rat GMB after transport.

#### **P225 Next Generation Sequencing of a Novel Astrovirus Detected in a Research Colony Of Mice**

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Murine astrovirus 1 (MuAstV1) is commonly found in both immunodeficient and immunocompetent research mice worldwide. Recently, we serendipitously identified a novel astrovirus in a mouse research colony as a result of an investigation into observed antibody cross-reactivity to an unrelated MTLV serology assay. Initial metagenomic next-generation sequencing (NGS) of feces from the serologically cross-reactive sentinel mice produced 3 small (~200 bp) sequences which had closest nucleotide homology to an unclassified rat astrovirus. A PCR assay, which targeted a conserved region among the available related rat astroviruses, was used to verify the presence of the novel astrovirus virus within the research colony and the cell culture used to produce the MTLV antigen. To better understand the genetic and protein relatedness of this novel astrovirus to other astroviruses, we used enrichment techniques and NGS to sequence both the novel astrovirus in the research colony as well as the astrovirus in the cell culture. A complete sequence was determined for the novel astrovirus from the research colony. This virus has 89% nucleotide homology to another recently reported astrovirus (MuAstV2) and should be considered another strain of the same species. The largest contig identified was 6,461 bps and contained 47 open reading frames (ORFs) when their minimum length set at 75 nucleotides. The largest ORF is composed of 824 amino acids and has 39% homology to a Mamastrovirus 3 capsid polyprotein (VP90). Sequencing of the cell culture-associated virus generated 2 contiguous sequences (3942 bps and 2579 bps) representing 61% and 40% of the MuAstV2 genome and share 92% and 89% homology respectively with the mouse colony-associated strain. NGS was a useful tool for determining the sequence of a novel astrovirus found in a research vivarium and cell culture. A better understanding of both the genetic and amino acid differences among astroviruses will be important for fine tuning the development of diagnostic assays.

### **P226 Phenotypic Characterization of the Spontaneous Mutation Tremor Exhibiting Psychomotor and GABAergic Transmission Impairment**

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The tremor mutant phenotype is the result of a recessive mutation that arose spontaneously from a colony of Swiss mice. Around postnatal day 21 (P21), mutants can be identified by the tremor, ataxia, motor incoordination, and tonic seizures. The mutation has been transferred into C57BL/6 background by successive backcrossing for at least 10 generations. Genetic mapping with microsatellite markers distributed through the mouse genome showed that the mutation is on chromosome 14, between 33.21 and 38.21 cM, making *Egr3* (early growth response 3) the main candidate gene. To characterize the mutant strain phenotype, we applied a behavior test battery consisted of 6 tasks to evaluate different aspects of psychomotor, cognitive, and sensory impairment. Tests were conducted in order of increasing invasiveness. The open field test (OFT) was used to analyze locomotion time, general activity, irritability, auricular reflex, tail flick, corneal reflex, response to touch, hindquarter fall, surface-righting reflex, grasping strength, tremor, and Straub tail reaction. In addition, elevated plus maze (EPM), balance beam test, T maze spontaneous alternation task, forced swim test (FST), and tail suspension test (TST) were performed. The behavioral characterization was performed on 8-wk-old C57BL/6 mutants and wild-type (WT) mice. Animals were euthanized by decapitation and the neurotransmitter gamma-aminobutyric acid (GABA) was measured in striatum, frontal cortex, and hippocampus by HPLC. In comparison to WT, mutants showed Straub tail reaction, reduced rearing and grooming frequency, ataxia, tremor, and motor incoordination. Hyperactivity and negative responses to anxiety were also observed, but there was no change in the spatial memory of the mutant. GABA levels were increased in the striatum and hippocampus of mutant mice. Our findings suggest that the mutation tremor could be involved into an impairment of the GABAergic transmission since it has been demonstrated that *Egr3* gene plays an important role in the regulation of GABA-A receptor in the central nervous system.

### **P227 Cytotoxic *Escherichia coli* Strains Encoding Colibactin and Cytotoxic Necrotizing Factor Colonize Laboratory Common Marmosets (*Callithrix jacchus*)**

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While most *E. coli* strains are commensal, some strains encode virulence factors that enable the bacteria to cause intestinal and extra-intestinal infections. Colibactin, encoded by a genomic island (*pks* island), and cytotoxic necrotizing factor (CNF), encoded by the *cnf* gene, are genotoxic and can modulate cellular differentiation, apoptosis and proliferation. Some commensal and pathogenic *pks+* and *cnf+* *E. coli* strains have been associated with inflammation and cancer in humans and animals. The present study characterizes the presence of *pks* and *cnf* in rectal and extra-intestinal isolates of *E. coli* from marmosets housed in three separate colonies. Bacterial cultures were performed on rectal swabs and extra-intestinal samples. Isolates of *E. coli* were identified and characterized biochemically. Specific PCR for *pks* and *cnf* gene amplification, and phylogenetic group identification were performed on all *E. coli* strains. Medical records were evaluated for correlation of health status with *pks+* or *cnf+* *E. coli*. A total of 140 *E. coli* strains were isolated from 121 out of

all 138 marmosets in the three colonies. In Colony A, 32 strains of *E. coli* were isolated from 26 of the 31 animals, 54.8% of the animals had *pks+* *E. coli*, and 51.6% had *cnf+* *E. coli*. In Colony B, 33 strains of *E. coli* were isolated from 32 of the 33 animals, 6.1% of the animals had *pks+* *E. coli*, and 9.1% had *cnf+* *E. coli*. In Colony C, 75 strains of *E. coli* were isolated from 63 of the 74 animals, 43.2% of the animals had *pks+* *E. coli*, and 35.1% had *cnf+* *E. coli*. Colony B had significantly fewer *pks+* and *cnf+* isolates than either Colony A or C (Fisher's exact test,  $p < 0.001$ ). The presence of *pks+* or *cnf+* *E. coli* did not correlate with health status. Both *pks+* and *cnf+* *E. coli* strains belonged mainly to phylogenetic group B2. Colibactin and CNF cytotoxic activities were confirmed using a HeLa cell cytotoxicity assay in representative isolates. Our findings indicate that colibactin- and CNF-encoding *E. coli* colonize laboratory marmosets and can potentially cause clinical and subclinical diseases that impact marmoset models. The prevalence (6-55%) of these potentially virulent *E. coli* strains can vary between different marmoset colonies.

### **P228 Rhesus Macaque (*Macaca mulatta*) Cerebrospinal Fluid Flow Rate and Volume Determined by Plasma and Cerebrospinal Fluid Pharmacokinetics Following Intraventricular Administration of Inulin**

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Cerebrospinal fluid (CSF) is often a critical element of pharmacokinetic (PK) and pharmacodynamic studies using rhesus macaques. Two elements critical to the design and interpretation of these studies are the CSF flow rate and volume. To determine these values, a rhesus CSF Ventricular Reservoir and Lumbar Port model, which permits humane serial CSF sampling, was used following intraventricular administration of Inulin, a high molecular weight polysaccharide relatively unaffected by absorption or secretion. The plasma and CSF inulin PK were evaluated. Three animals, previously implanted subcutaneously with a CSF ventricular reservoir, a lumbar port, and IV ports, received Inulin (2 mg total) intraventricularly. Serial paired plasma and lumbar CSF samples were then collected for 0-24 h. To evaluate the contribution of dietary Inulin, 3 additional macaques were fasted for 18-22 h, and plasma was collected. Inulin concentrations were quantified via ELISA. PK parameters were calculated using noncompartmental methods. The daily diet was analyzed for Inulin via Official Method No. 997.08 of the AOAC International. Intraventricularly administered Inulin was well tolerated in all subjects. Mean CSF PK clearance was 0.018 ml/min +/- 0.003 and the volume of distribution was 10.17 ml +/- 0.635. Inulin was quantifiable in all pre-dose plasma samples and increased over the sampling period precluding PK plasma interpretation. The dietary evaluation established a mean fasting baseline Inulin of 0.152 ug/ml +/- 0.058. Analysis of the feed detected 5 Inulin types ranging from 1100-1440 mg/100g. The CSF flow rate of 0.018 ml/min in rhesus macaques was determined by Inulin clearance. The volume of CSF is independent of body weight; therefore, the approximate total CSF volume of 10.17 ml was determined by the volume of distribution. Inulin concentrations were present in the plasma from the diet.

### **P229 Surveillance of *Trichomonas muris* in Mice by Direct Microscopic Exam and PCR**

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*Trichomonas muris* (TM) is a protozoan, which resides in the cecum and colon of mice. While TM is historically considered a commensal and nonpathogenic, recent studies demonstrate that it increases the

chemosensory tuft cells in the intestine as well as modulates the immune landscape of the colon in mice. Importantly, *TM* colonization influences various research models including protection from *Salmonella typhimurium* infection, aggravated inflammatory response in T cell transfer model, and increased tumor burden in APC<sup>min/+</sup> mice. While the emerging evidences indicate the potential effect of *TM* on animal models, its presence is not routinely monitored in sentinel mice. Therefore, the prevalence of this protozoan is currently unknown. Hence, the objective of this study is to determine the prevalence of *TM* in mouse colonies. As it is readily transmitted through fecal-oral route, cecal contents and fecal pellets from sentinel mice were collected during terminal necropsy and direct microscopic examination was performed. The morphology of the trophozoites was confirmed by Trichrome and Giemsa staining. By screening the sentinel mice housed in different buildings over a year, it was found that cecal content from 25% (33/133) of the sentinel cages were positive for *TM* trophozoites. However, only 8.2% (11/133) of the fecal samples tested positive for *TM*. Of the mice testing positive for *TM* in cecal contents, only 33% (11/33) of them tested positive for *TM* in the fecal examination. Interestingly, pseudocysts were most often found in the fecal samples, which can make it difficult for antimortem diagnosis and differentiation of *TM* from other cyst forming organisms. Hence, a PCR-based method was used for detection of *TM* in fecal samples. A primer set targeting 270 base pair of 28s rRNA of *T. muris* was designed and the PCR assay conditions were optimized. The primer set reliably identified the *TM*-positive samples in a subset of mice. Further studies to compare the sensitivity and specificity of the PCR assay versus direct microscopic examination are underway. Based on these results, we will ascertain if a PCR based method in addition to direct microscopic examination can be used to reliably identify *T. muris* in mouse colonies.

### P230 Characterization of Motor and Reflex Development in a Novel Ferret Model of Encephalopathy of Prematurity

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There is an ongoing need for animal models in which to test therapeutic interventions for infants with neurological sequelae of prematurity. The ferret (*Mustela putorius furo*) is an attractive species in which to model preterm brain injury because, like preterm human infants, they are born lissencephalic and develop gyrencephalic brains postnatally. Postnatal white matter maturation and complex cortical folding in newborn ferrets also occur in a similar pattern to that observed in the human brain during the third trimester, which includes the development of the cortical subplate that is prominent in human brain development. In human infants, inflammation caused by prenatal or postnatal infection, coupled with superimposed hypoxia and/or hyperoxia, appears to be important in the pathogenesis of preterm neonatal encephalopathy. Preclinically, these processes are commonly modeled by administration of bacterial lipopolysaccharide (LPS), which presensitizes the brain to hypoxia and produces a white matter injury that mechanistically reflects the white matter damage seen as a result of encephalopathy of prematurity in human infants. To characterize behavioral testing paradigms in a ferret model of encephalopathy of prematurity, cross-fostered P10 ferret kits were randomized to IP doses of 2-5mg/kg dose of LPS (Ultra Pure LPS from *Escherichia coli* 055:B5, 1mg) or saline vehicle, before being returned to their jills for 4h. LPS-injected animals subsequently underwent consecutive hypoxia-hyperoxia-hypoxia (60 min at 9%, 120 min at 60%, 30 min at 9%) in a humidified chamber with a target intra-hypoxic rectal temperature of 37°C. Saline controls received an identical period of normoxia. Behavioral tests including negative geotaxis, cliff aversion, and spontaneous righting and walking (righting reflex), were performed 3x/week from P21-P42, and used to generate a composite behavioral score. Between P42 and P70, the kits were examined weekly using an automated catwalk system to determine gait and motor function. Mean composite behavioral score increased from 2.3 at P21 to 13.0 at P43 (n=6-23 per time point). From P42 to P70, injured ferrets

(n=20) displayed a wider base of support (BOS) with the forelimbs relative to the hindlimbs compared to controls (n=14,  $P = 0.007$ ). This wider forelimb BOS coincided with significantly greater relative intensity of paw pressure in the forelimbs ( $P = 0.04$ ), as well as a 50% decrease in hind paw area ( $P = 0.01$ ), with no evidence of ataxia. Motor development in the newborn ferret in the first 5 wk after birth is easily quantifiable using tests traditionally applied in rodent models of brain injury. Motor development is closely linked to myelination over that time period, and injury at P10 is associated with motor deficits consistent with premature brain injury.

### P231 Silicone and Carbon Particle Migration Behavior in a Porcine Knee Model

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A new nonabsorbable suture comprising an outer sheath of ultra-high molecular weight polyethylene (UHMWPE), an inner polyester sheath, and a medical-grade silicone/salt filled core has been developed to address shortcomings of current high strength sutures. Despite its use on other sutures, a potential concern existed as to whether the silicone core in this suture could have a deleterious clinical impact. Our purpose was to determine if silicone debris had an observable intraarticular or extraarticular impact, or migrated into the lymphatic system. Using a porcine model, 2 study groups were created: 1 with suture particles created by rupturing hand tied knots of suture, while the other had vitreous carbon particles. Twelve Yorkshire pigs were randomly assigned to both groups. Half of the study materials were placed bilaterally by arthrotomy into the stifle joint and after capsular closure the remaining half was placed on the joint capsule before skin closure. Six wk post-implantation the stifle joints and regional lymph nodes were examined macroscopically and microscopically. No silicone debris migration was observed microscopically; however, carbon particle migration was noted in 100% of the iliac and 50% of the inguinal lymph nodes. The suture particle group showed lymph node inflammation in 25% of the iliac and 42% of the inguinal, but none of the popliteal lymph nodes. In the carbon particle group, 100% of the iliac, 75% of the inguinal, and 8% of the popliteal nodes showed inflammation. Carbon particles in the porcine knee migrated into 2 of the regional lymph nodes with some regularity (iliac 100% and iliac 50%), but no silicone particle migration nor macroscopic joint damage was observed. These data suggest that the clinical use of this suture may not pose any problems related to particulate from the silicone core.

### P232 Identification of Immunophenotyping Biomarkers and Characterization of Peripheral Blood Lymphocytes Populations Distribution in Minipigs

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We developed a complete panel of flow cytometry biomarkers for characterization of the major populations of porcine peripheral blood mononuclear cells (PBMCs). We subsequently analyzed distributions of these immune cells in blood samples of 4 breeds of minipigs. There were a number of common features associated with distribution of the major PBMCs in blood samples of different breeds of minipigs (Hanford, Yucatan, Sinclair, and Gottingen) (n=10 per gender per breed). T lymphocytes were the predominant population of PBMCs (around 70% of total PBMCs with drops to 61% in 8-mo-old male Sinclair and to 56% in 8-mo-old female Yucatan). Natural Killer (NK) cells were composed of 3 populations of PBMCs that were CD16<sup>+</sup>CD8<sup>-</sup> and CD16<sup>+</sup>CD8<sup>+</sup> and CD16<sup>-</sup>CD8<sup>+</sup>, respectively.

CD8<sup>+</sup> lymphocytes represented the majority body of NK cells with CD16<sup>+</sup>CD8<sup>-</sup> lymphocytes to be the least fraction (no more than 8% of total NK cells). Although Alpha-Beta ( $\alpha\beta$ ), as well as Gamma-Delta ( $\gamma\delta$ ) T-cell receptors (TCRs), were identified in Natural Killer T (NKT) cells,  $\alpha\beta$  T cells comprised no less than 75% of NKT cells. In summary, a novel flow cytometry approach has been developed to characterize the major porcine PBMCs in one single assay. This assay will facilitate immune investigations during nonclinical pharmacology and toxicology studies.

### **P233 Tricaine Methanesulfonate Is Stable and Effective for Zebrafish (*Danio rerio*) Anesthesia After Longterm Storage**

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Tricaine Methanesulfonate (MS222) is widely used for anesthesia and euthanasia of zebrafish. Although it is recommended to prepare fresh solutions, researchers often mix and store concentrated stock solutions to reduce occupational exposure, environmental waste, and for convenience. While this is common practice, published guidelines are infrequent and often vague. Thus, the objective of this study was to evaluate the stability and anesthetic efficacy of MS222 with longterm storage and to develop specific storage parameters. Stock solutions (100 mg/mL MS222) were mixed and stored in opaque jars at 4°C and -20°C for 2 and 6 mo. Stability of the solutions was analyzed using liquid chromatography-ion trap mass spectrometry and compared to fresh MS222. Fifty adult (male and female) wildtype AB zebrafish (*Danio rerio*) were randomly anesthetized with 1 of the following MS222 solutions (150 mg/L) to evaluate anesthetic efficacy: 1) 4°C, 2 mo; 2) 4°C, 6 mo; 3) -20°C, 2 mo; 4) -20°C, 6 mo; 5) freshly prepared. Time to cessation of swimming, loss of equilibrium, and lack of response to von Frey stimulation, return of equilibrium, and resumption of swimming were compared between groups. Two fish from each group were euthanized at 24 h and 2 wk post-anesthesia and histopathology was performed. All solutions were determined to be stable under both storage conditions. No clinically significant differences were observed between the fresh and stored stock groups during anesthetic testing. No evidence of anesthetic-related histologic changes were noted. Therefore, 100 mg/mL solutions of MS222 can be stored in opaque jars at 4°C or -20°C for 6 mo and be used to effectively anesthetize zebrafish.

### **P234 Short-term Fasting to Refine the Experimental Reproducibility of TNBS-induced Colitis**

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Inflammatory bowel disease (IBD) represents a complex interaction between the immune system and specific tissue regions of the gastrointestinal tract. Animal models of inflammatory bowel disease are critical and necessary tools for determining the activity, safety, and tolerability of novel drugs before clinical development. The goal of this study is to optimize the 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced mouse model of colitis to ensure low inter-animal variability that would lead to reduction in treatment group sizes without compromising statistical power. Eight-wk-old female BALB/c mice were randomized to nonfasting or fasting groups (n=6) prior to the intrarectal chemical challenge. In order to induce colitis, 1 milligram of TNBS in 100 microliters of 50% ethanol was intrarectally delivered to each animal. To ensure ease of deliverance, animals were fasted. Food was withdrawn for 8 h during the light cycle to allow emptying of the colon and food was restored immediately following the TNBS challenge. These mice were also orally dosed twice a day daily for 9 d with a vehicle or test compound. Mice were clinically monitored for

the incidence of inflammation and other clinical signs indicative of pain and distress. IBD is usually associated with inflammation-induced cachexia, therefore body weight was measured daily. Stool consistency, fecal blood, and rectal bleeding were also scored daily. Colons were collected and length measured following euthanasia at the end of the 9 d. By doing a comparative study of fasted mice versus nonfasted mice using this model, we were able to prove that fasting does not adversely impact animal welfare, more focused intrarectal TNBS delivery, and resulted in lower interanimal variability of measured outcomes.

### **P235 Blood Glucose Levels in Mice during Serial Blood Collection Are Not Elevated Following Tail-tip Snip under Brief Isoflurane Anesthesia**

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Tail-tip snip is a common technique to collect small amounts of blood in mice. Most commonly this method is used for serial measurements of glucose by performing 1 single cut and manipulating the clot for following collections up to 2 h. The standard for this procedure is that the snip is performed without the use of anesthetics due to the elevation of glucose. However, while there are multiple published reports of elevated glucose during longterm isoflurane or injectable anesthesia, there are none evaluating very short-term isoflurane. Similar anecdotal rationale is also used to justify blood collection without anesthesia when evaluating WBC. The principle of refinement requires scrutiny of animal procedures to assure they are the least painful/stressful as possible, without compromising research objectives. We tested the hypothesis that brief anesthesia during initial tail laceration will not elevate blood glucose or WBC during 2-h serial blood collection. Eight-wk-old CD-1 mice were housed in ventilated racks with autoclaved bedding. Group A (n= 5) mice were anesthetized in the anesthesia chamber with 2.5% isoflurane and 2% oxygen for 2-3 min, and with bupivacaine applied after cutting approximately 2 mm of the tail-tip with a scalpel blade; Group B (n= 5), mice were manually restrained (scruffed) and 2 mm of the tail-tip was removed. Approximately 10-15 ul blood was collected at time 0, 30 min, 60 min, 90 min, and 120 minutes. Blood was collected from Group A mice prior to recovery from anesthesia, but for all other time points the blood was collected while mice were awake and scruffed. Glucose was measured using a glucometer. After 24 h all mice were anesthetized with isoflurane and blood was collected using a retroorbital technique under anesthesia, and mice euthanized prior to recovery. There were no statistical differences in any time point, between Group A and Group B glucose measurements or in WBC lymphocytes measured 24 h later. The results of this study indicate that there is not a rationale for withholding anesthesia during initial tail-tip laceration for serial blood glucose measurement.

### **P236 Evaluation of Cryopreservation Time and Method for Optimal PDX Tumor Growth**

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Mouse models bearing patient-derived xenografts (PDX) often faithfully recapitulate human disease, making them valuable tools for evaluating standard-of-care drugs, preclinical efficacy testing, pharmacodynamic studies, and biomarker discovery. Optimal methods for preservation, reanimation, and serial passage of tumor materials are essential to high-quality PDX models. Here, we compared the take and growth rates of multiple PDX models (BR1367F, BR0901F, LG1306F, LG1208F) generated from fragments archived for 12- or 24-mo. For each model and at each time point, we compared 2 cryopreservation methods of tumor fragments from the same passage and donor mouse. The first method involved the cryopreservation of a large (200-300mm<sup>3</sup>) piece of tumor tissue sufficient to trocar five NOD.Cg-Prkdc<sup>scid</sup>/l2rg<sup>tm1Wjl</sup>/SzJ (NSG) mice.

The tissue was thawed, homogenized by mincing, and 30µl of tumor slurry implanted subcutaneously to the right flank of each mouse by trocar needle. The second method involved the cryopreservation of 5 small fragments (~10-30mm<sup>3</sup> per piece) which were divided amongst 5 NSG mice for subcutaneous trocar injection. Body weights, clinical observations, and tumor volumes were measured weekly. We found the use of tumor tissue preserved as smaller fragments greatly improved the overall engraftment success of all models tested thus far (BR1367F, 1/5 [20%; large] vs. 4/5 [80%; small]; BR0901F, 0/5 [0%; large] vs. 5/5 [100% small]; LG1306F, 3/5 [60%; large] vs. 5/5 [100% small]) irrespective of the duration of cryopreservation (12- or 24-mo). Our preliminary data for both time points suggest that small fragments improved the time to engraftment and tumor growth rates. These data suggest the size of the tumor fragment at the time of cryopreservation impacts subsequent tumor kinetics, with smaller intact fragments being optimal for tumor take and growth. This study has important implications for ensuring the quality of PDX engraftment projects, particularly those using cryopreserved tumor products and PDX tumor model passages.

### P237 Evaluation of Thermoneutral Housing Effects on Disease Progression in a GvHD Model

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The thermoneutral zone is the temperature range in which an organism can maintain core body temperatures at a normal metabolic rate. For mice, thermoneutral temperature is approximately 30-32°C. However, standard housing temperatures for laboratory mice according to the *Guide* is 20-26°C. The additional energy expenditure required for thermoregulation at subthermoneutral housing temperatures may have physiological consequences, including altered immunological responses. The goal of this study is to determine whether housing temperatures affect disease progression in mice with graft-versus-host disease (GvHD). Female NOD.Cg-Prkdc<sup>scid</sup>>Il2rg<sup>tm1Wjl</sup>/SzJ (NSG) mice, aged 6- to 8-wk old, were injected intravenously with ~10<sup>7</sup> peripheral blood mononuclear cells (PBMCs) to induce GvHD and assigned to the following groups: control A (22°C standard/cool housing temperature with a dome shack), control B (22°C standard/cool housing temperature with no shack), experimental A (30°C warm housing temperature with a shack), and experimental B (30°C warm housing temperature with no shack). Mice were housed in groups of 5 on shaved Aspen and wood chip bedding. Results were determined by comparison of disease activity index (DAI) scores, flow cytometry, and survival curves between groups. DAI scores were significantly higher for the cold-housed groups. There were also significantly higher levels of human CD45+ cells in experimental A group compared to control B group. All other cell populations evaluated were not significantly different between the groups. Our data show a significant difference in disease progression and survivability between warm- and cold-housed groups. Based on these findings, standard housing temperatures may have confounding effects on experimental GvHD studies.

### P238 Chronic Elevation of a Noncytotoxic Human-amylin Agonist Disrupts Metabolic Fuel Regulation in Transgenic Mice

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<sup>1</sup>School of Biological Sciences, University of Auckland, Auckland, New Zealand; <sup>2</sup>Centre for Advanced Discovery and Experimental Therapeutics, University of Manchester, Manchester, United Kingdom The American Heart Association reports that more than 380 million people globally have diagnosed diabetes. Obesity and diabetes have become major global concerns. They are characterized by hyperphagia and insulin resistance. Insulin resistance is a central mechanism in obesity and type 2 diabetes. Elevated blood amylin, termed hyperamylinemia, occurs in patients with insulin resistance, obesity,

and diabetes, but whether it plays a significant role in the pathogenesis of these conditions remains unresolved. To determine the metabolic effects of chronic hyperamylinemia in FVBn transgenic mice overexpressing a noncytotoxic human amylin agonist [<sup>25,28,29</sup>triprolyl]-hA in their β-cells (L44), we measured weekly food intake, body weight, and blood-glucose values and collected tail serum for hormone measurements. Increased food intake became evident in L44 mice (n=28) from ~day 80 and peaked at ~day 195, coinciding with peak blood glucose (~day 190), after which both declined. Following these peaks, elevated body weight and food intake persisted throughout the remainder of the lifecycle. We performed continuous indirect calorimetry in L44 transgenic (n=14) and matched nontransgenic littermates (n=6) during the period from ~day 150 to day 250 and evaluated activity, food consumption, O<sub>2</sub> consumption (VO<sub>2</sub>), and CO<sub>2</sub> production (VCO<sub>2</sub>). VO<sub>2</sub> and VCO<sub>2</sub> values were used to calculate the RER (respiratory-exchange ratio) and energy expenditure. L44 transgenic mice displayed a consistent trend across the entire 24-h period towards increases in VO<sub>2</sub>, VCO<sub>2</sub>, RER, and energy expenditure (all *P* < 0.0001), consistent with an elevated propensity for greater carbohydrate oxidation. We have addressed the question, "What is the response to chronic overproduction of a non-aggregating amylin agonist and how might it contribute to the pathogenesis of obesity and diabetes?" by constructing a novel transgenic mouse model (L44) that over-expresses the nonaggregating agonist, [<sup>25,28,29</sup>triprolyl]-hA in its β-cells. We found that these include pathogenic effects on systemic metabolic regulation (body weight, food intake, and blood glucose), levels of metabolic hormones (amylin, insulin, leptin, and adiponectin) and disruption of fuel regulation.

### P239 Evaluating Heating Methods for Mice

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Heat therapy can be used in many different ways for mice. It can be used after anesthesia to speed recovery, to treat hypothermia for mice found in wet cages, and to aid intravenous dosing. We evaluated different methods of heat therapy to determine its effectiveness at increasing mice temperature as well as increasing the overall temperature of the cage. We compared heat lamps with 75-, 100-, and 150-watt bulbs, an electric heating pad, and a slide warmer, using a standard, bedded shoebox style cage with each heating modality. The bedding inside each cage was a mixture of Aspen wood chips and shavings. We measured intracage temperature over a 10-minute period as well as rectal temperatures of mice placed on heat therapy for 10 min. Temperatures inside the cage without the heat therapy averaged 25.3°C. Over a 10-min period, the average temperature of a cage placed on the slide warmer increased 10°C compared to 5°C for cages placed on the electric heating blanket and 3-7°C for cages under the heat lamp. As expected, the higher wattage bulbs produced high intracage temperatures. Rectal temperatures for mice were similar between the different bulb wattages and the electric heating blanket however temperatures of mice from the slide warmer were not much warmer than control mice. Naïve mice had an average temperature of 35°C. Rectal temperatures for mice warmed on the slide warmer for ten minutes averaged 36.7°C compared to 37.06°C for the electric blanket and 37.2-37.9°C for the heat lamp. Overall we recommend that slide warmers not be used for heat therapy as they minimally increased the mouse's rectal temperature compared to other heating methods. Electric heating pads and heat lamps provide appropriate heat therapy for a 10-min period, however, longer durations should be evaluated prior to implementation to ensure mice do not overheat. Alternatively, cages can be placed halfway on/near the heating element to allow mice to self-regulate their proximity to the heat source as needed.

### **P240 Plasma Bupivacaine Levels in Sheep Implanted with a Wound Diffusion Catheter following Thoracotomy for Ventricular Assist Device Study**

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Neonatal or young small ruminants administered repeated small doses of local anesthetics can develop systemic toxicity. Bupivacaine has been shown to have greater cardiotoxic effects in comparison to other commonly used amide local anesthetics, such as lidocaine and mepivacaine. Bupivacaine is the local anesthetic of choice for lambs and adult sheep undergoing thoracotomy for ventricular assist device implantation at our institution due to its long duration of action (6-8 h). Current literature reports a safe dose of bupivacaine in small ruminants as 2 mg/kg. To date, no studies have investigated systemic levels of 0.25% bupivacaine following administration via a wound diffusion catheter for local anesthesia in sheep. Dorset crossbred lambs (n=3, 26.7 ± 5.9 kg) and adult sheep (n=1, 92.7 kg) received intermittent boluses of 0.25% bupivacaine (5 ml (12.5 mg) for lambs, 8 ml (20 mg) for adult sheep) approximately every 6-8 h for 3 d following thoracotomy for local anesthesia as part of their multimodal analgesic regimen. Sheep are dosed based on the volume of bupivacaine required to bathe the length of the incision while preventing excessive volume administration and subsequent seroma development. The average bolus dose of bupivacaine administered was 0.42 ± 0.16 mg/kg. A total of 12 plasma samples per animal were collected including 1 baseline control sample collected the day prior to bupivacaine administration. Plasma samples were collected approximately 6 h after bupivacaine administration in lambs and 1 h following bupivacaine administration in the adult sheep. The average plasma bupivacaine level was 50.3 ± 48.1 ng/ml (range 0-364 ng/ml), which is well below the reported safe dose of bupivacaine in small ruminants. Effective pain management was achieved in all sheep as a result of multimodal analgesia administration.

### **P241 Sex and Stimulus Movement Modulate Response Acquisition in a Species-dependent Manner**

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African green monkeys (*Chlorocebus aethiops sabaeus*) may serve as an alternative laboratory primate model to commonly used macaques species. However, questions remain as to the ideal behavioral training parameters and whether differences in response acquisition exist as a function of sex and/or species. The present study addressed some of these questions by analyzing acquisition of an autoshaped touchscreen response of cynomolgus macaques (*Macaca fascicularis*) and African green monkeys. In the procedure, a neutral stimulus was paired with food pellet deliveries. Touching the stimulus was not required, but repeated pairings engendered responding in the majority of subjects. Posthoc analyses were conducted to address whether response acquisition differed as a function of sex, species, stimulus movement, or some combination of these factors. Cynomolgus males (n=8) acquired the response faster with a moving stimulus, whereas cynomolgus females (n=8) acquired the response faster with a stationary stimulus. In African green monkeys, the males (n=17) acquired the response more reliably with a stationary stimulus and the females (n=6) acquired the response equally well across conditions. These results suggest that acquisition under conditions of stimulus movement may be differentially affected by sex and the species under study. African green monkeys do appear to provide a suitable and readily available alternative, but important differences should be appreciated. Additional experiments will help determine how sex and species interact with behavioral phenomena discovered and elaborated almost exclusively using males and macaques.

### **P242 Transcription Activator-Like Effector Nuclease-mediated Knockout of Tumor Suppressor Gene *p19<sup>arf</sup>* Causes Ocular Defects without Tumorigenesis in FVB Mice**

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The *p19<sup>arf</sup>* gene encodes a well-known tumor suppressor *p19<sup>arf</sup>* (*p14<sup>arf</sup>* in humans) which stabilizes p53 by interacting with *mdm2* (*hdm2* in humans). Previous studies reported that conventional *p19<sup>arf</sup>* knockout (KO) mice generated by replacing the exon 1 $\beta$  with Neomycin-resistance (*Neo<sup>R</sup>*) gene, exhibited persistent hyperplastic primary vitreous (PHPV), an ophthalmic disease characterized with accumulation of perivascular cells and cataract. However, *Neo<sup>R</sup>* has been reported to cause undesirable sideeffects and complicate the manifestation of target gene-related phenotypes, raising a concern on the role of *p19<sup>arf</sup>* observed in the KO mice. To elucidate the precise function which *p19<sup>arf</sup>* performs, we generated a new *p19<sup>arf</sup>* KO mouse line using a *Neo<sup>R</sup>*-free Transcription Activator-Like Effector Nuclease (TALEN) method, and examined them for basic and ophthalmologic phenotypes for 26 wk. Ninety-five percent (19/20) of KO mice exhibited cataract whereas none of wild-type and heterozygous (HT) mice showed the pathology. Among the mice with cataract, 31% (6/19) and 69% (13/19) of KO mice showed unilateral and bilateral lesions, respectively. Cataract first appeared 4 wk after birth in KO mice and prevented observation of optic nerve and blood vessels using fundus photography. The pathology worsened until 6 wk of age without further aggravation thereafter. In histopathological analysis, increased number of vacuoles in lens, rupture of lens capsule, and detachment of neuroretina from the retina pigment epithelium were detected in the diseased eyes. In conclusion, we generated a novel *p19<sup>arf</sup>* KO FVB mouse line and observed severe cataract from the pre-pubertal stage of development, confirming the crucial function of *p19<sup>arf</sup>* in the eyes. Additionally, our findings proved the usefulness of our mice, providing them as a useful animal model for study of cataract in humans.

### **P243 Male SCID Mice As New Animal Model for Inflammatory Breast Cancer Research**

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In vivo studies on breast cancer research are carried out on female immunosuppressed mice; however male breast cancer is a rare disease and its prevalence is increasing in the last decades. Therefore, we evaluated the capacity of male compared to female SCID mice to develop inflammatory breast cancer (IBC) tumors by inoculating the canine and human IBC cells lines (IPC-366 and SUM149). Twenty-four 6-8-wk-old male and female mice Fox Chase SCID Beige CB17.Cg-Prkdc<sup>scid</sup>Lyst<sup>tg</sup>/Crl were used. A suspension of 10<sup>6</sup>IPC-366 and SUM149 cells were injected subcutaneously into the fourth inguinal mammary gland in male and female mice. Mice were inspected twice a week for the development of tumors and when tumors were detected, they were weekly monitored by palpation and measured by calipers. Once tumors reached 1500 mm<sup>3</sup> of volume mice were sacrificed and tumors were homogenized for steroid hormone analysis. IPC-366 reproduced tumors in 90% of male mice compared with a frequency of 100% of female mice after 2 wk of cell injection. Interestingly, SUM149 reproduced tumors in 40% of male mice compared to 80% of female

mice after 3 and 4 wk of inoculation respectively. In male mice models, both cell lines had the ability to metastasize in lungs, however, metastatic rates were higher in female mice than in male mice (IPC-366: 90% versus 20%; SUM149: 80% versus 50%). Hormonal analysis revealed that male tumors had higher T and SO4E1 levels compared to female tumors. Despite male mice reproduced tumors with less frequency than female mice, these results showed that male mice could be a useful as a tool for in vivo IBC research.

#### **P244 Expression of NF- $\kappa$ B Targeted Genes in Psoriasis Linked Transgenic Murine Keratinocytes**

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Psoriasis is a poorly understood debilitating inflammatory skin disorder. CARMA2sh associated with CARD domain containing BCL10 and MALT1 (CBM complex) are regulating activation of transcription factor NF- $\kappa$ B. Mutation in CARMA2 gene affects the regulation and activation of NF- $\kappa$ B, which controls the immune and inflammatory response, cell survival, and cell proliferation. Distinct characteristic feature of psoriasis has been highly expressed when there is a missense mutation in CARMA2. CARMA2 is a molecular target for the treatment of psoriasis and other inflammatory disorders due to deregulated activation of NF- $\kappa$ B. Recently a novel CARMA inhibitory serine/threonine kinase which inhibits the ability to induce NF- $\kappa$ B was identified. Our objective is to explore the influence of CARMA inhibitory kinase on CARMA2sh and to check the efficacy of NF- $\kappa$ B activation in transgenic keratinocytes. Two CARMA2 point mutations associated with psoriasis (Gly117Ser and Glu 138Ala) were created by site-directed mutagenesis and generated transgenic murine psoriatic model using mouse embryonic stem cells. Transgenic keratinocytes were cultured and infected with lentiviral vector contain CARMA inhibitory serine/threonine kinase to investigate the expression levels of NF- $\kappa$ B targeted genes. BCL10 is a key regulator of the NF- $\kappa$ B-inducing activity of wild (wt) and psoriasis-associated CARMA2sh mutants. CARMA inhibitory serine/threonine kinase binds with CARMA2sh and phosphorylated, which inhibits the capacity to regulating activation of NF- $\kappa$ B stimulates BCL10 degradation were analyzed using gene expression and western blotting analysis. Results revealed that the BCL10 reduction was observed at protein level and there is expression level of NF- $\kappa$ B is not at significant level. Our study leads to a vital role for CARMA2 inhibitory kinase in triggering the degradation of BCL10 as a mechanism for reducing NF- $\kappa$ B-activating stimuli conveyed through the CBM complex.

#### **P245 Titration of Antigen Doses for Optimal Immune Responses in Rodents**

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Successful generation of therapeutic antibodies from rodents usually requires immunizing animals with recombinant protein antigens, as DNA antigens usually generate weak immune responses, while linear peptide antigens can induce antibodies that may not recognize native proteins. However, producing large quantities (in the milligram range) of high-quality recombinant antigen can be challenging, especially for multipass membrane proteins. The optimal dose of protein antigen used for priming and boosting animals depends on a variety of parameters, including immunogenicity of the antigen itself, immunization species, adjuvant, frequency, and route of dosing. While lower doses of antigen have been thought to drive more stringent selection (in terms of affinities) of antibodies in vivo, it is difficult to determine a priori how low we can go before adversely affecting total antigen-specific immune response. To analyze relationships between antigen dose amounts and quality of resulting immune responses, we immunized 36 Sprague Dawley rats with 3 protein antigens (of

varying levels of immunogenicity) at 4 different dose groups (using 3 rats per group) starting off at the highest priming dose of 100 ug and 7-10 boosts of 50 ug. Immunizations were administered subcutaneously near shoulders and base of tail, intraperitoneal, and hock. Groups 2, 3, and 4 had successively half the priming (50 ug, 25 ug, and 12.5 ug, respectively) and boosting doses (25 ug, 12.5 ug, and 6.25 ug, respectively). Blood was collected through retroorbital bleeds. We analyzed antigen-specific serum IgG titers, and generated hybridomas from all 4 groups. Interestingly, for 2 out of 3 antigens, lowering antigen doses 4-fold had no effect on serum titers and more importantly, numbers of antigen-specific clones obtained. Immune response to the third antigen (which had the weakest immunogenicity) was significantly decreased with lowered doses of antigen. These results indicate that except for weakly immunogenic antigens (high homology across target and host species for example), low antigens doses can be used without adversely affecting immune responses.

#### **P246 Seroprevalence of Rodent Pathogens in Wild Rats from St. Kitts**

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Peridomestic and wild rats may pose an animal biosecurity risk to laboratory rodent colonies due to the possibility of pathogen spillovers. Thus, routine pathogen surveillance in the wild population is essential to maintain a microbiologically defined rodent colony health status. A pilot surveillance study was conducted to gather information on the exposure of selected pathogens in wild rats inhabiting the Caribbean island of St. Kitts. Serum samples collected from 22 of 29 rats captured were tested for the presence of antibodies to various rodent pathogens using a rat serology panel. The rat species were identified as *Rattus norvegicus* (11/29; 37.9%) and *Rattus rattus* (18/29; 62.1%) based on amplification and sequencing of the mitochondrial cytochrome b gene. Exposure to 11 of 19 (57.9%) pathogens tested in the panel was detected, and 21 of the 22 (95.5%) rats sampled were positive for 1 or more pathogens tested. Presence of antibodies to the following pathogens was detected: mouse adenovirus type 2 (16/22; 72.7%), Kilham's rat virus (15/22; 68.2%), cilia-associated respiratory bacillus (13/22; 59.1%), reovirus type 3 (9/22; 40.9%), rat parvovirus (4/22; 18.2%), rat minute virus (4/22; 18.2%), rat theilovirus (2/22; 9.1%), infectious diarrhea of infant rats (1/22; 4.5%), *Clostridium piliforme* (4/22; 18.2%), *Mycoplasma pulmonis* (4/22; 18.2%), and *Pneumocystis carinii* (1/22; 4.5%). Antibodies to Hantaan virus, lymphocytic choriomeningitis virus, Toolan's H-1 virus, mouse adenovirus type 1, pneumonia virus of mice, rat coronavirus/sialodacryoadenitis virus, Sendai virus, and *Encephalitozoon cuniculi* were not detected. This study provides the first evidence of exposure to various rodent pathogens in wild rats on the island of St. Kitts.

#### **P247 Contaminated Shipping Materials Identified as the Source of Rotavirus Infection in Exported Mice**

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Over a 4-wk period in 2017, our institution received notification from 7 different institutions that mice exported from our specific-pathogen free (SPF) barrier facilities had tested positive for mouse rotavirus (MRV). The exports originated from several different buildings across multiple campuses. Our institution excludes MRV in all of our barrier facilities and has historically been free of this virus. Extensive testing of our rooms from which the exported mice originated did not detect the presence of rotavirus. The single commonality identified among the 7 shipments was the use of shipping boxes acquired from 1 vendor. These shipping boxes arrived at our institution prepackaged with unsterilized feed and bedding which we hypothesized was the source of the rotavirus. To test this hypothesis we housed naïve sentinel mice



in clean cages with feed and bedding transferred from 29 unopened unused shipping boxes. Sentinel mice were exposed to this bedding and feed for 14 d then evaluated by MRV serology and PCR. Twenty-four of the 29 sentinels were seropositive and 14 of 29 were PCR positive. These results provided direct evidence that MRV detected by recipient institutions originated from the contaminated feed or bedding within the shipping boxes. To our knowledge, this is the first report of contaminated materials in shipping boxes resulting in a rotavirus infection of mice during export.

#### P248 Minipig Model for the Evaluation of Microneedle Delivery

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Porcine skin has been recognized as a close match for human skin due to similarities in physiology and anatomy. Intradermal (ID), microneedle-assisted drug delivery can provide several benefits, including increased patient compliance, unique pharmacokinetics (PK), and improved compound stability as formulated in the solid-state. The purpose of the study was 2-fold 1) to evaluate suitability of different pig breeds for ID injection and 2) to assess prototype microneedle array application and skin irritation in a preferred porcine species. ID injection studies were carried out in Yucatan, Micro-Yucatan, Sinclair, and Hanford miniature pigs. Using n=6 injections per breed of pig, injections were qualitatively assessed based on criteria of needle insertion, formulation leakage, ID wheal formation, and time to injection. PK studies were conducted in a cohort of 4 anesthetized female Yucatan minipigs weighing approximately 40 kg. Isoflurane, 5% to effect (O<sub>2</sub>, 2%), inhaled, was investigated as an alternative to injectable anesthesia (Telazol, 8-10mg/kg, intramuscular). Skin preparation pre-microneedle application involved hair clipping to remove the bulk of the hair, shaving to remove stubble, and skin scrub to remove dead skin. Once needles were removed, the 5-point Draize scoring was used to monitor erythema and edema over 72 h. In addition, injection sites were dyed with 2% Genticin Violet to quantify microneedle array insertion efficacy by counting individual microneedle insertions. Blood samples were collected pre-dose at 15 min., 30 min, 1 hr, 2 h, 4 h, 6 h, 8 h, 24 h, 30 h, and 48 h post-dose. The Yucatan minipig was selected as the preferred minipig model for microneedle evaluation based on high reproducibility of successful injections, that is, manageable piston backpressure and lack of formulation leakage. A novel microneedle array was successfully inserted in Yucatan minipig skin with >90% insertion efficiency and demonstrated only a transient skin erythema with ≤2 Draize scores. Overall, these preclinical studies demonstrated that Yucatan minipigs are suitable for both hollow and solid microneedle system assessments and present a valuable tool in the preclinical evaluation of microneedle-assisted drug delivery.

#### P249 FOLH1/GCPII Inhibitors in Inflammatory Bowel Disease: Evidence of Local Mechanism of Action

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Inflammatory bowel disease (IBD) is a group of debilitating chronic inflammatory conditions affecting an estimated 3.1 million people in the U.S., with one-third of patients unresponsive to available therapies. The folate hydrolase gene (*FOLH1*), which encodes for the enzyme glutamate carboxypeptidase II (GCPII), is highly overexpressed in human IBD where it is associated with robustly increased GCPII enzymatic activity (300-1000%). We have previously shown that administration of a potent, selective, and non-orally bioavailable GCPII inhibitor, 2-(phosphonomethyl)-pentanedioic acid (2-PMPA; IC<sub>50</sub>=300 pM), via either intraperitoneal or oral dose routes, significantly reduces disease severity in the dextrose-sodium sulfate

(DSS) model of colitis (n=20, C57BL/6NHsd). However, it is unclear if 2-PMPA is exerting its protective benefits in this model through a local or systemic action, which is essential for translational development. A known limitation of the DSS-colitis model is that over the course of DSS-exposure the epithelial barrier becomes increasingly compromised and develops heightened permeability that is not representative of human disease. To assess the local versus systemic activity of 2-PMPA, we have now performed early biomarker studies in which C57BL/6NHsd (n=20) mice were exposed to DSS + oral 1 mg/kg 2-PMPA once daily for 3 d. Colon was harvested on day 3, a time point prior to epithelial barrier disruption, and biomarker analysis was performed. We found that DSS induced increases in mRNA expression of IL-1β, a proinflammatory cytokine implicated in the pathogenesis of both human and murine colitis. This increase was significantly attenuated with 2-PMPA treatment (*P* < 0.05). Additionally, trends towards improvement were identified for other known players in IBD including VEGF and iNOS, both of which are involved in inflammation and associated neovascularization. Parallel studies are in progress to ascertain whether 2-PMPA has a direct effect on colon epithelial cells in vitro. In summary, we have previously demonstrated that *FOLH1/GCPII* is a therapeutic target in human IBD. Our current findings show that 2-PMPA has direct local effects in DSS-colitis and supports that an orally administered, GI-restricted, GCPII-inhibitor may be a translationally relevant IBD therapeutic.

#### P250 Pharmacokinetics and Efficacy of Oral Mirtazapine in Guinea Pigs (*Cavia porcellus*)

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Guinea pigs are cecal fermenters requiring frequent and consistent feed intake to ensure normal gut motility. Transport, age-related disease, diet changes, and other sources of chronic stress can reduce their appetite, leading to gastrointestinal stasis which can be life threatening in this species. Mirtazapine, a tetracyclic antidepressant, is used in dogs and cats to treat nausea and inappetence, and has been shown to increase feed intake in cats. It has anecdotally been used as an appetite stimulant in guinea pigs, but a therapeutic dose of mirtazapine has not yet been established for this species. Six healthy male guinea pigs were administered mirtazapine at 1.88, 3.75, or 7.5 mg orally once daily for 4 d, where each guinea pig received all doses over 3 different sessions separated by a 7-10 d washout period, in a randomized crossover design. Blood for serum pharmacokinetic analysis was collected prior to the first dose of each session and at time points 0.5, 1, 2, 8, 12 and 24 h after the first dose was administered. Body weight, feed intake, and fecal output were recorded every 24 h for each guinea pig during the dosing sessions and washout periods. Significant differences in weight gains, feed intake, and fecal output were not present, as compared to 3 male guinea pigs of similar age given saline only, suggesting that once daily mirtazapine does not have a significant effect in young, healthy guinea pigs. The pharmacokinetic results showed peak plasma levels being reached at 30 min and returning to 0 at 8 h, demonstrating that dosing every 24 h is not appropriate for this species. Further studies with dosing performed every 8 h is planned to more clearly elucidate the effects of mirtazapine in this species.

#### P251 Comparison of Different Anticoagulants for Multiple Electrode Aggregometry

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Normal platelet function may become compromised through the administration of various drugs, diseases, or genetic factors. Knowledge of acquired or inherited platelet disorders in laboratory animals may help researchers better understand the pathogenesis of

human platelet dysfunction and promote the development of improved therapeutic strategies. Multiple electrode aggregometry (MEA) can be used to analyze platelet function by measuring the velocity of platelet aggregation in response to platelet agonists such as adenosine diphosphate (ADP) and collagen. Hirudin is the manufacturer-recommended anticoagulant for the MEA testing apparatus. However, hirudin blood collection tubes are not readily available in most laboratories thus prompting the investigation of alternative anticoagulant blood collection tubes. We hypothesized that hirudin and lithium-heparin tubes would provide similar results, while sodium citrate tubes would produce variable results given its ability to chelate calcium, an important regulator in the coagulation cascade. Ten-ml whole blood samples were collected from 4 naïve adult male beagle dogs using the cephalic and jugular veins. Samples were divided into hirudin, lithium-heparin, and sodium citrate anticoagulant tubes in preparation for analysis. Platelet function was analyzed using the MEA apparatus by measuring the velocity of platelet aggregation in response to platelet agonists. Platelet counts were also performed for each animal. Results of the MEA test are reported as area under the curve with arbitrary units; this unit was normalized to each animal's platelet count. For the ADP agonist, canine platelet function did not statistically differ when blood was collected into hirudin or lithium-heparin tubes ( $0.40 \pm 0.05$  or  $0.34 \pm 0.03$ ;  $P = 0.254$ ). The sodium citrate samples compared to the hirudin samples differed significantly ( $0.40 \pm 0.05$  versus  $0.22 \pm 0.03$ ;  $P = 0.010$ ). When testing the collagen agonist, canine platelet function was different between blood collected into hirudin tubes versus lithium-heparin tubes ( $P = 0.038$ ), but this difference was much more significant when comparing hirudin samples to the sodium citrate samples ( $P = 0.001$ ). Lithium-heparin and sodium citrate tubes are universal and more practical for blood collection compared to hirudin tubes. This study revealed that lithium-heparin tubes can be used as a reliable alternative to hirudin tubes for MEA.

#### **P252 Historical Background Control Data in the RccHan:WIST rat in Chronic Toxicity Studies**

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Historical background control data supports improved interpretation of lesions in chronic toxicity studies. A chronic toxicity study was performed to generate historical background control data in the RccHan:WIST rat model. One-hundred and thirty male and 130 female RccHan:WIST rats were pair-housed under standard housing and husbandry conditions with ad libitum access to a lower energy diet (16% protein, 4% fat) and water. Rats were administered tap water (10 mL/kg body weight) by once weekly gavage. Body weight, food consumption, morbidity, and mortality were monitored throughout the in-life phase of the studies. Interim necropsies were performed at 1, 3, and 6 mo in 20 animals per gender for generation of historical control data at common acute or subchronic study time points. At the conclusion of the study period, remaining animals were submitted for necropsy and microscopic evaluation of select organs was performed. Neoplastic and nonneoplastic lesions were characterized and complete blood count/serum chemistry was performed. A detailed analysis of survival, body weight, clinical pathology, and histopathology results will be presented. These data support the use of the RccHan:WIST rat as a valuable toxicology model and will assist in interpretation of lesion data in future studies with this model.

#### **P253 Maternal and Embryo-fetal Historical Background Control Data in the Hsd: Sprague Dawley Rat**

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Maternal and embryo-fetal historical background control data

supports improved interpretation of lesions in reproductive toxicity studies. A maternal and embryo-fetal toxicity study was performed to generate historical background control data in the Hsd:Sprague Dawley rat model. One-hundred previously nulliparous and virgin time-mated female Sprague Dawley rats, allotted in 4 subsets of 25 rats each, were housed under standard housing and husbandry conditions with ad libitum access to a standard diet (18% protein, 6% fat) and water. Rats were administered tap water (10 mL/kg body weight) by oral gavage once daily from gestational day 6 to 17. Body weight, food consumption, and clinical observations were monitored throughout the in-life phase of the study. On gestational day 20, animals were euthanized and submitted for Cesarean section and necropsy. A macroscopic postmortem evaluation was performed on all animals, including counts of corpora lutea and implantations and uterine weights. Fetuses were removed, weighed, sexed, and examined externally for defects as well as soft tissue abnormalities and skeletal abnormalities. Placentas were examined and weighed. One-hundred dams were pregnant and 100 litters were evaluated. There were 1,488 fetuses examined externally; 698 had fixed visceral evaluations and 790 fetuses had skeletal evaluations. A detailed analysis of maternal data, including body weight, food consumption, and pregnancy outcome will be presented. Fetal data including uterine and placental weights, in addition to fetal examinations, including external, soft tissue (visceral), and skeletal will also be presented. These data support the use of the Hsd:Sprague Dawley rat as a valuable toxicology model and will assist in interpretation of lesion data in future studies with this model.

#### **P254 Evaluation of High Doses of Buprenorphine for Greater Effectiveness and Extension of Analgesia in Mice**

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Appropriate dosing of analgesics requires the researcher to adhere to a proper dosing schedule. Traditionally, small doses of buprenorphine (0.05–3.0 mg/kg SQ) have been used which must be administered every 6 to 12 h. A more convenient dosing schedule may increase compliance, thereby increasing the welfare of the rodents after surgical manipulation. Simbadol is a buprenorphine injection for cats. Unlike other sustained-release buprenorphine, Simbadol contains no slow-release element and increases the concentration of buprenorphine at the time of administration (6 x the normal concentration) for a 24-h analgesic duration. We attempt to determine if higher doses of buprenorphine can extend the functional duration of analgesia in mice. C57BL/6J and CD1 mice were evaluated for baseline nociceptive response by exposing them to a hot plate stabilized at 50° C and measuring the time between exposure and response (either vocalization, a jump, or a hind paw lick). The following day the same mice were treated with a single dose of 1 of 9 doses of buprenorphine (0.05, 0.1, 0.5, 1.0, 2.0, 3.0, 5.0, 7.0, or 10.0 mg/kg), then challenged post-injection on a 50° C hot plate at 30 min, 4 h, 8 h, 24 h, 48 h, and 120 h (n=10 for each group; 5 males, 5 females). A previous study revealed a high therapeutic index for subcutaneous buprenorphine administration in mice, as the authors were unable to calculate an LD50 for mice even at doses as high as 300 mg/kg. Our results suggest that for acute, A-δ fiber nociception, doses above 2.0 mg/kg may be necessary for appropriate analgesia; at doses of 3 mg/kg, CD1 mice may experience prolonged analgesic duration; and, unexpectedly, at doses between 5 and 10 mg/kg, mice may experience hyperalgesia at time points as far as 120 h post-injection. The main side effect observed was hypoactivity in doses above and including 7 mg/kg. Other side effects were not observed or measured due to the invasive nature of measurement (temperature, heart rate). We recommend using a dose of 3.0 mg/kg in CD1 mice and 5.0 mg/kg in C57BL/6J mice to extend the duration of analgesia for Type A-δ fiber-associated pain up to 24 h. Further studies are necessary to determine whether these doses are appropriate for Type C fiber-associated pain, as well as to determine the significance of potential side-effects. We feel the risk of limited side

effects is outweighed by the benefits of enhancement of analgesia at the higher doses of 3–5 mg/kg.

### **P255 Comparison of Microbiota among Multiple Sites in Cranially Implanted Research Macaques**

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Macaques are commonly used in cognitive neuroscience research due to similarities between the macaque and human brain. Cephalic recording chambers (CRCs) are commonly implanted to facilitate electrode placement for neuronal recordings. Bacterial colonization of CRCs is common and occasionally results in clinical sequelae, such as meningitis and brain abscessation. The goal of this study was to compare the microbiota colonizing CRCs, the peri-CRC skin margin, the oral cavity, and feces and to determine the likely source of CRC bacterial colonization. Paired samples of CRC exudate, feces, and swabs of the oral cavity and implant skin margin were collected from 18 rhesus macaques (15M, 3F, age range 4–19; n=79 samples). DNA was extracted from samples using the DNeasy Powerlyzer Powersoil kit. The V4–V5 region of the 16S rRNA gene was amplified by PCR and sequenced using 300 paired-end reads. Alpha diversity of observed operational taxonomic units (OTUs) was significantly lower in CRC exudate communities ( $\mu=60.5\pm 53.1$ ) as compared to fecal ( $\mu=230.4\pm 39.1$ ,  $P=0.006$ ) and oral communities ( $\mu=108.6\pm 21.4$ ,  $P=0.024$ ) but not skin communities ( $\mu=125.5\pm 80.9$ ,  $P=0.054$ ). Principal coordinate analysis of unweighted Unifrac distances revealed closely clustered bacterial populations from fecal samples and oral swabs, while CRC exudate and skin margin samples were more intermixed. Comparison of unweighted Unifrac beta-diversity within samples from individual macaques identified that CRC communities were significantly different from fecal, oral, and skin communities in 85%, 27% and 26% of macaques, respectively (Bonferroni-corrected  $P\leq 0.01$ ). Overall, our data indicates that the majority of bacterial species colonizing CRCs arise from skin and oral communities, rather than from fecal contamination. Strict peri-implant margin skin sanitization protocols and regular dental hygiene may be beneficial targets for limiting bacterial colonization of CRCs.

### **P256 In Vivo Evaluation of Gold Nanoparticle Conjugated Grafts Using an Ovine Anterior Cruciate Ligament Model**

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Roughly 200,000 Americans will require surgical repair of their anterior cruciate ligament (ACL) every year. A majority of ACL repairs today involve the use of an autograft, but due to the morbidity of graft harvest, there is an increased interest in allograft use. However, studies have shown up to 23% of allograft patients will require a revision surgery. One reason for this is allografts are not as rapidly remodeled and incorporated into host tissue as autografts. Recently the use of gold nanoparticles has arisen as a potential solution to this problem. Gold nanoparticle attachment modifies the surface structure and encourages cellular attachment and proliferation. This increased surface energy promotes attachment of proteins including those necessary for cellular attachment. We hypothesized that the attachment of nanoparticles to an ACL autograft will increase cellular incorporation. To test our hypothesis 6 Polypay sheep had their ACL surgically removed and replaced with a decellularized human gracilis tendon. The tendon was crosslinked using the previously published 2 mM 1-ethyl-3-[3-dimethylpropyl]carbodiimide and 5 mM N-hydroxysuccinimide method with or without the addition of 20 nm gold nanoparticles. Descriptive analysis of histologic changes, quantitative analysis of revascularization, and semiquantitative

histological scoring of the bone tunnel portion of grafts were performed after 8 wk. The results of this study revealed both the experimental and control grafts to be biocompatible however there were no statistically significant differences between the experimental and control groups. Several promising trends were present nevertheless. The joint fluid for the experimental grafts had a greater percentage of normal synovial cells, and the experimental graft had less graft breakdown and a greater amount of blood vessel formation on average. In conclusion, this study demonstrated the biocompatibility of a gold nanoparticle tissue scaffold in a large animal model.

### **P257 In Vitro Effects of Demethylating Agents Decitabine and 6-thioguanine in Canine Osteosarcoma**

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The role of epigenetic aberrations in carcinogenesis have become more transparent. Hypermethylation occurs at CpG islands causing gene silencing, while hypomethylated regions allow transcription of genes. Dysregulation can lead to abnormal proliferation, migration, and cell invasion. These aberrations have been shown to play an important role in osteosarcoma, which is the most prevalent bone cancer in young adults and dogs. The disease in these species shares a number of characteristics, making dogs an ideal comparative model. We hypothesized that demethylating agents would increase apoptosis and cell death while decreasing viability and global DNA methylation in canine osteosarcoma cell lines. We evaluated two demethylating agents at biologically achievable dosages, decitabine and 6-thioguanine, in 2 canine osteosarcoma cell lines: primary tumor line D17 and metastatic line Abrams. Cells were treated at 0- and 24 h-time points; apoptosis/viability were evaluated at 6, 12, 24, and 48 h after treatment. Changes in global methylation were confirmed using a genome analysis method. The Abrams line had a significant increase in apoptosis with all concentrations of 6TG and decitabine. Decreased viability and cell death were noted in all treatment groups. The low dose of decitabine, however, caused an early increase in proliferation. The D17 line had a significant increase in apoptosis at 12 h with decitabine and 12 and 24 h with 6TG. An increase in cell death and corresponding decrease in cell viability was not observed until the 48 h-time point. High dose 6TG actually caused an early increase in proliferation. Measures of cell death/life were different between drugs and cell lines used in our experiment. However, increases in apoptosis and cell death in the metastatic line is an attractive finding. These results support the use of demethylating agents as additional therapeutic agents for priming or combination chemotherapy in osteosarcoma. Based on safety studies in other canine tumor types, our group plans to initiate a prospective clinical trial in tumor bearing dogs using demethylating agents during standard of care treatment.

### **P258 Effects of Dietary Supportive Care on Bioluminescent and Fluorescent Imaging in *Mus musculus***

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In vivo optical imaging is widely used in preclinical studies to evaluate drug efficacy on metastatic models. Implementing optical imaging at the onset of metastasis, the background signal must be reduced as low as possible to accurately detect low signal lesions. It is well-established that chlorophyll containing diets have significant auto-fluorescence and -luminescence that interferes with detectability of small lesions. Current literature recommends a complete purified alfalfa-free diet for at least 4 d prior to imaging for background reduction. These efficacy

studies may require nonpharmaceutical dietary supportive care to improve quality-of-life and many of these feeds may show unacceptable levels of fluorescence and luminescence, limiting supportive care options. We evaluated 10 commercially available feeds (test feeds) and compared to a standard purified alfalfa-free diet (control diet) to characterize the background signal. Each feed was imaged by both 2D bioluminescence and fluorescence. For fluorescence imaging, background levels were assessed by choosing the excitation/emission (Ex/Em) filter pairs for the predominate imaging protocols for GFP (465 nm/520 nm), mCherry (570 nm/620 nm), AF680 (675 nm/720 nm), and AF750 (745 nm/800 nm) probes. Three of the test feeds had decreased or equivalent auto-luminescence signal compared to the control diet; one of which provided additional high-caloric support. All test feeds which contained either only sucrose or a sterile water source showed reduced fluorescence compared to the control diet at all tested wavelengths. Additionally, for wavelengths imaging GFP, all 10 test feeds showed reduced fluorescence compared to the control diet, including 5 nutritionally complete gel-based diets. A fortified water gel supplement showed reduced or equivalent fluorescence at AF680 and AF750 wavelengths and one nutritionally complete gel-based diet showed decreased fluorescence at the AF680 wavelength. These supportive care options may help prolong study endpoints and increase wellbeing in rodent metastatic models undergoing optical imaging procedures.

#### **P259 Analysis of Gut Microbiota Diversity from a Commercial C57BL/6NTac Production Barrier**

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The role of the microbiome in health remains a significant area of research for many investigators with newly discovered links to various disease states. It is well established that changes in the gut microbiota of mouse models can have a major role in experimental variability. For animals sourced from commercial providers, housing in different facilities and/or with different husbandry methods may affect gut microbiota profiles. To assess sampling methodologies for profiling gut microbiota diversity of commercial mice, we collected fecal samples from male and female C57BL/6NTac mice in a single commercial specific pathogen free C57BL/6NTac production barrier and subjected them to 16s RNA sequencing to assess gut microbial diversity. In terms of richness, between 363-432 OTU were detected in animals within this production location (n=60). Although age (6 versus 7 wk, n=30) did not affect microbiome profiles, microbiome diversity was significantly different between male and female mice (n=30). The phyla Firmicutes and Proteobacteria were enriched in female mice whereas males had a higher prevalence of Bacteroidetes and Tenericutes. Female mice were enriched for 22 OTUs and males enriched for 10 OTUs corresponding to specific genera. Pooling within-sex fecal samples prior to sequencing (pools of 3, 6, or 10 cages; n=3-6) did not have a significant effect on microbiome beta diversity compared to individual cage results (n=14-16), with a comparable representation of resultant phyla. This study supports that gender-based differences in gut microbial diversity exist in commercial rodent populations and that the representative gut microbiota of a commercial production barrier can be monitored using a within-sex pooled sample approach. This study provides sampling methodology validation for characterization of fecal microbiome diversity of C57BL/6NTac mice from a commercial barrier in order to assist researchers in understanding how the gut microbiota of commercial animals may contribute to experimental robustness and reproducibility.

#### **P260 Blood Pressure Reference Intervals for Ketamine-sedated Rhesus Macaques (*Macaca mulatta*)**

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Appropriate calculation and use of reference intervals has widespread clinical and research implications. Unfortunately, reference intervals for blood pressure in one of the most commonly used nonhuman primate species, the rhesus macaque (*Macaca mulatta*), have never been calculated. While anesthetic drugs and noninvasive methods of blood pressure measurement both have known effects on blood pressure values, their use provides the safest, fastest, and most widely used approach to clinical evaluation and blood pressure collection in this species. Noninvasive blood pressure measurements were collected from more than 100 healthy, ketamine-sedated, adult rhesus macaques, representing both sexes, aged 8 to 16 y old, of varying body condition scores, with 2 types of sphygmomanometers, and at 3 different anatomic locations. Reference intervals were calculated for each device, in each location, establishing normative data beneficial to clinical veterinarians assessing animal health, and encouraging researchers to use noninvasive methods. The reference interval calculated for the mean arterial pressure using a standard oscillometer was 52-124 mmHg at the brachium, 57-134 mmHg on the pelvic limb, and 54-124 mmHg at the tail. The reference interval calculated for the mean arterial pressure using a high-definition oscillometer was 38-129 mmHg at the brachium, 52-147 mmHg on the pelvic limb, and 57-128 mmHg at the tail. Age, body condition score, sex, type of sphygmomanometer, and location of cuff placement were all found to significantly influence BP measurements, providing important information necessary for the appropriate interpretation of noninvasive blood pressure values in rhesus macaques.

#### **P261 A Field Strain of Minute Virus of Mice Exhibits Age and Strain-specific Pathogenesis**

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The influence of mouse strain, immune competency, and age on the pathogenesis of a field strain of minute virus of mice (MVMm) was examined in BALB/c, C3H, C57BL/6, and SCID mice experimentally infected as neonates, weanlings, and adults. Sera, mesenteric lymph node, spleen, peripheral lymph node, thymus, jejunum, colon, heart, lung, salivary gland, liver, pancreas, kidney, gonad, bone marrow, brain, feces, urine, and nasopharyngeal lavage fluid were harvested at 7, 14, 28, and 56 d after oronasal inoculation and evaluated by serology, quantitative PCR, and histopathology. Seroconversion to recombinant viral capsid protein 2 was consistently observed in all immune competent strains of mice, regardless of age inoculated, while seroconversion to the viral nonstructural protein 1 was consistently detected only in neonate inoculates. Viral DNA was detected by quantitative PCR in multiple tissues of immune competent mice at each time point after inoculation, with the highest levels observed in neonate inoculates at 7 d after inoculation. In contrast, viral DNA levels in tissues and bodily excretions consistently increased over time in immune deficient SCID mice regardless of age inoculated, with mortality observed in neonatal inoculates between 28 and 56 d after inoculation. Overall, productive infection was more frequently observed in immune competent mice inoculated as neonates as compared to those inoculated as weanlings or adults, and immune deficient SCID mice developed persistent, progressive infection, with mortality observed in mice inoculated as neonates. Importantly, the

clinical syndrome observed in experimentally infected SCID neonatal mice recapitulates the clinical presentation reported for the naturally infected, immune deficient NOD  $\mu$ -chain knockout mice from which MVMm was initially isolated.

### P262 Experimental Infection of Mice with *Veronaea botryosa* Models Human Phaeohyphomycosis

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*Veronaea botryosa* is a ubiquitous, saprobic, dematiaceous mold capable of causing cutaneous and subcutaneous lesions in humans, as well as systemic infection in a variety of other species. In the last decade, *V. botryosa* has been associated with emergent systemic fungal infections in aquatic animals, including cultured sturgeon (*Acipenser* spp.), captive amphibians, and wild reptiles. Recently, intraspecific variability among *V. botryosa* isolates from different clinically affected hosts and geographic regions was found using repetitive extragenic palindromic PCR fingerprinting (rep-PCR); however, little is known regarding zoonotic potential of the different genetic clades, and no animal model currently exists to investigate *V. botryosa* phaeohyphomycosis. In this study, immune competent Hsd:ATHymic Nude-Fox1nu heterozygote (nu/+) and immune deficient homozygote (nu/nu) mice were inoculated by subcutaneous injection (SC) or orogastric gavage (OG) with 1 of 3 representative *V. botryosa* strains recovered from white sturgeon (*Acipenser transmontanus*), green sea turtle (*Chelonia mydas*), and human hosts, previously typed via rep-PCR. Mice were observed daily for signs of morbidity and mortality, and dissemination of the fungus in surviving mice was investigated by culturing splenic samples 30 d postinoculation. Additionally, histological analysis of the injection site, regional lymph node, salivary gland, spleen, liver, mesenteric lymph node, and gastrointestinal tract was performed to assess fungal associated changes at the site of inoculation, as well as dissemination to other tissues. No mortality was observed in any of the treatment groups, and there were no histological changes observed in the OG-exposed animals, and fungus was not recovered from splenic samples. Mice did not develop systemic phaeohyphomycosis, but histopathological changes at the site of injection mimicked the condition seen in humans with fungal hyphae and local inflammation.

### P263 A Next-Generation Multiplexed Fluorometric Immunoassay for Serodiagnosis of Nonhuman Primate Infectious Diseases

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Multiplex immunoassays including multiplexed fluorometric immunoassay (MFIA) based on Luminescence polystyrene (PS) beads have been in use for more than 15 y for routine serosurveillance of nonhuman primate (NHP) colonies. A new NHP MFIA using the next generation magnetic MagPlex microspheres was developed. MagPlex beads have several advantages over PS beads including no prefiltration of samples, no leaky expensive filter plates, and improved washing efficiency. For example, MagPlex is quick and easy to separate from the solution using a magnetic separator. Antigens for several common infectious agents in NHP colonies including simian immunodeficiency virus (SIV), simian retrovirus (SRV), simian-T-lymphotropic virus (STLV), herpes B virus, and measles virus were part of the 19-member NHP MFIA bead panel. Whole virus or purified recombinant antigens were individually coupled to different color coded bead sets. In addition, several system and sample suitability controls including tissue control beads to determine the sample related nonspecific antibody binding, species-specific IgG and anti-IgG beads, were added to respective panels to validate individual runs of the

MFIA. Efficacy of this next generation MagPlex MFIA was compared to PS MFIA in a validation study using 16 known positive sera from naturally or experimentally infected macaques (*cynomolgus* or *rhesus*) for 1 or more of the above mentioned infectious agents. A similar number, 16 known negative macaque sera were used from specific-pathogen free colonies. All samples were tested by 2 different technicians on 3 different days for a total of 6 runs. A total of more than 3,000 assays were performed and analytical performance of the rodent MagPlex MFIA assay including selectivity and limit of detection was found to be comparable to or better than those obtained by PS MFIA. Overall diagnostic sensitivity of NHP MagPlex MFIA was 99% compared to 100% for PS MFIA. Diagnostic specificity of both NHP MagPlex and PS MFIA were nearly 100% suggesting that MagPlex MFIA is an acceptable alternative assay for serodiagnosis of adventitious infectious agents of NHP colonies.

### P264 One Size Does Not Fit All: Tailoring Your Mouse Injectable Anesthesia Methods

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Injectable ketamine-xylazine is a popular anesthetic, but variability has been anecdotally reported. There are large strain differences between inbred/outbred, and genetically engineered mouse models (GEMM) can have altered responses to anesthesia. Researchers need more information about using cocktails to quickly induce anesthesia. This project seeks to titrate several cocktails for 3 strains to allow strain-specific dosing that achieves uniform performance. Female mice (n=80 total) from 3 commonly used strains were selected: C57BL/6, BALB/c, and C.B17-SCID. Each group underwent 1 of the following IP protocols: ketamine(K) 100mg/kg + xylazine(X) 15mg/kg; K 100mg/kg + X 15mg/kg reversed with atipamezole(AT) 1mg/kg; K 100mg/kg + X 15mg/kg reversed with AT 3mg/kg; K 75mg/kg + dexmedetomidine(D) 0.5mg/kg, reversed with AT 1mg/kg; K 75mg/kg + D 0.5mg/kg reversed with AT 3mg/kg; and K 100mg/kg + X 20mg/kg + acepromazine(Ace) 3mg/kg. Mice were monitored for loss of righting reflex (LORR), loss of pedal reflex (LOPR), recovery of pedal reflex (RPR) and recovery of righting reflex (RRR.) Reflexes were checked every 5' from LORR until RPR. Reversal with AT was done at ~30' of LORR. We can see using Fisher's Exact Test that K/X/Ace consistently worked throughout all strains as the best to achieve LORR ( $\bar{x}$ : 2.75' C57BL/6, 3.75' BALB/c, 1.8' C.B-17 SCID), LOPR ( $\bar{x}$ : 7.75' C57BL/6, 9.0' BALB/c, 7.1' C.B-17 SCID) and deep anesthesia until RPR ( $\bar{x}$ : 64.25' C57BL/6, 55.25' BALB/c, 62.1' C.B-17 SCID) and RRR ( $\bar{x}$ : 73.0' C57BL/6, 66.0' BALB/c, 108.1' C.B-17 SCID.) K/X 100/15 had variable results in C57BL/6 for LOPR and did not elicit LOPR in BALB/c, which achieved LOPR with K/D 75/0.5, while none of C57BL/6 mice did. In terms of AT reversal at the ~30' LORR mark, 3mg/kg yielded faster RPR than 1mg/kg. Selection of the most appropriate cocktail must be done according to strain and the need for restraint to perform minor procedures or major surgery. Reversal with AT 3mg/kg is advantageous to elicit RPR/RRR and shorten anesthesia until mice are fully ambulatory. Usage of K/X/Ace is so far the most reliable cocktail to use throughout the 3 strains tested.

### P265 Characterization of NipSnap2 as a Potential Mediator of Mitochondrial Permeability Transition

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The loss of cells underlies the basis of many diseases. A common cause of cell death is the mitochondrial permeability transition (MPT), which is characterized by a sudden increase in inner mitochondrial membrane permeability leading to ATP depletion, mitochondrial

swelling, rupture, and ultimately cell death. This process is mediated by the MPT pore, a nonselective channel in the inner membrane. However, the components of the pore itself are not well defined, with a matrix protein called cyclophilin-D (CypD) being the only defined regulator of the MPT pore. To identify novel pore components we conducted proteomic analyses of CypD-binding proteins and identified a putative mitochondrial protein called NipSnap2. More recently, an shRNA-based screening study suggested that knockdown of NipSnap2 could attenuate oxidative stress-induced MPT and cell death. Consequently, we hypothesized that NipSnap2 is a crucial component of the MPT pore. Initial immunofluorescence studies using GFP-tagged constructs in fibroblasts demonstrated the presence of a mitochondrial localization sequence in NipSnap2 and confirmed its localization to mitochondria. To assess NipSnap2's role in MPT, mouse embryonic fibroblasts were cultured and were transfected with a control siRNA or a siRNA targeted against NipSnap2 to decrease protein levels. Alternatively, a parallel set of cells was infected with a control virus or a virus coded to produce NipSnap2, elevating levels of the protein. Verification that NipSnap2 protein levels were altered in the respective groups was determined using Western blot analysis. Sets of transfected and infected cells then underwent a calcium retention capacity (CRC) assay, an index of MPT. Depletion of NipSnap2 by siRNA did not significantly affect CRC when compared to control cells. Overexpression of NipSnap2 also did not greatly alter CRC. Together, these results suggest that NipSnap2 is not an essential component of the MPT. Future studies will include cell death assays to determine cellular viability in the face of altered NipSnap2 protein levels. Additional studies in NipSnap2 knockout cells, which are completely devoid of NipSnap2 protein, will also be implemented.

#### **P266 Changes in Visual Cues Affect Morris Water Maze Performance in Juvenile Rats**

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External visual cues are necessary for place navigation during Morris water maze testing. Posters used for external visual cues were replaced following facility renovations, after which juvenile rats took subjectively longer to complete the water maze testing. We performed a retrospective analysis of control animal study data to compare performance prior to and after the change in visual cues. For the comparisons, 8 to 10-wk-old Sprague Dawley rats were used in 2 toxicology studies. In the first study, 3 posters with different shapes and conspicuous graphics were used for visual cues, with 2 posters hung from the ceiling and 1 placed above the rim of the water maze pool. These posters were replaced by 4 new posters of different colors, similar shape, and less prominent graphics that were all placed just above the rim of the water maze pool. Forty rats were used in the study with original posters, while 50 were used in the study with the new posters. Over 4 consecutive d, each rat was given 4 trial runs per day, starting from a different quadrant of the pool for each run. The time to reach a submerged platform (latency), path length, cumulative distance, and swim speed were documented using commercially available video tracking software. Comparison of these values between studies was accomplished using repeated measures analysis of variance with trend testing. The results showed both male and female rats displayed an overall increase in latency and swam at a faster speed when the new posters were used. Male rats demonstrated an overall increase in path length and cumulative distance with the new posters, while females demonstrated a slower decrease in both path length and cumulative distance between days with the new posters. Additional evaluation is necessary to determine the etiology of these differing results; however, it can be concluded that changes in external visual cue number, position, and character significantly altered test performance in juvenile rats.

#### **P267 Repeated Intraperitoneal Administration of Methylcellulose Is Associated with Systemic Accumulation of Foam Cells**

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Methylcellulose (MC) is a nontoxic hydrophilic compound derived from cellulose that is frequently used as an emulsifier and thickener in a variety of food products. While MC is considered an FDA "generally recognized as safe" compound when administered by the oral (PO) route, there is limited data describing the effects of repeated systemic delivery of MC, despite frequent use in preclinical studies. For example, MC is a commonly used vehicle for the early evaluation of antiepileptic drugs as it facilitates solubilization of hydrophobic agents. We determined if repeated intraperitoneal (IP) administration of MC was sufficient to induce histopathologic lesions in corneal kindled mice (CKM), a commonly used preclinical model of temporal lobe epilepsy. Male, 4 to 5-wk-old, Hsd:NSA(CF-1) mice (n=3-6/group) underwent corneal kindling or sham kindling for 4 wk. Mice received a 3 s (60 Hz, 3 mA intensity) stimulation twice daily until acquisition of kindling criterion, defined as 5 consecutive Racine stage 5 seizures. Upon acquisition of the fully kindled state, mice were then randomized to receive either 0.5% MC or sterile saline (IP or PO) twice weekly for 6 wk. Body condition, appearance, and behavior were assessed throughout the compound administration period by a veterinarian blinded to treatment. After 6 wk of treatment, animals were euthanized and necropsied. Histological evaluation revealed systemic accumulations of MC-laden cells and secondary inflammation only in IP MC-treated mice (n=7/7). MC-laden cells were present in kidney, liver, mediastinal lymph nodes, mesentery, aorta, and choroid plexus, with lesions present in all animals that received IP MC. Animals that received MC PO (n=2) or saline via either route (IP n=6 or PO n=3) had no lesions. While there were no significant effects of IP MC treatment on body weight gain, appearance, or general behavior, our histopathologic findings suggest that repeated IP MC administration does elicit systemic effects and may confound any interpretation of investigational drug-induced pathologic lesions. These data highlight the necessity of considering and evaluating the effects of repeated and chronic administration of diluents and vehicles used in preclinical drug studies.

#### **P268 Ulk1-dependent Autophagy of $\alpha$ -globin in $\beta$ -thalassemia**

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Macroautophagy occurs constitutively at a low level, but is accelerated by cellular stressors, such as starvation, protein disorders, or DNA damage. Unc-51-like kinase 1 (Ulk1) is a protein that is central for some autophagy processes. In the genetic disorder  $\beta$ -thalassemia, mutations in the  $\beta$ -globin (*HBB*) gene cause a buildup of free  $\alpha$ -globin, which forms intracellular precipitates that impair erythroid cell maturation and viability. Protein quality control systems, including autophagy, proteasomal system, and chaperones, mitigate  $\beta$ -thalassemia pathophysiology by degrading toxic free  $\alpha$ -globin. As animal research technicians, we were maintaining the mouse *Ulk1/Hbb* colony for related experiments. Homozygous *Ulk1*<sup>-/-</sup> mice with the heterozygous  $\beta$ -thalassemia (*Hbb*<sup>Th3/+</sup>) mice were interbred and once the dam gave birth, the pup's weight, phenotype, litter size, gender, and toe sample were collected for identification and genotyping. All the genotypes were born with expected Mendelian ratios, but the *Ulk1*<sup>-/-</sup> *Hbb*<sup>Th3/+</sup> mice exhibited a high rate of perinatal death. Only 15% of *Ulk1*<sup>-/-</sup> *Hbb*<sup>Th3/+</sup> mice survived for 30 d (n=15), compared to 80% of *Ulk1*<sup>+/+</sup> *Hbb*<sup>Th3/+</sup> mice (n=20) ( $P < 0.05$ ). To examine the consequences

of *Ulk1* loss in  $\beta$ -thalassemic erythroblasts, we transplanted d14.5 fetal liver cells from double mutants and controls (CD45.2) into lethally irradiated wild-type hosts (CD45.1; >92% engraftment). We show that loss of the autophagy activating kinase gene *Ulk1* with  $\beta$ -thalassemia heterozygous gene reduces autophagic clearance of  $\alpha$ -globin and exacerbates disease phenotypes. Systemic treatment with rapamycin for 30 d, an indirect *ULK1*-activating drug, reduces  $\alpha$ -globin precipitates and lessens pathologies in  $\beta$ -thalassemic mice, but not in those lacking *ULK1*.  $\beta$ -thalassemic-treated mice showed reduced thallemic hallmarks as improved blood count by increased RBC count by 27% ( $P < 0.001$ ), decreased reticulocyte count by 44% ( $P < 0.001$ ) and reduced spleen size ( $P < 0.01$ ).

#### P269 Survival Facial Vein Bleed in Neonatal ABCD1 Mice

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Mutations in the peroxisomal protein, ABCD1, result in X-linked adrenoleukodystrophy (X-ALD), with clinical manifestations that vary from a progressive spinal cord dysfunction to a fatal form of childhood inflammatory demyelination in the brain known as childhood cerebral ALD (cCALD). It has been previously reported that *ABCD1* is highly expressed in human monocytes and neutrophils. In *Abcd1* deficient (*Abcd1* KO) mice, changes were identified in these cell populations as early as postnatal day 4 using a nonsurvival blood collection technique. A literature search revealed that there are currently no validated and published methods for survival blood collection in neonates. Thus, to develop a model of survival blood collection to monitor leukocyte changes over time, 11 *Abcd1* KO and 6 WT neonates were weighed on postnatal day 5 and then bled via the facial vein. The conscious neonates were gently restrained by the scruff to create tension over the mandible and 3mm lancets were used to superficially puncture the facial vein. The samples were collected via capillary blood collection tube, as volumes were as low as 20 $\mu$ l. Clinical observation parameters such as bruising, overall general health, and dam rejection were recorded 24 h post-collection. On postnatal day 8, weight and clinical observations were collected again. Weight gain from day 5 to day 8 increased by an average of 41% for the WT neonates and an average of 43% for the KO neonates. There was no rejection from the dam and no abnormal clinical observations were noted at either time point. These findings suggest that a successful survival facial vein bleed is possible in neonatal mice without any adverse effects. The establishment of this technique will enable monitoring of leukocyte development in multiple mouse models of disease.

#### P270 Anesthetic Effects of a Medetomidine, Midazolam, and Butorphanol Mixture and Atipamezole Antagonism in Rabbits

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An anesthetic mixture of medetomidine, midazolam, and butorphanol (MMB) has been used in mice and rats. However, there is little information regarding its effects in rabbits. We investigated anesthetic effects of MMB by different administrative routes, as well as antagonistic effects by atipamezole in rabbits. Six male Japanese White rabbits were subcutaneously (SC) and intramuscularly (IM) administered with 0.15 mg/kg medetomidine, 1.0 mg/kg midazolam, and 1.5 mg/kg butorphanol. The doses given intravenously (IV) were

0.1 mg/kg medetomidine, 1.0 mg/kg midazolam, and 1.5 mg/kg butorphanol. We measured 6 reflexes (body righting reflex, corneal reflex, and withdrawal reflexes of 4 legs by noxious stimulus using hooked forceps) every 5 min after administration of MMB. When all reflexes were nonexistent, we determined the surgical anesthesia starting time. The length of reflex absence determined the surgical anesthetic duration. The recovery time was determined when all reflexes were recovered. Another 6 male rabbits were administered MMB by IM. Respiratory rate, O<sub>2</sub>-saturation, and heart rate was measured every 5 min upon administration, and before and during anesthesia. A sensor clip was placed along the rabbits' ear artery. Such parameters were also measured in non-anesthetized rabbits kept in a rabbit holder. Thirty min after administration of MMB, atipamezole (0.75 mg/kg) was injected by IM. Recovery time was measured. After administration of MMB, starting times were 16.7 min, 11.7 min, and 5 min by SC, IM, and IV routes, respectively. The IV administration showed a significantly faster starting time compared to the other administration routes. The SC administration showed a later starting time compared to the IM route. Surgical anesthetic durations were 27.5 min, 35.0 min, and 36.7 min by SC, IM, and IV routes, respectively. There were no significant differences among the 3 different routes. Recovery times were 140 min, 132 min, and 76 min by SC, IM, and IV routes, respectively. The recovery time after IV administration was significantly faster than the other routes. Our results demonstrated that the IM administration route seemed the most suitable in rabbits. After administration of MMB by IM route, O<sub>2</sub>-saturation decreased slightly compared to nonanesthetized rabbits. Heart rate and respiratory rate were decreased, but stable. After the injection of atipamezole, the recovery time was 16.5 min. Overall, we found MMB to be a useful anesthesia for rabbits.

#### P271 In Situ Single Pass Intestinal Perfusion in Mice for Studying on Drug Intestinal Permeability

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To date, the most commonly used model for predicting absorption process in humans is Caco-2 cell monolayer system. Although there is a good correlation between drug permeability in Caco-2 cell lines and drug absorption in humans, when it comes to carrier-mediated transport or monitor intestinal metabolites, Caco-2 permeability study can only work within the limit. In situ single-pass intestinal perfusion (SPIP) is a comparatively direct approach of measuring compound absorption. The aim of this study is to test the feasibility of using SPIP in mice for both assessing the intestinal permeability of test compound and monitoring its metabolite. A segment of jejunum (8 cm) from CD-1 mice (n=4) was isolated and perfused with the perfusion buffer containing midazolam (50  $\mu$ M, 0.2 mL/min) for 60 min. The correlated mesentery vein was cannulated with 24 G catheter filled with 100 IU/mL of heparin. Following 30 min of equilibrium, the intestinal perfusion buffer and the blood sample from the mesentery vein was collected every 5 min for over 60 min. All these samples were quantified using liquid chromatography-mass spectrometry. The permeability of midazolam is  $15.4 \pm 0.3 \times 10^{-4}$  cm/s (from lumen) and  $3.7 \pm 0.5 \times 10^{-6}$  cm/s (from mesenteric vein). The intestinal permeability is consistent with the literature. The major metabolite of midazolam, 1-hydroxymidazolam, was monitored for 60 min. The cumulative amount of 1-hydroxymidazolam from the intestinal perfusate and blood is  $11.25 \pm 3.3$  pmol/cm<sup>2</sup> and  $1.54 \pm 0.62$  pmol/cm<sup>2</sup>, respectively. In summary, we successfully set up a SPIP mice model for assessing compound's intestinal permeability and monitoring its metabolites. SPIP mice model is a valuable tool in the drug development, not only due to multiple transgenic strains of mice are available, but also due to mice have high similarity to human in transporter expression profiles and intestinal enzymes.

## Platform Sessions

### PS1 Is It Time for an Animal Program Price Index?

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Universities frequently use the Higher Education Price Index (HEPI) for financial planning as it tracks the main higher education cost drivers. However, the HEPI does not include research costs because not all higher education institutions have research programs. The absence of an index meaningful to higher education research enterprises has left animal programs without an analogous basis for modeling financial strategic planning, increasing the risk for inaccurate assumptions and flawed planning. The Yale Animal Resource Center has conducted surveys since the late 1990s to profile and benchmark costs of U.S. academic institution animal research programs. A longitudinal data analysis identified trends that allowed a simplified comparison of cost changes to public price/growth indexes and confirmed that the current triad of increasing costs (inflation), decreasing buying power of the dollar, including the “research dollar” as measured by the Biomedical Research and Development Price Index (BRDPI), and plateauing research funding creates a financial environment in need of understanding the drivers in the cost trends of an animal program’s “basket of goods and services.” An approach similar to the HEPI approach to higher education’s basket of goods and services was used to create baskets of goods and services specific to animal research programs. The baskets include salary and benefits, equipment, materials, husbandry/sanitation supplies, building/equipment maintenance, medical supplies, services, and business expenses. As for the HEPI, surrogate item costs available in public databases from the Bureau of Labor Statistics and various cost indexes were used to create the baskets. An index predicting cost trend associated with an animal program operation provides a more accurate, evidenced-based approach to building 5- and 10-year animal program financial models. Using an accurate, index-based cost to build financial models helps guard against developing and perpetuating unrealistic financial models, which can undercut program development and erode investigator and staff morale and the quality of research in animal programs.

### PS2 Comparison of Academic Animal Program Organization, Operations, Services, and Costs in the United States and European Union

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The Yale Animal Resource Cost and Benchmarking survey, conducted in U.S. academic research institutions about every other year since the late 1990s, was modified to capture similar thematic information from League of European Research University (LERU) member institutions from Denmark, England, Finland, France, Germany, Ireland, Italy, Netherlands, Scotland, Spain, and Switzerland. Preliminary data analysis suggests that: a) like U.S. programs, most LERU programs have mice and rats, but fewer programs have monkeys, b) LERU vivaria have about equal amounts of housing and procedure space, while U.S. facilities tend to have twice as much housing as procedure space within the vivaria, and c) per diem rates have similar compositions, with ~50% covering salary and fringe, followed by supplies (~25%), facility costs (~10%) and other expenses (~15%). Unlike some U.S. programs, the LERU programs tend not to over-recover mouse care costs, but ~60% of both US and LERU programs under-recover mouse care costs. However, while the vast majority of U.S. programs under-recover medium NHP care costs, in LERU

programs the split is more even between break even and under-recover. On average LERU programs have a small positive net-operating balance, while U.S. programs average a large deficit. In LERU programs less than 50% of institutions cover an animal program deficit, while almost 100% of such deficits in U.S. programs are covered by the institution. Deficits not covered by institutions in LERU programs tend to be allowed to accumulate, are covered by program reserves, or are covered by a loan to the program. In setting per diem rates, LERU programs rely more on cost accounting, care more about having competitive rates with peer institutions, and are less influenced by animal user groups than U.S. programs. Outsourced services are similar, with virology and serology services being most frequently outsourced. The full analysis details how the European and U.S. financial environments compare and elucidates the various financial pressures under which animal-based research operates in the 2 environments. Preliminary conclusions are: a) LERU programs are more accountable to their budgets and b) diversity amongst programs is more institution to institution and the state/country matters less.

### PS3 Highlighting Workplace Inefficiencies: Welcome to the Matrix

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Have you ever wondered precisely how many staff members are needed to perform each and every task within your animal facility? What happens when someone calls in sick and you’re left scrambling to put out a dozen fires from the previous day? Most biomedical research facilities use an ancient platform to determine their needs, which is typical of the “this is how it has always been” method. What if there was another way? Over the course of 24 mo, we focused on a project looking to answer the age-old question, how many people do we actually need to properly take care of our animals? The end result of the project fundamentally changed the operations of the whole unit. The Matrix, as the name implies, is a matrix where the user inputs the current census, and the software informs you precisely how long tasks should take, how many full-time employees are needed, and other extremely useful information. If you’re currently using lean management or continuous improvement methodologies, the Matrix also highlights waste as never before. You might just have the equivalent of 2 full-time employees cleaning biological safety cabinets or washing the floors. When the Matrix identifies this waste, you can forecast exactly how much time you will save if you hold a short improvement project on that area. At the conclusion of the project, management was able to pinpoint the precise location of inefficiencies, and thereby remove or improve a variety of processes that ended up saving the department hundreds of thousands of dollars.

### PS4 The Husbandry and Care of a Research Colony of Betta Fish (*Betta splendens*)

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*Betta splendens* have become a model for understanding the genetic and neurological basis for aggression. While a mainstay in the aquarium hobby, Betta fish have rarely been used in a laboratory setting. Unlike most other laboratory fishes, they present a number of physical challenges for large cohorts as they must be singly housed to prevent fighting and require static water for breeding. Maintaining appropriate water quality required buffering RO water to the correct pH and conductivity as well as twice weekly 100% water changes to compensate for frequent feedings, resulting in reduced ammonia levels. These are challenges not seen as commonly in recirculating systems used for zebrafish because there is often no establishment of a bio filter within static tanks so water quality can quickly reach dangerous levels. Since the colony is breeding, feeding practices over a variety of life stages were developed, including the use of paramecia, brine shrimp, and several commercial dry feeds. Similar feed



structuring is used for growing fish in our Zebrafish Core, with brine shrimp being a mainstay for all species. Upon seeing another betta, males show acute aggression, which can wane if they become accustomed to viewing each other. Since part of the research includes an assessment of aggression, housing of this species required the placement of dividers between tanks to ensure that they can only see forward and not develop that acclimation. Furthermore, due to the lack of professional vendors, laboratory bettas are purchased from a variety of breeders which can bring unwanted pathogens with them. While other laboratory fishes are often kept in groups of the same strain, individual bettas are particularly more valuable so steps are taken to ensure they do not succumb to the various diseases seen. Multiple treatment regimens for commonly seen conditions were developed, requiring the stocking of an aquarists' pharmacy to include a broad spectrum treatment for the control of diseases caused by *Ichthyophthirius* (ich), *Costia*, *Trichodina*, *Chilodonella*, *Oodinium*, and fungal infections (malachite green and formalin); neomycin sulfate; minocycline; and a nonantibiotic treatment for bacterial and fungus infections. We currently house more than 100 adult bettas and have a thriving breeding program.

#### **PS5 Fine Tuning a Rodent Clinical Health Program through Analytics: Balancing Workload and Managing Effort**

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In 2015, our organization developed and established a single rodent clinical health program in an effort to unify practices and establish reasonable workloads and expectations for the veterinary technologists and animal care specialists who routinely manage clinical health issues. The system was designed or use on a tablet and uses a central database for all rodent cases across 8 buildings and 3 U.S. states. Technicians document and communicate about cases in real-time. Pulling analytics from our database for 2017, we calculated average values for each technician performing rodent clinical care. The data was limited to ulcerative dermatitis cases, a common condition with a fairly uniform distribution in rodent facilities. Values for new cases per day, clinical visits per case, clinical visits by day of the week, and time to resolution for ulcerative dermatitis were calculated and compared. Results identified an outlier among our rodent clinical health team. The ratio of new cases/clinical visits suggested that he rechecked ulcerative dermatitis cases more frequently than his peers despite having significantly longer days to resolution. We estimated that 30% of the clinical visits being conducted by this individual were unnecessary. The analytics provided by our rodent clinical health program were critical in identifying this outlier, so we could provide coaching, as well as monitoring performance moving forward. While this tool was particularly helpful for managing this individual employee, it is most useful to look at the caseloads of the entire workgroup and adjust assignments based on case load. This data has also been used to justify incremental animal care positions for facilities with growing rodent populations.

#### **PS6 Electronic Management of Large Animal Social Housing through an Inhouse Digital Solution**

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At our institution, socially compatible large animals are socially housed by default, but often become separated temporarily at some point during their study due to anesthesia or surgical procedures. It is not uncommon for a single animal to undergo multiple separations and/or be grouped with different animals during the study. Our medical record system and social housing records have traditionally been paper-based. One of the challenges of paper-based social housing records is the administrative burden of documenting grouping and separating animals. This typically requires completing duplicative information on multiple animal records. Paper-based systems do not

easily lend themselves to viewing of a group of animals and in our experience were not effective for managing the dynamic nature of social housing. Over the past few years our department has been developing electronic recordkeeping methods for a variety of animal facility operations. To address this, we added an element to our digital large animal clinical case tracker that adds the functionality of scanning animal barcodes or selecting animals to add them to groups, buttons to separate them from their cohort, and data entry options to document single-housing rationale. Single-housed animals are flagged in the system which prompts periodic reevaluation. We added social group information to our dashboard view of each holding room which indicates which animals are grouped and which are individually housed. Migration from paper-based social housing records to this digital method has greatly improved accuracy, consistency, simplified our social housing documentation.

#### **PS7 Validation of New LED Red Lighting and Its Effect on the Circadian System of Rats**

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Red light is often used to observe rodents while in a dark phase because studies have shown that laboratory rats are insensitive to long wavelength light. In contrast, even brief exposure to shorter wavelength light during their subjective night is sufficient to cause shifts in their endogenous circadian system. Therefore, it is important to use proper lighting so as not to disrupt normal circadian rhythm which has effects on systems such as the metabolic cycle and hormonal fluctuations. During the commissioning of a new vivarium, an LED red light system was validated to ensure it would not modulate the circadian systems of the animals. The factory setting for the red lights had a peak wavelength of 635nm rated at 1,150 lumens and the white light was rated for 2300-2500 lumens. Eight-wk-old male Sprague-Dawley rats (n=6/group) were acclimated for 2.5 wk in the vivarium, housed in separate cubicles that were on a partial reverse light cycle (lights on at 2400 h, lights off at 1200 h). On the test day, rats were euthanized 4 h after the onset of the dark phase. Two cubicles were subjected to either 144.24 lux (highest setting) or at 3.23 lux (lowest setting) LED red lights for 60 min before euthanization via decapitation, while the other cubicles were left in complete darkness. Brains and blood were collected for subsequent testing of *c-Fos* mRNA levels and corticosterone hormone measurement. We found that the rat's circadian system was responsive to an hour at either red light intensities, as evidenced by increased *c-Fos* mRNA (neuronal activity marker) in the suprachiasmatic nucleus (SCN). We also found increased *c-Fos* mRNA in various regions of neocortex, including the primary motor and somatosensory cortex, suggesting that the rats were behaviorally responsive to the red light. In conclusion, minimal use of an LED red light during the rat's dark phase caused alteration of the rat's circadian system. As our data shows, red light can have unexpected consequences, and for sensitive studies, it is essential to understand the potential impacts in the circadian rhythm of even a small amount of red light exposure.

#### **PS8 Lighting: An Extrinsic Environmental Factor in Laboratory Animal Science**



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Lighting, an extrinsic environmental factor in the laboratory animal facility, influences animal circadian, neuroendocrine, and

neurobehavioral regulation and, ultimately, scientific outcomes. Adherence to proper light and lighting protocols, as outlined in the *Guide*, is essential for the health and well-being of laboratory animals and leads to improved scientific outcomes. Recently, the National Institutes of Health (NIH) published guidelines, now endorsed by the greater biomedical research community, that help to enhance rigor and support research that is reproducible, robust, and transparent. Previous investigations from ours and other laboratories on numerous species have demonstrated that even small changes in intensity, duration, and wavelength (color) of light at a given time of day significantly influence circadian rhythms of animal metabolism and physiology. Here we discuss our present knowledge, as well as current and potential future practices regarding monitoring of various lighting technologies, including the emerging technology of light emitting diode (LED) lighting. Based on these published studies, as well as new neuroendocrine, metabolic, and physiologic findings from our previous and recent 2018 GLAS-supported rodent investigations, we propose a novel, simple, and concise species-specific metric, or standard set of reporting parameters, with emphasis on rodents, to include comprehensive details and measurements of lighting and lighting protocols; light meters and photo optics; within cage/environment radiometrics/photometrics; and, animal retinal photopigment-weighted illuminances for both the visual and nonvisual systems associated with light regulation of circadian rhythms of animal metabolism and physiology. Measures for the metric are relatively easy and straightforward to achieve and require little additional time, effort, and financial resources on the part of institutions. Light, as an extrinsic factor in which the laboratory animal is housed and raised, may now be better accounted for in the experimental design, potential influences on investigative results, and for sharing with other researchers for improved reproducibility in laboratory animal research. Further, in keeping with the *Guide* and the new NIH initiative, this metric enhances our ability to monitor and report lighting parameters that may lead to improved scientific outcomes.

#### PS9 A Cost-effective Approach to Anesthetic Waste Gas Scavenging

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When animal users use isoflurane anesthesia there is always potential for exposure to anesthetic waste gas. There is very little data published regarding typical exposure limits specific to isoflurane. However, the current recommendation for exposure limits is 2 parts per million/hour. This limit is based on a study performed by the National Institute for Occupational Safety and Health conducted on other halogenated gasses in 1978. Any researcher using isoflurane is strongly encouraged to do so in a biosafety cabinet that is ducted to the outside as this promotes little to no exposure to anesthetic waste gas. This recommendation may not be feasible for all researchers. They may not have access to a ducted cabinet as they are using specialized equipment (such as imaging) which may not be located in or near a cabinet. We recommend the use of a portable active scavenging unit in these instances. Active scavenging units are expensive, bulky, and use filters that are costly to replace and difficult to monitor for saturation. Commercially available active scavenger units are quite expensive ranging from \$2,700 to \$3,800. A solution to providing researchers and their staff with adequate resources to reduce exposure to anesthetic waste gas was accomplished by building our own portable active scavenging unit. We were able to construct a lightweight (<2kg) active scavenger using items purchased from local and online sources at a fraction of the cost of commercial units. The weight of our unit is <2kg as opposed to 6.5kg of our commercial unit. Additionally our unit is battery operated and can be placed anywhere on the work station making it even more portable. We opted to utilize small charcoal canisters that are easy to obtain and monitor for saturation. The total cost of our scavenger was <\$100. We plan to provide an active scavenger with every anesthesia unit we distribute across our animal facilities.

#### PS10 Addressing Challenges Associated with Managing Dogs and Mice Experimentally Infected with Rabies Virus

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Laboratory animals experimentally infected with rabies virus present unique management challenges for animal facility personnel, veterinarians, and the IACUC. Animals infected with rabies virus pose a potential health risk to employees, present obstacles to performing veterinary and experimental procedures, and require more intense animal welfare and protocol oversight. To mitigate these difficulties, our institution developed methods, protocols, and standard operating procedures (SOPs) to ensure that research projects with rabies-infected dogs and mice follow contemporary animal welfare and occupational health standards. Our scientific, veterinary, husbandry, biosafety, and occupational health teams worked together to achieve a safe and humane approach; this, however, required several key steps. First, our IACUC approved clearly established humane endpoints, which the research and veterinary teams reviewed closely and maintained in appropriate facility locations. Second, we developed our personal protective equipment (PPE) SOPs and decontamination methods in concert with our biosafety and occupational health teams to ensure practices were consistent with industry standards. After receiving medical clearance, staff members trained to don and doff PPE per SOPs that conformed to the most recent edition of the Biosafety in Microbiological and Biomedical Laboratories (BMBL) manual. Third, we developed techniques and methods to safely anesthetize and perform procedures on infected animals or to move infected animals between pens or rodent cages without direct contact. The research and veterinary teams first conducted procedural drills with noninfected animals to ensure safety and efficacy prior to any work with rabies virus in an animal. Lastly, we conducted prestudy meetings with research staff, animal care staff, and other investigators who shared the same facility to provide safety guidance and welfare assurance. We also reassessed our program to ensure our anesthetic and endpoint protocols met the highest possible standards. This integrated approach to rabies research with dogs and rodents ensured safe, humane, and high-quality research outcomes.

#### PS11 The Influence of Daytime Exposure to Blue-enriched LED Light on the Nighttime Melatonin Signal and Circadian Regulation of Murine Metabolism and Physiology

glas

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Light and lighting protocols for laboratory animal facilities, as outlined in the *Guide*, are important to both biomedical researchers and animal care personnel. Light entrains the master circadian pacemaker located in the suprachiasmatic nucleus (SCN) of the brain, which controls all metabolic, physiologic, and neurobehavioral processes of the body, particularly the nighttime circadian melatonin signal, in a near 24-h circadian manner. Previously we demonstrated in rats that blue-enriched light (460-480 nm) from light-emitting diode (LED) lighting at daytime (bLAD) increases the amplitude of the nighttime circadian melatonin signal by 7-fold compared with broad-spectrum (300-700 nm) cool white fluorescent (CWF) lighting, resulting in improved animal health and wellbeing. Here we tested whether adult male and female nude mice (CrI:NU(NCr)*Foxn1*<sup>tm1</sup>; n=6 per group), an important model in cancer and metabolism studies, exposed to bLAD, compared

to CWF, lighting amplifies the circadian nighttime melatonin signal. Animals in an IACUC-approved protocol were maintained in an AAALAC-accredited facility for 8 wk on a common lighting regimen 12L (300 lux; 123  $\mu\text{W}/\text{cm}^2$ ; lights on 0600 h):12D (0 lux) on either CWF (control) or bLAD (experimental) lighting, and were assessed for arterial blood acid/gas, metabolic, and neuroendocrine hormone levels at 6 circadian time points. Results revealed that adult mice maintained in bLAD vs. CWF lighting had lower ( $P < 0.001$ ) dietary (-15.4  $\pm$  0.3%) and water intake (-13.1  $\pm$  0.1%), and body growth rates (-10.1  $\pm$  0.4%). Plasma nighttime melatonin levels were over 5-fold higher in the bLAD- vs. CWF-exposed mice, while integrative mean levels of plasma total fatty acids, glucose and lactic acid during the 24-h day were significantly lower by as much as 21.7  $\pm$  0.2% ( $P < 0.001$ ), consistent with a more healthful phenotype. The present findings suggest that daytime exposure to high-blue emission LED light, compared to CWF light, has a marked positive impact on the circadian regulation of neuroendocrine, metabolic, and physiologic parameters associated with the promotion of animal health and wellbeing that may influence scientific outcomes.

### **PS12 Color or Intensity: Environmental Lighting Preferences of Laboratory Rats**

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Tinted thermoplastic shelters (blue, red, amber) are often provided to rodents as a place to hide. Red tinting alters the spectral environment within the cage, effectively reducing the amount of blue/green wavelengths of light while reducing overall light intensity (light levels of 200 lux reduced to 15-30 lux). Rats prefer red environments and find bright lighting aversive. However, due to the concurrent decrease in light intensity with red environments, we cannot determine whether animals are choosing red environments based on spectral environment or low lux levels. We hypothesized that rats would prefer low light red caging and that this preference will be stronger in albinos. Eight breeder pairs of Long Evans and CD rats were randomly assigned into 1 of 4 treatments groups: red cage-200 lux, red cage-25 lux, clear cage-200 lux, or clear cage-25 lux. Offspring from these breeders were cohoused at weaning (1 CD, 1 Long Evans) into same-sex pairs in the same environment they were born in ( $n=3$ ). Cohoused pairs were placed within a preference apparatus that provided free access to all 4 environments for 3 consecutive d. Prior to testing, rats were randomly exposed to each of the 4 environments and connecting tubing for 12 h (6 h light and 6 h dark/environment). Rats were tested in the preference apparatus 3 times, once during each critical stage of development: juvenile (4-6 wk of age), puberty (7-9 wk), and adulthood (10-12 wk). Video was continuously recorded and scored for rat location using instantaneous scanning methods at 15-min intervals for the 3 test days. Data were analyzed using a 3-way ANOVA with post hoc Tukey tests. During the light cycle, CD rats preferred the 25 lux environments with no distinct preference for cage color. Long Evans rats avoided the red-200 lux caging but did not show a clear preference for the other environments. When lights were off, both CD and Long Evans rats were observed more often in the clear-200 lux cage. Lighting preference appears to differ between rat stocks but both show a clear avoidance of the red-200 lux cage.

### **PS13 Continuous Monitoring of Animal Behavior and Physiology Provides Unique Insight into the Impact of Cage Changes and Environmental Enrichment**

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Animal behavior and physiology are influenced by common laboratory procedures and choice of enrichment. Procedures can have

both acute and long-term effects, which may impact scientific study outcomes. Given that cage change schedules and choice of environmental enrichment vary widely across laboratories, our goal was to understand the duration and magnitude of impact on animal behavior and physiology. By continuously monitoring motion and breathing rates of mice in the home cage, we performed retrospective data analysis on the effects of cage changes: 1) across multiple years, 2) in different mouse strains, 3) during varying times in the day, and 4) in response to different forms of environmental enrichment. Response to cage changes was assessed in >250 pair housed female C57Bl/6 mice or single housed C57Bl/6J, BALB/cJ, or C3H/J male and female mice ( $n=15/\text{sex}$  and strain). Response to environmental enrichment was assessed in 10 male C57Bl/6J mice housed singly in cages containing either mixed seeds on corncob or soft cob bedding. Cage changing produced distinct and reproducible alterations in spontaneous motion and breathing rate patterns postprocedure lasting approximately 2-4 d. Response to cage changes over time and during different times of the day were investigated in single-housed C57Bl/6J male and female mice ( $n=9-15$ ). The type of environmental enrichment also produced distinct alterations in motion with daytime motion peaking sharply following cage changes with mixed seed enrichment. In contrast, mixed bedding-containing cages produced a blunted daytime motion increase that lasted several days. In summary, we demonstrated that continuous monitoring of motion and breathing rate provides meaningful longitudinal insights into animals' responses to routine cage changing procedure and environmental enrichment. These results strongly suggest that careful consideration is required in determining when routine procedures are performed during a study and what type of environmental enrichment is used.

### **PS14 Continuous Glucose Monitoring Reduces Stress and Improves Metabolic Data Quality in Mice**

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Glucose homeostasis is a primary readout used to assess effects of pharmacological, environmental, or genetic manipulations in most metabolic studies. In mice, it is widely accepted that blood glucose (BG) values are obtained on a background of stress caused by handling, restraint, and sample collection. However, the potential confounding effect of stress can be difficult to assess and is often either trivialized or ignored in the literature. Mice are usually fasted prior to metabolic tests like glucose or insulin tolerance tests (GTTs and ITTs) to avoid interference from postprandial glucose and hormone excursions. Fasting durations vary, but 4-6 h or overnight (16-20 h) are commonly used. Using continuous glucose monitoring (CGM) by radio telemetry, which continuously measures arterial BG, body temperature, and physical activity in mice for up to 6-8 wk, we studied 6 female C57BL/6J BomTac mice, to investigate the responses to common experimental conditions. Overall, we found that BG levels were exceptionally sensitive to acute stress, and activities, like weighing, changing cages, or measuring BG from the tail, caused it to increase by 25-50% and stay elevated for up to 1 hr. Notably, we measured BG from the tail at standard intervals during a GTT, and found that this caused a significant increase in BG throughout the test, compared to a GTT with no tail sampling. However, the most dramatic effects were the response to an overnight fast. BG declined gradually, reaching clinical hypoglycemia (3.9 mM) after 11 h, and dropping further to 2.7 $\pm$ 0.5 mM at 14 h of fasting. Mean body temperature was normal for 11 h, followed by 7 h of hypothermia, with an overall temperature drop of 6.2 $\pm$ 1 C, suggesting that the mice were in torpor for several hours. We conclude that stress-induced glucose excursions can be a major confounder in metabolic studies, and that they can be either avoided or accounted for by using CGM. Furthermore, fasting beyond 7-8 h in mice is a severe metabolic stress and should only be considered as a direct metabolic challenge. In terms of blood glucose levels, fasting for 4-6 h appears to provide the most physiologically relevant baseline for further metabolic testing.

**PS15 Measuring Immune System Perturbations associated with the Use of Buprenorphine in Laboratory Mice**

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Buprenorphine (Bup), both in regular and sustained-release (SR) formulations, is one of the most commonly used analgesics for laboratory mice. There is often concern by investigators that buprenorphine use may alter the host immune response which could influence research results. To assess the immune perturbations associated with SR-Bup, 20 6 to 8-wk-old female CD-1 mice were immunized with ovalbumin (OVA) on day 0. They were placed in groups of 5 and treated with saline, SR-Bup (0.6 mg/kg every 48 h), Bup (0.5 mg/kg daily) or SR-vehicle (0.5 ml every 48 h) for 18 d. Mice were immunized again on day 18 with OVA and euthanized 3 d later. Blood was collected for serum antibody titers to OVA, and spleens were collected for isolation. Splenocytes were isolated and stimulated with OVA and cultured for 72 h. TNF- $\alpha$ , IFN $\gamma$ , and IL-10 production were measured in cell supernatant by enzyme-linked immunosorbent assay. Serum antibodies to OVA were also measured by ELISA. The cytokine levels were significantly elevated in the saline, SR-Bup, and SR-Bup vehicle-treated mice compared to unstimulated splenocytes. The Bup stimulated splenocytes were only slightly elevated compared to unstimulated splenocytes. All treatment groups produced a robust serum OVA antibody response. These findings suggest there is minimal impact on the host immune response as SR-Bup has a similar immune response as saline treatment, and Bup may have a suppressive effect on cytokine production, but that does not impact antibody responses. These findings should be considered when deciding an appropriate postoperative analgesic regimen.

**PS16 Chemotherapeutic Tolerability and Estrogen Dose Response in the B6;129-Rag2<sup>tm1Fwa</sup>IL2rg<sup>tm1Rsky</sup>/DwlHsd (R2G2) Mouse Model**J Naden<sup>\*1</sup>, M Melton<sup>2</sup>, A Bast<sup>2</sup>, J Gardner<sup>2</sup><sup>1</sup>Veterinary Science, Research and Support, Envigo RMS Inc, Indianapolis, IN; <sup>2</sup>Horizon Discovery, St. Louis, MO

The B6;129-Rag2<sup>tm1Fwa</sup>IL2rg<sup>tm1Rsky</sup>/DwlHsd (R2G2) knockout mouse was developed by backcrossing an IL2rg (common gamma) knockout model to a recombinase activating gene (RAG2) knockout model. This model was developed to provide another immunodeficient option for the oncology and immunology fields. The literature supports better tolerability of DNA damaging oncology treatments for models that do not carry the SCID mutation. We have already reported that the R2G2 mouse model is more tolerant of whole body radiation than a model with the SCID mutation. Here we describe a study examining chemotherapeutic tolerability of common DNA damaging oncology drugs including 5-fluorouracil (5-FU), doxorubicin (Doxo), and cyclophosphamide (CTX) (n=10 per group) in female R2G2 mice. 5-FU was given at 30, 60, or 100 mg/kg, intraperitoneally, twice weekly for 5 wk. Doxo was given at 2 or 5 mg/kg, intraperitoneally, once weekly for 3 wk. CTX was given at 100 or 140 mg/kg intraperitoneally, once weekly for 3 wk. Body weight and survival were recorded weekly and complete blood count and clinical chemistry were performed at the end of the study. Results show that the R2G2 mouse model tolerates higher doses of these chemotherapeutic drugs than doses found in the literature for SCID models. A separate study was completed to examine estrogen tolerance. Exogenous estrogen tolerance is another common concern in oncology research as some immunodeficient mouse models cannot tolerate the subcutaneous estrogen pellets, developing negative secondary effects resulting in removal from study. We performed an estrogen pellet dose-response study in female R2G2 mice using 4 doses of 60-d release 17- $\beta$  estradiol pellets at 0.18, 0.36, 0.72, and 1.7 mg/pellet (n=10 per group). Body weight and survival were recorded weekly for 60 d, and complete blood count and clinical chemistry were performed at the end of the study. R2G2 mice show dose-dependent effects of estrogen on morbidity and mortality. These data will allow researchers to determine the optimal dose for

use in the R2G2 model. In conclusion, these data support that the R2G2 mouse model may be a good alternative to SCID models when administering DNA damaging chemotherapies or when estrogen supplementation is required for xenograft growth.

**PS17 Patient-derived and Cell Line Xenograft Growth in the B6;129-Rag2<sup>tm1Fwa</sup>IL2rg<sup>tm1Rsky</sup>/DwlHsd (R2G2) Mouse Model**

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We describe growth of multiple patient-derived (PDX) and tumor cell line xeno-(CDX) and allo-grafts in the B6;129-Rag2<sup>tm1Fwa</sup>IL2rg<sup>tm1Rsky</sup>/DwlHsd (R2G2) immunodeficient mouse model. The PDXs examined include colorectal and head and neck cancers. The CDXs studied include esophageal (OE33 and FLO1) and stomach cancer (AGS). Tumor growth data was also collected from 2 allografts of murine colorectal cancer (CT26) and B-cell lymphoma (A20) cells. Colorectal PDX tissue was subcutaneously implanted bilaterally into 5 male R2G2 and 5 male NSG mice. Growth was comparable between the R2G2 and the NSG mouse models, however the standard error was much lower in the R2G2 strain. Head and neck PDX 626 and 635 was transplanted in 2.2 mm<sup>2</sup> tissues into 4 sections of each of 2 R2G2 mice each (n=2/PDX), and 100% of mice developed either 1 or 2 tumors. The human esophageal adenocarcinoma OE33 cells were implanted into the left and right flanks of 3 each of R2G2, athymic nude, and SCID mice. There was a 100% take rate in R2G2 mice, 0% in SCID mice, and 17% in athymic nude mice. The human esophageal adenocarcinoma FLO1 cells were examined in 2 studies. In both studies, cells were injected into both flanks of R2G2 and SCID mice. Study A also examined growth in athymic nude mice. In study A, no tumor growth was seen in athymic nude or SCID mice, whereas the take rate was 100% in R2G2 mice. In study B, the take rate was 100% in both the R2G2 and the SCID mice, although differences were seen in growth rate. Human gastric adenocarcinoma AGS cells were implanted in both flanks of 4 each of R2G2 and SCID mice. The take rate was 75% in R2G2 mice and 0% in SCID mice. Head and neck squamous cell carcinoma SQ20b cells were implanted in 20 R2G2 mice and take rate was 90%. Growth of 2 allogeneic tumor lines was also examined. The mouse colon carcinoma CT26 cells were implanted in ten R2G2 mice and took in 100% of the mice. Mouse B-cell lymphoma A20 cells were implanted in ten R2G2 mice and take rate was 100%. Both allografts grew to 1000mm<sup>3</sup> by 13 d post-implantation. These data provide evidence that the R2G2 mouse model is a valuable tool for oncology programs including cell line tumor models research, with high take rates and quick growth of allogeneic models.

**PS18 Early Life Oral Cholera Toxin Vaccine Lowers Risk of Cancer**BJ Varian<sup>1</sup>, T Poutahidis<sup>2,1</sup>, G Haner<sup>1</sup>, A Hardas<sup>2</sup>, V Lau<sup>1</sup>, S Erdman<sup>\*1</sup><sup>1</sup>DCM, MIT, Cambridge, MA; <sup>2</sup>Laboratory of Pathology, Aristotle University of Thessaloniki, Thessaloniki, Greece

During the past 40 y, there has been an inexplicable increase in chronic inflammatory disorders including cancer in humans. Earlier studies in mice showed disrupted host gut microbes and immunity associated with westernized living practices led to higher risk for cancer of lymphatics and other nonintestinal tissues in later generations, highlighting the potential roles for gut microbiota and the need for health remedies to counteract such heritable risks of modern lifestyles. Here we tested whether a safe and simple oral vaccination strategy with an immune adjuvant during infancy is sufficient to counteract heritable cancer risk associated with a carcinogenic microbiome. As predicted, CD1 stock mice harboring the carcinogenic microbiome spontaneously developed a high frequency of lymphoma in 100% (10/10 males and 10/10 females) of mice examined, plus hepatocellular carcinoma in 80% (8/10) of males, and mammary carcinoma in 60% (6/10) in females, upon necropsy at 9-mo old. In contrast, we found that feeding 10ug of sterile *Vibrio cholerae* exotoxin

subunit B for a total of 3 times every other week via gastric gavage starting at 4 wk of age was sufficient to significantly inhibit cancers (lymphoma, liver, and mammary,  $P < 0.05$ ) development when compared with matching controls at 9-mo of age in our mouse models. Beneficial effects were transplantable to other animals using purified lymph node cells alone, confirming an immune-mediated mechanism. Taken together, we concluded that oral vaccination with cholera toxin B during early life helps stimulate health-protective immune responses and counteract cancer development later in life in host animals. In summary, immune adjuvants derived from bacteria may serve as potential vaccines to lower risk of cancer later in life.

### PS19 Long-term Impacts of Early Life Injury on Monoiodoacetate Induced Osteoarthritis in Adult Rats

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One in 10 newborns are born premature and receive an average of 14 +/- 4 painful procedures daily in the hospital. The developing neonatal nervous system readily alters in response to sensory stimuli due to neuroplasticity and immature descending inhibitory mechanisms. These early-life, injury-induced alterations can lead to increased severity of subsequent painful events. The effect on chronic osteoarthritis (OA) pain is unknown. The objective of this study was to assess the impact of early repetitive needle prick (RNP) injury on subsequent OA pain during adulthood. Sprague Dawley rat pups were placed into early life injury (n=18 male, 16 female; PND1)/no injury (n=16 male; 14 female; PND1) groups at birth. Each animal received a series of RNP or tactile (T) stimuli from postnatal d 1 to 7. Gait assessments, reflexive tests, and behavioral assays were performed at regular intervals. At 17 wk, OA (RNP+OA; T+OA, n=9 male, 8 female, 17-wk-old) and control (RNP+C, n=9 male, 8 female, 17-wk-old; T+C, n=7 male, 7 female, 17-wk-old) groups were created. OA was induced using 2mg monoiodoacetate (MIA) injected into stifle joint. During 6-wk period following OA-induction, RNP+OA animals had reduced ipsilateral limb use, compared to others, characterized by: decreased standing weight distribution on the ipsilateral limb ( $P < 0.0001$ ), reduced maximum contact area ( $P < 0.0001$ ), reduced intensity ( $P < 0.0001$ ) and longer swing phase ( $P < 0.0001$ ) during walking. Mechanical hypersensitivity was greater in RNP+OA groups when compared to all other treatment groups ( $P < 0.02$ ). RNP+OA animals showed less horizontal exploration ( $P < 0.05$ ) and spent less time in the center of the open field area ( $P < 0.02$ ) compared to controls. On the elevated plus maze, RNP animals spent more time on open arms. We have shown that early RNP injury appears to heighten pain due to OA-induced by MIA, over MIA alone in mature rats, as defined by clinically relevant limb use, with similar trends reflected in reflexive behaviors and complex behaviors measured. Future studies should assess the underlying mechanisms responsible for these chronic effects of RNP on later chronic pain.

### PS20 Comparisons of Co-culture and Mono-culture of Annulus Fibrosus and Nucleus Pulposus from Canine Intervertebral Discs

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Intervertebral disc degeneration (IVDD) is a leading cause of back pain and disability. There are no known regenerative treatments. Etiopathogenesis of IVDD is poorly understood and appears to be multifactorial. Inflammatory and degradative disease mechanisms are consistently associated with it. However, specific pathways and potential interactions between annulus fibrosus (AF) and nucleus

pulposus (NP) have not been fully elucidated, and valid animal models that closely resemble human IVDD are lacking. Dogs develop spontaneous IVDD similar to humans, and clinical IVDD is another significant canine health concern. This study was designed to test the hypothesis that co-culture of AF and NP would be associated with significantly higher levels of inflammatory and degradative metabolite production compared to separate AF and NP mono-cultures. With ACUC approval, we evaluated IVDs from non-chondrodystrophic dogs (n=6, euthanized for reasons unrelated to this study) to elucidate interactions between AF and NP. Lumbar IVDs were collected aseptically and 4mm explants were created from each AF and NP. Explants were assigned to co-culture or mono-culture. Cultures were maintained for 21 d; media were collected and refreshed every 3 d. Media were tested for inflammatory and degradative metabolites using commercially available assays. Production levels were compared for statistically significant ( $P < 0.05$ ) differences. Significant differences in IL-6, KC, MMP2, NO, and PGE<sub>2</sub> levels were noted between co-culture and NP monoculture. Significant differences in IL-8, KC, MMP2, MMP3, NO, and PGE<sub>2</sub> levels were noted between co-culture and AF mono-culture. IL-6, MCP1, MMP2, and MMP3 were significantly lower in co-culture while KC and PGE<sub>2</sub> were significantly higher in co-culture. Significant differences in IL-6, IL-8, MMP2, MMP3, NO, and PGE<sub>2</sub> levels were noted between AF and NP mono-cultures. Collectively, these data suggest that key metabolites known to be involved in IVDD are preferentially produced by AF or NP, and that the interactions between tissues significantly influence levels of production. Ongoing translational studies will be aimed at further elucidation of these important interactions towards understanding and addressing IVDD mechanisms.

### PS21 Electrophysiological Assessment of Cardiac Complication in Chronic Diabetic Minipigs

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Abundant evidence shows that patients with type 1 diabetes are at high risk for several cardiovascular disorders. Our objective was to assess potential cardiac electro-pathophysiology changes linked to chronic insulin-dependent diabetes in the Yucatan miniature swine. Diabetic animals were divided into 5 different groups based on duration of diabetes (Group 1, 4.1-4.8 y; Group 2, 5.1-5.7 y; Group 3, 6.0-6.1 y; Group 4, 6.8-6.9 y; Group 5, 3.3-3.9 y). Routine measurements of electrocardiograms, including HR, RR, PR, QRS, QT, and QTc, were done. A heart rate correction for the QT interval (QTc) was calculated using the Fridericia method [ $QTc = QT / (\text{cubed root of } RR)$ ]. Mean heart rate was decreased for the diabetic groups compared to the mean heart rate for normal animals. The mean PR interval was increased in all diabetic animals compared to normal animals and the effect increase with the duration of diabetes. The mean QRS interval was increased for all of the diabetic animals compared to normal animals. There were no pronounced QTc abnormalities in this study when comparing diabetic to the normal animals, although one animal did have a QTc prolongation of 43 msec. In addition, 1 animal had a prolonged PR segment (224 msec) associated with frequent ventricular escape complexes. This abnormality in rhythm would possibly go along with the duration and severity of the diabetes. In conclusion, chronic diabetes in Yucatan miniature swine manifests with progressive effects on heart rate, PR interval, and QRS duration. This indicates that the diabetic minipig could provide a good model to test preventative approaches for progressive cardiac therapies in diabetes, using electrocardiography segments as markers of early heart damage.

### PS22 Coelomic Distention and Lethargy in Colony of *Xenopus (Silurana) tropicalis*

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Over a period of 2.5 y, multiple frogs in a colony of western clawed frogs (*Xenopus (Silurana) tropicalis*) were reported for a similar presentation including bloating, coelomic distention, and lethargy. All frogs were used for oocyte collection and had been injected with human chorionic gonadotropin (hCG) to stimulate ovulation approximately 1-4 wk prior to presentation. All frogs were submitted for necropsy after being found sick or dead in the tank. Sick frogs were euthanized via buffered MS-222. On gross necropsy a variety of lesions were seen, including subcutaneous edema and coelomic cavities containing free-floating oocytes with no clear structure to the ovaries plus or minus serosanguinous/hemorrhagic fluid. On histopathology, there were varying degrees of hepatocyte vacuolization and hepatic melanomacrophage aggregates. The most significant finding was numerous round, purple, amorphous, proteinaceous globules, (vitellogenin), seen on the serosal surfaces of multiple internal organs, as well as within intravascular spaces of multiple organs including the lung, heart, spleen, liver, oviducts, and kidneys. The significant presence of vitellogenin, an ovarian yolk protein, in the systemic vascular system is suggestive of a coelomic uptake, most likely by the lymphatics system. The moderate to severe amount of vitellogenin and red blood cells seen on several serosal surfaces of organs within the coelomic cavity is indicative of either the over-production or release of vitellogenin from the increased catabolism of oocytes and increased production from the liver due to exogenous hormone administration. These signs are consistent with Ovarian Hyperstimulation Syndrome (OHSS). The suggested theory of OHSS in *Xenopus* spp. is that the loss of vitellogenin from the ovaries into the coelomic cavity leads to hemorrhage followed by edema of tissues due to acute inflammation, which then triggers osmoregulatory shock, coelomic distention, subcutaneous edema, and eventually death. OHSS is an iatrogenic condition and has been reported in *Xenopus* spp., as well as in humans, due to exogenous hCG injections.

### PS23 Voluminous Regurgitation and Inappetence in a Golden Retriever with Duchenne's Muscular Dystrophy

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A 6-mo-old, male, intact Golden Retriever dog, affected with Duchenne's Muscular Dystrophy (DMD), was reported for an acute bout of voluminous regurgitation and inappetence of 18 h duration. Ten wk prior to presentation, the patient was on a Tamoxifen study and was discontinued 6 wk into the study due to exacerbation of the DMD phenotype (severe bilateral carpal hyperextension, severe bilateral hind limb plantigrade stance and continuous paraphimosis with penile trauma). The patient was also reported for intermittent regurgitation and nonproductive retching once a week for the 2-3 wk prior to presentation. The clinical manifestations of DMD and the reported clinical signs were managed with supportive care. At presentation, the patient was quiet, but alert with referred upper airway noise on auscultation. Abdominal palpation revealed no pain or abnormalities. Differential diagnoses for regurgitation for this patient were megaesophagus, foreign body, intussusception, or other GI obstruction. Differential diagnoses for the abnormal lung auscultation was aspiration pneumonia, pain, or panting. Three-view thoracic radiographs confirmed severe megaesophagus. The thoracic radiographs also showed alveolar-bronchiolar opacities in the cranioventral and caudodorsal portions of the chest with an ill-defined diaphragm. Euthanasia was elected due to the severity of clinical signs and poor prognosis. Gross necropsy confirmed severe megaesophagus and atelectasis of the left caudal lung lobe. A gastroesophageal intussusception was discovered immediately cranial to the diaphragm. The mesenteric blood vessels were engorged with diffuse congestion of

the small intestines. Megaesophagus and aspiration pneumonia are common sequelae of DMD in Golden Retrievers. However, gastroesophageal intussusception is a very uncommon condition in this, or any, population of dogs. Repeated bouts of regurgitation and nonproductive retching in a DMD dog should not be ignored as a complication of megaesophagus and radiographs or other advanced imaging should be considered for rapid diagnosis and institution of treatment as deemed fit.

### PS24 White Spots on the Liver

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Nox2 (Cybb) knockout mice lack phagocyte superoxide production which increases susceptibility to *Staphylococcus aureus* and *Aspergillus fumigatus* infections. The model is used to study chronic granulomatous disease and to evaluate the role of phagocyte-derived oxidants in inflammation. An investigator working with this model found that 50% of the mice presented with liver lesions during scheduled terminal blood collection. Mice ranged from 28 d to 4 mo of age. The mice were maintained in static microisolator cages on paper bedding and received antibiotic water to decrease the risk of opportunistic infections. Antemortem, there were no reported signs of ruffled coat, hunched posture, or other signs associated with ill thrift. Out of a cohort of 10 mice, a representative sample of 2, aged 28 d and 4 mo, was submitted for necropsy. Histopathology revealed moderate to severe necrotizing hepatitis, splenitis, and enteritis. Further investigations including bacteriology, and special stains were utilized to make a definitive diagnosis of bacterial septicemia associated with *Klebsiella oxytoca*.

### PS25 Ataxia and Disorientation in a Yucatan Minipig

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Intake examination of a 3.5-mo-old intact, male and experimentally naïve Yucatan minipig identified mild disorientation and stumbling. Altered mentation and gait abnormalities were noted immediately upon arrival at the facility. Veterinary examination found grade 2 ataxia in the hind limbs with occasional crossing of the front limbs, yet ambulation was in a straight line. The tail was limp with minimal voluntary movements, and superficial pain sensation was present. Anal tone and defecation were normal. Muscle tone and strength of the limbs were normal. Proprioceptive deficits were not identified. Abnormal posture included marked reluctance to raise the head from a lowered position. Demeanor was manic on arrival but quickly evolved to obtunded when housed between conspecifics. Heart and respiratory rates were increased. Ability to evaluate mucous membranes was limited but they appeared pink during brief visual observations. Complete blood count values were normal. Serum chemistry analysis showed hypernatremia (Na<sup>+</sup>: 160 mmol/L), azotemia (BUN: 39 mg/dL), and increased albumin (4.9 g/dL), consistent with dehydration. Differential diagnoses included conditions affecting the central nervous system such as shipping trauma; electrolyte imbalances; metabolic, toxic, and endocrine disorders; or encephalitic infectious diseases. Gross necropsy findings were unremarkable. Histology showed laminar cerebral cortical necrosis with eosinophilic perivascular cuffs, which is pathognomonic for salt toxicosis. Salt toxicosis occurs in pigs from sudden increase in salt intake, but more often from acute water deprivation. Clinical signs of salt toxicosis are secondary to cerebral edema with antemortem diagnosis based on history, clinical signs, and elevated serum or cerebrospinal fluid sodium levels. Water should be offered in small volumes but frequent intervals if deprivation is suspected. Prognosis is poor once neurological symptoms manifest.

### PS26 Unilateral Leg Lameness in a White Carneau Pigeon (*Columba livia*)

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An 11-y-old, 545 g male White Carneau pigeon (*Columba livia*) presented with acute onset left leg lameness. The animal was used in operant conditioning studies during the previous 4 y. Clinical history was unremarkable. These studies require feed restriction, good appetite, and health. Pigeons are removed from wire-bottom home cages and transported upside down in plastic pitchers to and from laboratory chambers. At presentation, the left leg was nonweight-bearing, and toes dragged and lacked pinch reflex and proprioceptive response. There was no swelling, and the pigeon had normal attitude and appetite. Due to acute onset, the differential diagnosis included fracture, dislocation, muscle or ligament injury, or nerve damage. Radiographs of the leg did not reveal any fractures or dislocations, narrowing the differential diagnosis to soft tissue (muscle, ligament, nerve) injury. The leg was wrapped and the pigeon examined daily and treated with carprofen (5 mg/kg IM) for 10 d. The leg was still nonweight-bearing but toes were correctly positioned and showed pinch reflex and proprioceptive response. Repeat radiographs on d 10 confirmed absence of fracture or dislocation but showed loss of left leg muscle mass consistent with disuse atrophy. The leg was rewrapped and the pigeon treated with dexamethasone (1 mg/kg IM) for 7 d, then tapered and discontinued. On day 23, the left leg was still nonweight-bearing, and pinch response was decreased. Weight loss had reached 12% despite normal appetite. Bloodwork on day 29 showed heterophilia, and carprofen (5 mg/kg IM) treatment was resumed. Despite frequent gavaging with slurried feed, weight loss continued. On day 31, the left leg was nonweight-bearing with toes dragging and no pinch reflex or proprioceptive response. On day 32, the pigeon was euthanized, necropsied, and tissues submitted for histopathology. Gross findings included hepatomegaly, pale kidneys, and tumor on left kidney. Histology revealed a T-cell lymphoma in the left kidney. Neoplasms are common in older pigeons, but lymphomas comprise only ~5% of tumors. The history and acute onset of symptoms confused the differential diagnosis. Tumor impingement of the left lumbosacral nerve plexus likely caused the lameness and sensory deficits.

### PS27 Abdominal Distention in a Rhesus Macaque (*Macaca mulatta*)

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A 4-y-old, female rhesus macaque (*Macaca mulatta*) presented with abdominal distention during access for a routine research procedure. Physical exam revealed the abdomen was moderately distended and soft. The animal was thin, with a body condition score of 2.5/5. Prior to presentation, the animal had a normal attitude and fecal production, although appetite was variable and the animal was underweight for its age. Abdominal radiographs revealed significant gas distention of the large intestines, specifically the proximal colon. A red rubber catheter was passed per rectum to attempt to relieve the gas distention but was unsuccessful in reducing air volume. Diagnostics including complete blood count, serum chemistry, blood culture, urinalysis, fecal exam, and culture were collected and were unremarkable. The animal was prescribed simethicone 20mg PO SID and monitored for fecal production and quality. Recheck abdominal radiographs showed increased large intestinal gas distention. A barium study was performed but was inconclusive in ruling out mechanical obstruction. The animal was prescribed milk of magnesia 10mg/kg PO SID, meloxicam 0.1mg/kg SQ SID, and the simethicone dose was increased to 40 mg BID as continued supportive care. A saline enema was administered followed by colonoscopy, which was ultimately unsuccessful and inconclusive. Due to poor prognosis, the animal was

euthanized and submitted for necropsy. Gross pathology findings revealed 2 strictures affecting the proximal colon, approximately 3.4 cm apart. The colon proximal to the strictures was distended with gas and some liquid feces, while the distal colon contained normal, formed feces. The strictures acted as a partial mechanical obstruction allowing for passage of liquid feces which solidified in the distal colon and obstructed passage of gas leading to abdominal distention in this animal. Histopathology showed multiple, chronic ulcers with abundant granulation tissue, fibroblasts, and collagen, as well as neutrophilic inflammation, within examined sections from the stricture. Chronic cicatrizing colitis, resulting in ulceration and fibrosis, is a condition that can affect rhesus macaques and appears to be the cause of the colonic strictures and clinical signs in this case.

### PS28 Sneezing and Inappetence in an Immunodeficient Laboratory Rabbit

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An approximately 5-mo-old, male, *IL2rg* knockout rabbit (*Oryctolagus cuniculus*) was examined for sneezing and inappetence. On physical examination, the rabbit was slightly underconditioned with mild serous nasal discharge and a body temperature of 105.7°F. A complete blood count revealed lymphopenia, consistent with a severe combined immunodeficiency (SCID) phenotype, and marked heterophilia suggesting a systemic infection. Differential diagnoses for the inappetence, sneezing, fever, and weight loss included a bacterial, viral, or fungal infection. PCR analysis and bacterial cultures on nasal swabs were positive for *Bordetella bronchiseptica* and negative for *Pasteurella multocida*. Supportive care was initiated with a course of trimethoprim sulfa, additional hay, meloxicam, and metoclopramide. Following 3 wk of treatment, thoracic radiographs were obtained due to poor response to therapy, which revealed multifocal opacities circumferentially centered around bronchial airways. Differentials included allergic, infectious, or inflammatory causes. Trimethoprim sulfa was continued empirically for 4 wk. The rabbit was reported again 1 mo later for sneezing and mild anorexia. Physical examination findings included lethargy without nasal discharge. Supportive care was provided for several days and clinical signs resolved. One mo later, the rabbit rapidly decompensated and expired. Necropsy and histopathology findings revealed severe bronchointerstitial pneumonia, with the presence of flocculent eosinophilic material in alveoli. Analysis of bronchoalveolar (BAL) fluid and tissue sections with GMS staining revealed numerous *Pneumocystis* organisms. PCR for *Pneumocystis oryctolagi* on tissue sections confirmed the diagnosis. Immunocompetent rabbits commonly harbor this fungal organism at low levels until weaning, at which time subclinical or transient respiratory signs may be seen, and the organism is generally cleared. However, immunosuppressed rabbits may be prone to severe pulmonary infections with *P. oryctolagi*, as well as respiratory bacterial infection. Since immunocompromised human patients often encounter respiratory complications from fungal infections, SCID rabbits might serve as a useful model for human *Pneumocystis* infection.

### PS29 Facial Swelling in a Southern Giant Pouched Rat (*Cricetomys ansorgei*)

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An adult 1.6 kg male, singly housed, Southern Giant Pouched Rat (*Cricetomys ansorgei*) presented with right-sided facial swelling. The rat was housed in an AAALAC-accredited institution and was part of a colony used for reproductive and behavioral research. Under general

anesthesia with isoflurane, a 3 x 2 cm swelling was palpated in the musculature at the level of the ear and jaw, and copious, putrid, yellow-white pus exuded from the ear canal. The rat was started on meloxicam (2mg/kg PO SID) and amoxicillin/clavulanic acid (20 mg/kg PO BID). Differentials included a primary otitis media, cheek pouch abscess, and cheek tooth root abscess. Two d later, the swelling had not improved and the rat was not consuming his oral medications. The rat was anesthetized for medical CT, which showed severe lysis and periosteal reactions along the entire length of the right mandible, apparently originating from the right lower incisor with secondary involvement of the cheek teeth. An oral exam was unremarkable. Although the rat was maintaining weight and appeared appetent, the animal seemed too painful to eat. The rat was transitioned to injectable meloxicam (1 mg/kg SC SID) and long-acting cefovecin (8 mg/kg SC). Due to poor prognosis associated with surgical debridement and marsupialization, and the preexisting mandibular instability, euthanasia was elected. After euthanasia, nano-CT was performed, which confirmed changes seen on medical CT. Necropsy showed a 1 x 1 cm encapsulated nodule that extended caudally from the right mandibular third molar, with severe inflammation in the muscle, fascia, periodontal ligaments, and bone. A pathologic fracture was visible. Anaerobic and aerobic cultures grew many *Fusobacterium necrophorum* and *Bacteroides fragilis* group, as well as a gram-positive branching rod resembling *Actinomyces*. These bacterial species are anaerobic oropharyngeal commensals commonly found in abscesses due to oral trauma. *Actinomyces bovis* is known in cattle to cause lytic regions of osteomyelitis surrounded by new periosteal bone and fibrous tissue, colloquially known as lumpy jaw. To our knowledge, this is the first time that an odontogenic infection or abscess resembling lumpy jaw has been reported in a Southern Giant Pouched Rat.

### PS30 Acute Lethargy in a Rhesus Macaque (*Macaca mulatta*)

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A 20-y-old intact female rhesus macaque (*Macaca mulatta*) was reported for laying at the bottom of her cage, unwilling to rise. On examination, the patient was sitting upright with a tucked abdomen but would intermittently lie down on the cage floor. The animal appeared to shift its weight from side to side, with occasional grinding of the teeth. This monkey had been previously diagnosed with endometriosis and for the past 5 y had been treated with monthly medroxyprogesterone (40mg) injections. Due to its history, the animal was empirically treated with meloxicam (0.2mg/kg), was given its monthly injection 2 d early, and was placed on veterinary observation. The patient improved and was sedated 3 d later for physical exam, bloodwork (CBC/chem), and an ultrasound examination. Physical exam revealed a loss of approximately 12% body weight over the last 5 mo. Ultrasound demonstrated the presence of endometrial cysts and a thickened uterine wall, findings which remained unchanged from previous examinations. Blood work showed markedly elevated alkaline phosphatase (502IU/mL), mildly elevated glucose (128mg/dL), and an increased white blood cell count (17,400/ $\mu$ L) with neutrophilia (10,440/ $\mu$ L). In order to address a potential infection, the animal was treated with an empirical 10-d course of enrofloxacin (5mg/kg daily). Furthermore, cage-side glucose measurement of 298mg/dL was suggestive of diabetes mellitus. The monkey was sedated after the completion of the antibiotic course for bloodwork and radiography. Abnormal results included a glycosylated hemoglobin of 10.5, glucosuria, and a serum glucose level of 140mg/dL. Radiographs showed an enlarged uterus and incidental lumbar spondylosis. Based on the aforementioned results the patient was diagnosed with diabetes mellitus. While mild to moderate hyperglycemia is often presumed to be due to stress of sedation, it is important to monitor for potential signs of diabetes in medroxyprogesterone-treated animals, as diabetes mellitus in an uncommon sequela to longterm treatment with this drug.

### PS31 Moving Beyond 3Rs in IACUCs

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In every IACUC in the U.S., there are 2 constants: discussion on harm/benefit of a study design and how the 3Rs can decrease the harm. Each institution has a mechanism to record discussions at IACUC meetings and in many instances use forms, templates, or checklists. These tools are developed to facilitate a robust review and provide documentation satisfying USDA and federal funding requirements as well as institutional policies. There are recognized benefits to using forms, templates, or checklists; they compel completion of obligatory documents or information. Based on a recent review of the top 20 U.S. institutions receiving NIH funding, any prompts for ethical discussions were often limited to the 3Rs and harm/benefit analysis. Except for 1 institution, no other directions on ethical theory, such as telos, were found in the templates. However, there is a potential harm that arises to the nonhuman animal subjects from lack of substantive guidance on ethical principles or values to be considered. One solution would be consideration of voluntary institutional or departmental mechanisms for discussions employing ethical principles. Better integration of ethicists into review and debate of study protocols, even before submission to the granting body and IACUC is yet another possible solution. As practiced today, forms, templates, and checklists benefit IACUCs in assuring compliance. These same forms, templates, and checklists do not prompt rigorous ethical discussion beyond harm-benefit analysis and the 3Rs. For those of us who have accepted the charge by society to ensure humane, necessary, and ethical research, we must continue to seek a solution. Because if not by us, then by whom?

### PS32 How to Build an IACUC from Scratch

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We detail the journey of a Kentucky start-up CRO's unique challenges and solutions in building a new IACUC. The challenges included recruiting committee members from a region away from research-hubs, training a new IACUC quorum, assignment of responsibilities, coordination for a committee comprised of several members unaffiliated with the institution, and determining guidelines for its growth and continuing education. To begin our approach, as the company had several unique challenges, it became incumbent upon the company to self-educate by scheduling weekly meetings and discussions to determine what was needed of the institution to meet and exceed the Office of Laboratory Animal Welfare's (OLAW) standards and the best way forward to achieving those goals. Ultimately, it became a trail-blazing task to launch the efforts and begin the process, starting with recruiting, training, and establishing annual IACUC goals with a structured timeline for committee members. Additional guidelines were imposed on the committee to ensure congealing of the procedures over the course of its first 2 y. Our observations for reaching the stated objectives in the annual goal sheets were encouraging, with the majority of them being met or exceeded. The additional guidelines regarding protocol quorum review and committee training for retention of policies and procedures were almost always adhered to unless scheduling conflicts made it impossible. We observed that inadequate sources were available for the compliance troubleshooting necessary of atypical institutions such as ours. However, we found an abundance of adequate sources were available for designing and implementing training structures. After reflecting on our enterprise, we conclude that with adequate levels of self-education and planning throughout, it was possible to establish a successful and competent IACUC in a uniquely challenging setting. It is our institution's hope that describing our experience will provide new institutions, whether they be academic or commercial, some insights and will find the task of achieving IACUC independence less daunting.



### PS33 On the CUSP: A New Option for Addressing Administrative Burden

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Under the auspices of the Federal Demonstration Partnership, the Compliance Unit Standard Procedure (CUSP) Project offers an option to address administrative burden at the institutional level. The goal of this project is to create an online repository where institutions can share standard procedures used in animal care protocols with the broader animal welfare compliance community. A working group, representing over 40 institutions, has been formed to support site design and development. The working group is organized in to 3 teams, each focused on a different topic area: data import and export, data organization, and data storage and maintenance. The group has made significant progress in developing this resource over the past year and has received strong support from our regulatory partners. We will provide an overview of the CUSP project, including its uses and structure, as well as an update on the current status and what attendees should watch for moving forward.

### PS34 Continuing Professional Development In Laboratory Animal Research in the Netherlands

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The implementation of the European Union Directive 2010/63/EU has set new requirements for the training of people conducting animal experimental work. The revised act states that scientists, animal technicians, and caretakers must be competent to perform activities with animals and have species-specific knowledge. Continuing professional development (CPD) is required to ensure good practices are known and practical skills should also be retained or acquired if needed. The law does not state how to meet the requirements for CPD. We house rabbits, guinea pigs, hamsters, cotton rats, rats, mice, killifish, and zebrafish. We offer training for staff members/animal caretakers and we provide training modules for animal technicians, researchers, and residents of different national and international surgery departments. Training is provided by the microsurgery team, which consists of 4 skilled experimental microsurgeons. We offer a wide variety of techniques, ranging from handling, restraining, and injecting rodents up to specific techniques and an advanced microsurgery course. To ensure continued training of our facility staff, staff members are required to train in practical procedures at least twice a year. Credits are awarded per trained technique, and an annual minimum amount of credits is required. In addition, researchers and animal technicians from other departments also need to be competent to handle animals and perform experiments. Under the auspices of our local Animal Welfare Body we assess practical skills and determine the training needed, keep training records, and record acquired competencies. After training, supervisors will determine whether the trainee needs additional training or that the skills are sufficient to execute the experiment either under supervision or independently. We ensure that only competent persons handle the animals, which benefits the quality of the experiment and most importantly, the welfare of the animals. We will present our route to a reliable system to monitor and ensure training of all people involved in animal research and comply with the European Directive.

### PS35 The ILAR Roundtable: A Resource for the Research Community

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The Roundtable on Science and Welfare in Laboratory Animal Use was established to provide a forum to stimulate open dialogue, exchange of information, and collaboration among entities interested in promoting the development and awareness of cutting-edge laboratory animal research topics, and their translation into more effective, efficient, and humane use of animals in research, testing, and education. In existence for 5 y, the roundtable has produced a diverse portfolio of activities on a range of topics, including practical, scientific, educational, and regulatory. We provide concise information regarding each of the topics covered by the roundtable to date, including developments that have taken place since then and discuss the roundtable's purpose, areas of engagement (biomedical sciences, One Health, conservation, animal welfare and ethics, rigorous and reproducible science) and impacts while soliciting suggestions from the audience about its future.

### PS36 Working with Universities to Support and Encourage Engagement with the 3Rs

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The NC3Rs is an independent, scientific organization established by the UK government to discover, develop, and promote new ways of replacing, reducing, and refining the use of animals in science. In our first 10 y, we primarily worked with individual researchers, other research funding bodies, and industry companies on advancing the 3Rs. During this time, we invested over £60 million in university-based research, career development, infrastructure, and open innovation to deliver 3Rs and other impacts. From extensive consultation with university colleagues, however, it was reported that more could be done to support an active 3Rs community within their establishments. In response to this feedback, we developed 3 new approaches to encourage further engagement with the 3Rs. The first was to appoint regional programme managers who work within universities to boost their 3Rs activity. These specially trained, scientific staff provide expert advice on the 3Rs, organize workshops and other events targeted to local needs, and encourage 3Rs research and knowledge exchange at a regional level. The second initiative is a free video tutorial on the scientific importance of the 3Rs for use in initial training of researchers and animal technicians involved with animal procedures, as well as life sciences students. The third is a 3Rs self-assessment tool to allow higher education institutions to benchmark their 3Rs activities and progress, with a second shorter tool for individual research groups. The tools are secure, interactive, online resources that map scores longitudinally and provide tailored advice on how improvements can be made to encourage a more active 3Rs culture. Through these approaches, more than ever before, we are supporting universities to deliver on their commitments to the 3Rs, benefiting science, scientists, and animals.

**PS37 Changing the Culture on a University Campus: A Conversation about Biomedical Research**

KS Patterson\*

Laboratory Animal Resources, Montclair State University, Montclair, NJ

Historically, our campus has been closed about the use of laboratory animals in research. No mention was made in campus tours or brochures, and we were advised to not make it public that we had animals on campus. In the meantime, groups such as PETA and Beagle Freedom Project have gained more momentum and support. These groups are all we hear and see in the media. Why should the public not believe what they are saying when no one from the science community is speaking up? A large part of the problem is that researchers do not share what they really do and are afraid or apprehensive to discuss their work. There recently been a larger effort to lead global educational campaigns to reach both researchers and the general public about the importance of biomedical research. We aimed to continue this effort on our campus. This presentation discusses the small steps recently taken on our campus to educate students and present the true facts about laboratory animals used in biomedical research. We wrote a class entitled "Introduction to Laboratory Animal Science" and the charter class went through last fall. We are sharing our experience in the presentation of this class and other activities we are planning to engage and educate students in laboratory animal science.

**PS38 Transitioning Research Beagles into Retirement Using a Positive Reinforcement Training Program**

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We are committed to ensuring all animals have the highest level of care and welfare. A canine adoption program was developed to satisfy our desire to retire the research beagles and to be in compliance with Nevada Senate Bill 261 commonly referred to as the "beagle bill." After receiving feedback from initial adopters, we identified an opportunity to provide the beagles with some additional skills to help transition them into their new life. The training program focused on the following areas: harness and leash, basic manners, new locations and experiences, novel floor textures, and novel sounds. Permission was obtained from management to bring the dogs to our receiving dock which included a functioning bathroom, an office area, and garage doors. In addition, items were procured, such as fake grass, a carpet square, harnesses, leashes, a TV, and a radio. The training program was managed by 2 technicians that performed daily 20-m sessions for each dog which occurred up to 4 times a week. Training records were used to track the progress of each dog and to aid in communication between technicians. The 20 dogs involved in the program exhibited a large range in confidence levels at the start of the program but every dog left the facility with the skills necessary to easily transition into retirement. The success of the program was measured by positive feedback from adopters which led to a waiting list of staff who were interested in adopting 1 of our beagles. The program was so popular that additional staff, including nonvivarium staff, were invited to help perform the training sessions. It has been a beneficial program not only for the dogs but also rewarding and enjoyable for the employees who participated and contributed to the success of this effort.

**PS39 Using an Online Collaboration Tool Platform as an Alternative to Software Database Technology**

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IACUC administrators face continuous challenges as new policies,

regulations, and guidelines are implemented by institutional, regulatory, and accrediting bodies. The increase in regulatory responsibilities can hinder timely and efficient IACUC review processes when a software database technology is not a viable option. An animal welfare and compliance office, in collaboration with an information technology department, developed a process that uses a web-based platform to facilitate an interactive review system. This interactive system has been implemented across 3 campuses and has proven effective in allowing a timely and efficient IACUC review. While implementation of the collaboration tool presented some challenges, such as initial training of all of the IACUC members, nonaffiliated member access to the internal IACUC review webpage, and minor technological issues, the benefits have proven to be efficient, cost-effective, and a positive experience. The benefits of the system included reducing email volume for the animal welfare and compliance office, the IACUC, and the principal investigators; improvement in document control oversight; and the ability to have multiple IACUC reviewers simultaneously edit the same document. With increasing guidelines and regulations, the need for an electronic management system is essential for the proper maintenance of IACUC-related documentation. For institutions that do not have funding for and/or access to commercial software solutions, a web-based collaborative platform can be used to strengthen the communication between the animal welfare and compliance office, the IACUC, and principal investigators. This information is beneficial for institutions without software database technology capabilities, but need to accommodate the ever-expanding administrative research demand.

**PS40 Whose Line Is It Anyway? Establishing an In-House Rodent Registry**D Duvall<sup>1</sup>, AJ Watts<sup>2</sup><sup>1</sup>Animal Resources Program, University of Alabama at Birmingham, Birmingham, AL; <sup>2</sup>Institutional Animal Care and Use Committee, University of Alabama at Birmingham, Birmingham, AL

After multiple unsuccessful attempts to institute an in-house rodent registry, the Animal Resources Program (ARP) and IACUC took the initiative to create a user-friendly registry for investigators. Having access to lines on campus could help investigators determine if they could make use of animals from colleagues or import them from outside sources. Registry data consists of lines from breeding colonies on campus, commercial sources, imports from outside institutions, or lines generated by a company or the Transgenic Core. With more than 1,750 lines listed, the registry does not account for every line on campus, but is a powerful tool to assist the investigator looking for a line which may benefit their research. Data provided from approved IACUC protocols and the ARP animal order database was extracted through queries into an Excel spreadsheet. Information was then filtered to be searchable by species, strain, line, or nomenclature. Finally, the registry file was posted to a newly created secure web page for viewing by faculty and staff. When an investigator identifies a line they are interested in obtaining, they contact Rodent Registry via email and request contact information for the investigator with the animals. Based on animal availability and other research or legal factors, the PI's coordinate the details of the transfer with ARP. Even though this registry has the potential to save investigators time and money, reduce animal wastage from established colonies, as well as providing health benefits to campus animals, it has been met with both commendation and criticism. Currently, a more refined method for data collection from the IACUC and ARP animal order databases is being developed along with a secure searchable database to take the place of the Excel spreadsheet.

#### PS41 Impact of Partial Cage Division on Aggression and Behavior on Long-term Housing in Co-housed Male C57Bl/6 Mice

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Aggression within groups of co-housed male mice adds confounds to animal research. With this concern in mind, custom-designed partial cage dividers were developed to mimic burrow-like housing, with the goal to reduce aggressive and anxiety-like behaviors. Aggressive-like behavior in group-housed male mice was significantly reduced when housed in partially divided caging, however, the long-term impacts of the divider on aggression and anxiety-like behavior is unknown. To assess the long-term impact of partially divided caging on aggression and anxiety-like behavior, animals were raised with either partial cage dividers or in standard housing with no divider. Following 1 wk of acclimation in the vivarium, mice were weaned at 21 days old (Day 0) and randomly assigned to 1 of 2 groups: 1) standard cage; 2) cage with a partial cage divider. Animals were tested on rotarod, open field, novel object recognition, elevated plus maze, and Y maze beginning on d 40 through d 70. After no experimental intervention for 42 d, animals were video recorded over 12 h on d 133, 137, 151, 158, and 179, each spanning a light cycle change. Observers blinded to study design and hypothesis scored each video for number and type of aggressive behaviors, which were summed for each hour and analyzed. Mice were weighed and checked for bite wounds on d 133, 137, 151, 158, and 179. Results indicated a statistically significant decrease in aggressive behaviors of mice in partially divided cages compared to mice in standard cages, without changing behavioral responses to common tasks with the exception of significantly improved outcomes for anxiety based testing. We conclude that partial cage dividers reduce overall aggressive-like behavior in co-housed male mice, reduce anxiety, and do not alter typical behavioral testing responses.

#### PS42 Two Prevalent Individually Ventilated Caging Systems Show Comparable Efficacies in Detecting Murine Infectious Agents via Exhaust Air Particles

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Exhaust air particle real-time polymerase chain reaction (EAP-PCR) is used to detect murine infectious agents. Two IVC rack vendors, vendor A and vendor B, developed in-line EAP collection devices. Since the air flow and the mode of EAP capture in the 2 vendors' IVC racks differ, we compared their efficacies for detecting murine infectious agents using EAP-PCR. All materials used were decontaminated or sterilized. After rack decontamination, baseline samples were taken from each exhaust-side horizontal plenum opening (vendor A) and from the horizontal air-exhaust plenum (vendor B) and screened via real-time PCR for infectious agents to verify the cleaning procedure. Over 3 mo on each rack, singly kept male mice, primarily on a C57BL/6 background, and infected with *Helicobacter* (n=24 or 21), *Staphylococcus aureus* (n=9 or 5), *Pasteurella pneumotropica* (n=25 or 22), *Streptococcus beta-haemolytic* (n=30 or 23), *Klebsiella oxytoca* (n=1) and their sentinel positive for *Entamoeba* spp. were kept in the vendor A and vendor B system, respectively. In both systems, 47/60 (vendor A) or 47/70 (vendor B) cages contained mice (n=8 or 15 with negative mice). Both EAP capture media were evaluated for FELASA-listed infectious agents and *Bordetella bronchiseptica*, *Bordetella hinzii*, *Campylobacter* genus, *CAR bacillus*, *Corynebacterium bovis*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The baseline samples from the racks tested negative. Both EAP capture media detected *Entamoeba* spp., *Helicobacter* spp., and *Pasteurella pneumotropica* but not *Staphylococcus aureus*, *Klebsiella oxytoca*, and group B beta-haemolytic *Streptococcus*. We showed, for the first time,

that both EAP capture media comparably detected the infectious agents in naturally infected mice monitored over a 3-mo period. The EAP real-time PCR technology can serve as an adjunct method of HM, leads to the reduction of the number of mice used for routine HM, and contributes to the 3Rs.

#### PS43 Evaluation of Patterns of Use Preference of Gnawing Devices for Rats and Mice

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Gnawing has been demonstrated to be one of the best means of enrichment for laboratory animals. There are several gnawing devices available in the market, differing widely in size, texture, and materials. We investigated specific gnawing preferences of mice and rats. Knowledge of device preferences will allow for maximization of enrichment with proper fund allocation. This study is being conducted to guide enrichment practices for laboratory animals used throughout the industry. Gnawing behavior is especially observed in rodents because of their constantly growing incisors. Their incisors need to be regularly worn down to maintain length within safe limits. Rarely used incisors can pierce the skull of rodents and cause injuries and even death. If a gnawing device is present in the cage but is not preferred or useful to the rodent, there may be unnecessary loss of life. Selection of appropriate gnawing devices can avoid such wasteful fatalities and help research laboratories conduct unhindered research. Two common materials of gnawing devices are wood and plastic. We found the most optimum wood device and plastic device by comparing 4 devices from each category. Video scoring, weight loss analysis, and amylase paper saliva analysis were conducted to determine the most preferred device. In the first round, devices were compared with members of the same category (wood or plastic) to determine the best device within the respective category. The most preferred wooden devices was found to be tongue depressor. The most preferred plastic device was a commercially available flexible polymer chew. For the second round, the best devices from each category were placed in the cage together to identify which device was best preferred by the rats and mice. Video scoring showed tongue depressors to be significantly more preferable than the flexible polymer chew in mice.

#### PS44 Housing Environment and its Effects on Mouse Aggression

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Conspecific aggression is one of the leading causes of morbidity in laboratory mice at research institutions, creating animal welfare concerns and often leading to early experimental termination. Fighting in mice is difficult to control because of its unpredictable nature and poorly defined treatment protocols. Additionally, research into identifying sources of aggression exhibits variable and often contradictory findings. In an effort to uncover sources of aggression in mouse cages, we conducted a yearlong cross-sectional epidemiological study to determine the prevalence and identify predictive factors of aggression. Buildings and rooms across campus were chosen to maximize factor variability. Cages were then visually assessed one at a time on randomly selected racks within these animal holding rooms. The target variables were fighting and its related trauma. Independent variables included time of year, type of caging, bedding material, nesting material, other forms of enrichment, position on the rack, location of the rack in the room, presence of ear tags, sex, strain, and stocking density. Analysis was conducted using nominal logistic regression and generalized linear modeling. Fighting and related trauma were noted in approximately 15.3% and 2.8% of male mice housed in groups of 2 or more, respectively. Mice housed on the top of racks and in individually ventilated cages with corncob bedding showed increased levels of aggression when compared to those

housed on the bottom of racks or in static cages with aspen bedding. Other predictors of aggression included strain and time of year. This data suggests that aggression in mice is multifactorial and is influenced by factors thought to be controlled for, such as the external environment. This study also helps provide a framework for housing at risk or valuable animals used in research while generating hypotheses for experimental research into individual environmental factors.

#### PS45 Water and Dust Baths as Environmental Enrichment for Zebra Finches (*Taeniopygia guttata*)

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Zebra finches are becoming an increasingly important biomedical research model, most frequently in the field of neurobiology. Historically, laboratory animal literature has given less attention to zebra finches than to more traditional laboratory animal species, particularly with regards to their husbandry and welfare. However, with the increasing interest in these birds as research models, there is a greater need for focused studies concerning their care and keeping in the laboratory. Water and dust baths have been identified as methods of enrichment for zebra finches, but there is a dearth of evidence assessing the value of these baths in fulfilling species-specific behavioral needs. Seventy cull zebra finches were grouped randomly into mixed-sex cages of 10 birds each. Each cage was provided with either a water or dust bath for at least 3 h per day for 5 d, followed by 2 d with no bath access. The procedure was repeated using the alternate bath. Those that received water baths were given dust baths and vice versa, for the same period of time. All interactions with the baths were video recorded and retrospective analysis of behavioral parameters was completed to determine how and when the baths were used, and whether birds demonstrated a preference for 1 type of bath over the other. Zebra finches were found to interact readily with both water and dust baths. However, only water baths were used for bathing; dust baths were used primarily for foraging. Body condition and feathering scores were determined before and after testing with no significant change in either value. No significant feather plucking or fighting was seen in any context. These results support the notion that water baths are a valuable form of enrichment for zebra finches, and suggest that foraging options for this species should be further explored.

#### PS46 A Comparison of Mouse Reproductive Performance in 2 Types of Individually Ventilated Cage Systems

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We evaluated breeding efficiency in C57Bl/6J mice in a novel individually ventilated cage (IVC) design (product A) compared to a traditional IVC design (product B). Product A introduces an airflow from below the level of the bedding, creating a plenum in the cage where airflow is diffused through the bedding. One outcome of the design modification is this product can operate at a low-velocity airflow (e.g. 30 air changes per hour (ACH)). As mice often use scent cues in normal behaviors such as breeding, a low airflow was hypothesized to increase breeding efficiency. Further, the product A design keeps the bedding dry, preventing ammonia accumulation and affording a longer cage change interval. Thus, allowing the nest to go undisturbed until weaning, potentially increasing reproductive efficiency. Eight monogamous breeding pairs were housed per each cage system and were allowed to breed for 6 mo. Cages were changed on day 1, the day after pups were observed in the cage. Using the manufacturer recommend settings, a 14-d cage change at 60 ACH in product B was compared to a 21-d cage change at 30 ACH in product A. Various reproductive parameters, such as litter size, number of

litters, pup weaning weight, and cage performance were compared. Product B averaged 6.4 pups per litter and had 27 litters total while product A averaged 7.3 pups per litter and had 33 litters total. Average pup weight on d 28 was 14.7g (n=17) in product B and was 12.9g (n=23) in product A. In regards to cage performance, ammonia levels were measured by an electrochemical sensor and by wireless metal-oxide semiconductor sensors. Other cage performance observations found that product A reduced flooding incidences due to automatic watering valve failures. This study finds that product A, with a significantly modified design concept, to be a suitable environment for mouse breeding on a 21-d cage change schedule.

#### PS47 Pairing across Macaque Species: An Alternative Option to Achieve Social Housing

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The *Guide for the Care and Use of Laboratory Animals* emphasizes the need to socially house social species, including nonhuman primates. Social housing improves psychological well-being, promotes species-typical behavior, and reduces abnormal behavior. Most primate facilities focus on social housing primates of the same sex and species. However, when there is an odd number of animals of the same sex and species, or when there are repeated episodes of aggression toward conspecifics, alternative approaches need to be considered to provide social housing opportunities. Over the last 10 y, our facility has been cohousing mixed species of macaques to meet the social needs of our macaque colony. Using the same stepwise pairing protocol used for same species introductions, we have attempted 329 rhesus and cynomolgus pairings, of which 113 were successful. A successful pair is defined as stable cohousing of 4 mo or longer. Of the successful pairs, 5% were young animals (less than 5 y of age), 42% were a young animal paired with an adult, and 53% of the pairs were adult macaques. Our success at cohousing rhesus and cynomolgus macaques indicates that social housing mixed macaque species is a viable option for facilities that house nonhuman primates.

#### PS48 The Art of Wild Rat Wrangling

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Domesticated, standardized, pathogen-free research animal colonies have shaped standards of care, housing, health surveillance, and safe handling. Recently, questions have arisen about the relevance of clean, lab-reared rodents versus pet store or wild-caught animal models of human disease. An IACUC-approved research protocol queried whether results from the spontaneous Sprague Dawley epileptic seizure model would be recapitulated in wild Brown Norway rats. We were tasked with trapping wild rats on campus and housing them in our vivaria for behavioral-test studies. This posed several obstacles such as zoonotic adventitious pathogen exposure, adequate housing, and safe handling. Working closely with the campus IACUC, occupational health nurse, environmental health and safety officers, integrated pest management officer, and attending veterinarian, we developed a protocol for live capture of contaminated rodents for which we normally keep out of our vivaria. Using an aromatic bait and Sherman-like catch and release traps, we set at various locations across campus in the evenings when Colorado nighttime lows were > 40 degrees F. After donning approved PPE, traps were checked in the mornings. Challenges we faced while trapping included escapees, bait consumption without capture, aberrant species, predation, and capture of pregnant females. Upon successful capture, animals were transported to ABSL-2 quarantine, anesthetized, and samples were taken for a global panel of 50 pathogens of which 19 pathogens were detected. All animals were treated for multiple internal and external parasites. After stabilization and treatment, the lab was then cleared to

perform surgeries and behavioral testing within the ABSL-2 suite. Since domesticated strains are very intelligent, the “street smarts” of the wild rats and the unique hurdles they present were not underestimated. Successful introduction of wild-caught rodents into our vivaria resulted in reevaluation and refinement of our animal husbandry practices for the safety of the animals, the safety of our staff, and the protection of our colony animals.

#### **PS49 Benefits of Cage Enrichment in Breeding Colonies of C57BL/6J Mice**

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Cage enrichment promotes nesting instincts of laboratory mice and improves their well-being. We studied how enrichment affected pup survival and productivity in breeding colonies of the C57BL/6J strain. Five conditions were compared: cardboard shelters (tunnel or hut), paper nesting materials (vendor A or vendor B), and aspen shaving nesting material. Each group had 600 breeding trios mated at 6-8 wk of age and maintained for 30 wk; 20 new cages per group accrued each week. Diet, water, bedding, and husbandry practices were consistent among groups. Each group had similar weekly numbers of pups born per cage during a 5-mo period when there were at least 200 cages per group. During this time, the most pups were weaned from cages with tunnels: the per-cage weekly average significantly increased 18% over aspen chips ( $P = 0.0002$ ) and 11% over huts ( $P = 0.0473$ ). The fewest pups were culled from tunnel cages for reasons including runts, hair loss, and picked whiskers; weekly per-cage averages were reduced 60% compared to aspen chips ( $P < 0.0001$ ), 61% compared to vendor A ( $P < 0.0001$ ), and 45% compared to huts ( $P = 0.0180$ ). Vendor B's product reduced the weekly numbers culled by 32% relative to aspen chips ( $P = 0.0244$ ) and 34% relative to vendor A ( $P = 0.0118$ ). Vendor A's product was the only enrichment that significantly reduced pup mortalities; weekly numbers decreased by 67% relative to aspen chips, 68% relative to vendor B, 65% relative to huts, and 68% relative to tunnels ( $P < 0.0001$  for all). About 500 cages per group have completed a full breeding period at the time as of June 2018. Among these cages, vendor A and tunnel groups had the highest percentages of pups weaned (79% and 80%, respectively). Survival to weaning was 76% for huts, 75% for vendor B, and 69% for aspen chips. Pup mortality was lowest in vendor A cages (4%); rates were 8% for aspen chips, 9% for vendor B, 8% for tunnels, and 7% for huts. The fewest pups were culled from tunnel cages (4%); rates were 11% for vendor A, 15% for aspen chips, 10% for vendor B, and 10% for huts. Fewer runts and barbered pups accounted for the improved weaning success in tunnel cages. The results show the benefit of enrichment for breeding colonies and suggest possible additional improvements by combining enrichments.

#### **PS50 Development of a Successful Preterm Infant Model Using *Sus Scrofa domestica*.**

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Preterm infants have been shown to have metabolic and developmental deficits within their first postnatal week of life leading to an increase in neonatal morbidity. Our goal was to design an effective model using Yorkshire swine that would resemble preterm infants and allow the investigative group to carry out a nutritional support study. Three groups of preterm piglets with 8, 12, 13 piglets, respectively at gestational ages 105-107 d) were delivered via Cesarean section, resuscitated, and allowed to recover in a controlled environment. Each piglet was provided a group of caregivers, including members of the NICU staff, a veterinary technician, and

direct oversight of the NICU physician and veterinarian. Once the piglets were stabilized clinically based on predetermined parameters they underwent an anesthetic event, placement of a central line and placement of feeding tube within hours of the Cesarean. The piglets were again allowed to recover from these procedures and remained on study for 7-10 d. During the study length many challenges were faced in the medical management of premature piglets that we do not face with animals of a full gestational age. The care of the piglets underwent a great evolution over 3 litters through trial and error. The final major alterations were made for the third litter, which was the most successful. New recovery units were purchased which allowed us to control environmental conditions and eliminate piglet crowding to give us a better visual on each piglet. The Cesarean and recovery process was modified to maintain a controlled environment. We eliminated the need for an anesthetic event and central line placement on the first day of the procedure by use of umbilical catheterization. The veterinarian determined critical monitoring parameters, and were posted identifying when intervention was deemed necessary. The parameters included albumin, blood glucose, temperature, SpO<sub>2</sub> and mentation. Finally, nutritional support was changed by the group to support the growing piglets. The parameters that we changed gave us 100% survival to end point of the final litter. We determined that these changes were imperative to the success of the study and overall care and welfare of the piglets. Not only was this study incredibly challenging from a medical and husbandry aspect it also had a profound emotional impact on everyone involved in the study.

#### **PS51 The Caribbean Primate Research Center and Hurricane Maria: Lessons Learned**

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The Caribbean Primate Research Center (CPRC) was faced with one of its biggest challenges when Hurricane Maria devastated the island of Puerto Rico on September 20, 2017. Both CPRC facilities, Sabana Seca Field Station (SSFS) and the free-ranging island of Cayo Santiago, were severely affected. Each site had its own set of unique challenges, including the logistics of providing transportation, getting food and water to animals, equipment damage and lack of power for almost 10 months, all while our employees' were dealing with the fact that their homes and lives had been severely impacted. Simple tasks we take for granted, such as getting ice, using cell phones, and acquiring gasoline, became significant challenges. Though both sites were unique in the types of struggles they faced, one unifying goal was evident throughout our post-Maria reality: making sure that the CPRC took care of more than 4,000 rhesus macaques in the midst of the chaos. Access to produce on the whole island was limited, water availability at both places was also limited in some cases, and natural enrichment (leaves, brush, trees) was all gone at Cayo Santiago. Over the subsequent months, new challenges arose, including a lack of air conditioning as the excessive heat began to take a physical and mental toll on our employees and dependence on an unreliable generator became the norm. Despite it all, our work with various research groups has continued, and our team was recently awarded a GLAS grant. We saw no appreciable change in morbidity or mortality of our animals at SSFS, and all escapees from the damaged corral were accounted for. In the year since Hurricane Maria, we have been buoyed by an outpouring of support from the laboratory animal, primatology, and research communities, and as a result, the immense task of rebuilding both facilities has been well underway.

#### **PS52 Sexual Dimorphism of the Obesity Phenotype in C57bl/6j Mice**

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Women are generally cardiometabolically protected from obesity-

induced hypertension and type II diabetes compared to men despite a higher prevalence of obesity within the female population. This cardiovascular protection has also been observed in high-fat diet (HFD)-induced obese female mice but whether this protection extends to metabolic function has yet to be determined. We hypothesized that obese female mice would be metabolically protected from insulin resistance and glucose intolerance. To test this, 5-wk-old-male and female C57BL/6J mice were randomly placed on either a standard chow diet (18% kcal from fat) or a 60% HFD for 11 wk (n= 8-12/group). During the last week of the diet period, body composition and intraperitoneal insulin and glucose tolerance tests were performed to assess insulin sensitivity and glucose tolerance, respectively. On the last day of treatment, body weight was measured followed by euthanasia and adipose tissue collection. Throughout the treatment period, female mice maintained a lower body mass in comparison to their male counterparts. However, there were similar increases in adiposity between genders in response to HFD ( $P = 0.001$  diet,  $P = 0.316$  gender,  $P = 0.693$  interaction; 2-way ANOVA). Both obese male and female mice developed mild hyperglycemia ( $P = 0.021$  diet,  $P = 0.265$  gender,  $P = 0.463$  interaction), insulin resistance ( $P = 0.001$  diet,  $P = 0.444$  gender,  $P = 0.249$  interaction), and glucose intolerance ( $P = 0.001$  diet,  $P = 0.156$  gender,  $P = 0.685$  interaction). In contrast to males, however, HFD fed female mice did not develop hyperinsulinemia ( $0.6 \pm 0.1$  chow female,  $1.1 \pm 0.1$  HFD female,  $0.9 \pm 0.3$  chow male,  $3.7 \pm 0.5$  ng/mL HFD male;  $P = 0.001$  diet,  $P = 0.001$  gender,  $P = 0.006$  interaction). This suggests, that in contrast to our hypothesis, female mice develop obesity-related insulin resistance and glucose intolerance to a similar extent as males. There is, however, sexual dimorphism of the obesity phenotype in C57BL/6J mice related to control of circulating insulin levels that needs to be further explored. These overall findings suggest that C57BL/6J mice could serve as a good translational model in the study of obesity-induced metabolic dysfunction in both men and women.

#### PS53 *Corynebacterium bovis*: The Search for Its Bacteriophage Lysin for Use as a Potential Therapeutic

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*Corynebacterium bovis* is the causative agent of *Corynebacterium*-associated hyperkeratosis (CAH) in immunocompromised mice. The resulting skin pathology can be profound and may be associated with severe wasting, potentially making the animals unsuitable for research. The administration of antibiotics is effective in resolving disease, but does not eradicate the bacterium; however, antibiotic use may be contraindicated as it can affect tumor growth. Lytic enzymes (lysins) obtained from bacteriophages are being investigated as potential novel antimicrobial agents for a variety of bacterial diseases. The advantage of a lysin is its target specificity without affecting the host or engrafted tumor. The goal of this study was to identify *C. bovis*-specific phages and isolate their lysin to test as a potential therapeutic. *C. bovis* isolates, obtained from 1 human and 2 mice, were treated with mitomycin C to induce phage replication. All samples showed turbidity reduction over time, which suggested potential lytic activity. However, when plate-lysis assays were conducted no bacterial lawn clearance was observed. Thus, the presence of phage could not be confirmed by this method or by electron microscopy. Subsequently, using whole-genome sequencing technology, the genome of 20 *C. bovis* isolates obtained from humans, cows, and rodents were sequenced and analyzed for

phage. No intact phage sequences were identified in any of the isolates. Thus, despite the ubiquity of phages in almost all bacteria, it appears that *C. bovis* does not have any associated phage. Genomic analysis further revealed that all isolates had at least 1 confirmed clustered regularly interspaced short palindromic repeats (CRISPR) system, which may explain why no phages were found as CRISPRs serve as an adaptive immune system for prokaryotes. Additionally, toxin-antitoxin systems, shown to mediate defense mechanisms against phage infection, were detected in the majority of the isolates. Although highly unusual, *C. bovis* does not appear to have a phage system, making lysin therapy more difficult. However, lysins from phage of closely related organisms could have an effect on *C. bovis*, but this has not yet been explored.

#### PS54 Characterizing the Infectivity, Tissue Tropism, and Pathology of MuAstV2, a Novel Murine Astrovirus

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Astroviruses, a genetically diverse group of RNA viruses, are known to infect a wide range of mammalian and avian species. The first astrovirus in laboratory mice was described in 1985. Advances in molecular genomics and increased population surveillance has led to the identification of various astrovirus strains in feral and laboratory rodent populations, the significance of which is currently unknown. A common astrovirus infecting mice, murine astrovirus 1 (MuAstV1), is endemic in many research and production laboratory mouse colonies. We recently identified a novel astrovirus genetically distinct from MuAstV1 using metagenomics. This virus, murine astrovirus 2 (MuAstV2), is most closely related to astroviruses isolated from 2 feral rat species in China and a virus recently identified in feral mice in New York City. Our search for the virus was initiated following false positive results to MTLV in a multiplex immunofluorescence assay that used MTLV antigen generated in an immortalized murine AKR T cell line that was subsequently found to be infected with a closely related virus. MuAstV2 is readily transmitted by the fecal-oral route, and immunocompetent mice infected during the initial outbreak remained asymptomatic. Histopathologic evaluation of all organs submitted from these mice showed no significant morphologic changes. However, mild to moderate levels of viral nucleic acid were detected by in situ hybridization (ISH) in the enterocytes of the small and large intestine, and within mononuclear cells of Peyer's patches and mesenteric lymph nodes. We report on studies conducted to characterize the infectivity, tissue tropism, and pathology of this virus following oral inoculation of young adult C57BL/6NCrl and NOD-*Prkdc<sup>em26Cd52</sup>Jl2r<sup>em26Cd22</sup>/NjuCrl* (NCG) mice. The magnitude and duration of viral shedding was characterized by performing fecal qRT-PCR. Gross and histopathological changes, as well as viral tropism identified using in situ hybridization were examined at various time points post-inoculation. These findings as well as the potential impact of MuAstV2 on research will be discussed.

#### PS55 Comparing Mouse Sentinel and Exhaust Air Dust Health Monitoring Surveillance Programs

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To monitor rodent colony health in research facilities, soiled bedding

sentinel (SBS) animals are most commonly used. SBS testing may employ multiplexed fluorometric immunoassay (MFIA), PCR rodent infectious agent (PRIA) panel, endo-/ectoparasite testing, necropsy, or other methods. However, several pathogens have been proven to be unreliably detected or transmitted by soiled bedding providing an opportunity to develop an enhanced monitoring plan. Recently, exhaust air dust (EAD) testing via PCR has emerged as an adjunct method or replacement for rodent health colony monitoring. In an effort to improve colony health monitoring and potentially reduce sentinel animal numbers, we evaluated the efficacy of monitoring via EAD compared to SBS in an established SPF facility. We hypothesized that comparison of the 2 methods for health monitoring would show EAD to be just as or more sensitive than SBS monitoring. In a facility exclusively using individually ventilated cage (IVC) racks able to be fit with commercial EAD filter medium at the exhaust manifold, we monitored 3 housing rooms (2 standard barriers [SB] and 1 standard plus barrier [SPB] room) for 1 y. Quarterly testing of SBS via MFIA/PRIA and EAD filters via PCR was performed. Room configuration ranged from 4-6 single-sided 70 cage racks and 1-2 double-sided 140 cage racks per room. One live sentinel was tested per rack side (maximum 69 cages/sentinel) and 1 EAD was tested per rack (maximum 70 or 140 cages). The pathogens tested were all agents excluded in the SPB rooms. The SB included the typical agents excluded in most rodent SPF facilities, while the SPB rooms also excluded *Helicobacter* spp., *Pasteurella pneumotropica*, and mouse norovirus (MNV). Monitoring health with the full panel of agents for all rooms found that EAD PCR consistently detected MNV the same as the SBS, as well as additional detection of *Helicobacter* spp. and *Pasteurella pneumotropica* where the SBS did not detect the bacterial agents. This suggests that EAD is especially valuable in detecting these bacteria in the SPB rooms that exclude them. The findings of this comparison study indicate that an established SPF facility housed exclusively on IVC racks can reliably implement EAD PCR testing as an alternative to SBS monitoring.

#### PS56 Characterizing the Murine Immune Response following Infestation with *Demodex musculi*

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*Demodex musculi* infestations are rarely reported in laboratory mice. However, the sharing of genetically engineered mouse strains among institutions has led to the inadvertent introduction of *D. musculi* into many colonies. Until recently, detection methods were unreliable and testing for the parasite was not routinely performed as its presence in laboratory mice went unrecognized. We estimate a prevalence of approximately 10-30% in academic colonies based on PCR data from animals imported into our institution. While infestations are clinically inapparent in most mouse strains, *D. musculi* burdens are increased and clinical signs have been reported in several strains of immunodeficient mice. Though the immune phenotypes of animals in these reports were known and likely the cause of increased susceptibility, what, if any, effect *D. musculi* has on the immune system of immunocompetent mice is unknown. We hypothesized that infestation with *D. musculi* modulates innate and adaptive immune responses and the mite burden would be dependent on the immunophenotype of the infested strain. As Th2-mediated immune responses are known to increase resistance to parasitic infections, Th1-dominant strains will likely be more susceptible to infestation. We characterized the mite burden and immunologic changes in naïve Swiss Webster (outbred), C57BL/6NCr(Th-1 skewed immune response), and BALB/cAnNCr(Th-2 skewed immune response) mice following exposure to *D. musculi*. *Demodex*-infested NSG mice from a

previously established *Demodex*-positive colony were cohoused with naïve Swiss Webster, C57BL/6NCr, and BALB/cAnNCr mice. Age-matched mice (n=5) were euthanized for sample collection 14 and 28 d later. Mite burden was determined by PCR and comprehensive skin histopathology. CD4 and CD8 cell counts, T-cell activation markers (CD44, CD25, CD69, Ly6C) were evaluated using flow cytometry, and complete blood counts were performed. Not surprisingly, highly immunocompromised NSG mice had the highest mite burdens, which correlated with high PCR copy numbers, although no evidence of clinical disease was noted. No significant differences between infested and naïve animals in terms of T-cell activation or complete blood counts were observed in Swiss Webster mice.

#### PS57 The Effect of Buprenorphine and Buprenorphine Sustained Release on the Minimum Alveolar Concentration of Isoflurane in Mice

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Buprenorphine (BUP) and buprenorphine SR (BupSR) are used as preemptive analgesics in mice, potentially affecting anesthetic requirements. The purpose of this study was to determine the effects of BUP and BupSR on the minimum alveolar concentration (MAC) of isoflurane and on heart and respiratory rates. We hypothesized that BUP and BupSR would significantly decrease MAC, heart, and respiratory rates in isoflurane anesthetized mice. Forty-seven male and female C57BL/6 (6-15 wk of age) mice received either 0.2 mL of saline (control), BUP (0.1 mg/kg), or BupSR (1.2 mg/kg) subcutaneously 10 min before induction of anesthesia with isoflurane. Mice were maintained at 3 randomized, increasing, isoflurane concentrations and depth of anesthesia was assessed by response to a noxious stimulus after 10 min at each isoflurane concentration. A surgical plane of anesthesia was defined by the loss of hind limb withdrawal. MAC for each injection was calculated using quantal and bracketing techniques. A 1-way ANOVA at the MAC for each injection was used to compare MAC, heart, and respiratory rates between the injections. Significant differences were observed between BUP and BupSR on MAC ( $P < 0.001$ ). BUP reduced MAC by 18% in females (1.72±0.08% control; 1.41±0.09% BUP) and 26% in males (1.82±0.03% control; 1.33±0.15% BUP). BupSR reduced MAC by 16% in females (1.45±0.11%) and a 22% MAC reduction in males (1.42±0.13%). Sex did not significantly affect MAC. The heart rate of the mice receiving BUP was significantly lower ( $P < 0.001$ ; 359±24 bpm BUP) than the mice receiving the BupSR and control injections (455±68 bpm BupSR, 499±48 bpm saline) at similar planes of anesthesia. There were no significant differences in the respiratory rate (126±21 bpm BUP; 132±13 bpm BupSR; 122±22 bpm control). This study demonstrates that BUP and BupSR act similarly in male and female mice and decrease isoflurane requirements to maintain a surgical plane of anesthesia in mice.

#### PS58 Female-induced Ultrasonic Vocalizations in Male C57BL/6J Mice as a Proxy Indicator for Acute Pain

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Recognizing pain objectively in rodents is challenging, yet it is an essential component to minimizing pain and distress in these animals. Mice produce ultrasonic and audible vocalizations to communicate with conspecifics and this behavior has been studied as a modality for pain recognition with mixed results. Female-induced ultrasonic vocalizations (FiUSV) are ultrasonic vocalizations produced by adult males when presented to adult females or their urine. This is an affiliative behavior that may be reduced if the mice are in pain or distress. To determine if FiUSV can be used as a proxy indicator for

pain recognition, we compared ultrasonic vocalizations produced by male C57BL/6J mice in response to female urine at baseline, 1h, and 3h post administration of a sublethal dose of lipopolysaccharide (LPS; 12.5 mg/kg IP) or equal volume of saline. Pain was assessed by orbital tightness, posture, activity, and piloerection immediately after ultrasonic measurement. We hypothesized that painful or distressed male mice would have a decreased inclination to mate and therefore would produce fewer FiUSV. At baseline, 32 out of 33 mice produced FiUSV ( $149 \pm 127$  USV/2 min). There was no change from baseline at the 1- or 3-h time points in the saline-treated mice, whereas LPS-treated mice demonstrated significantly fewer FiUSV than baseline ( $P = 0.0078$ ), producing 0 USV at both time points. Mice treated with LPS showed signs of pain at 3 h but not 1 h according to orbital tightness, posture, activity, and piloerection. These findings show that FiUSV can be used as a proxy indicator for acute pain and a change from baseline can be detectable prior to onset of visual clinical signs.

#### PS59 An Affordable and Efficient Method to Decontaminate Laboratory Equipment Using Chlorine Dioxide Gas

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A significant concern in laboratory animal medicine is contamination due to pathogen outbreaks and how to adequately address the issue of small equipment decontamination. Many factors play a role in the selection of the decontamination method including cost, efficacy, and personnel time and safety. Chlorine dioxide (ClO<sub>2</sub>) gas is an effective method, but commercially available systems which include a large-scale ClO<sub>2</sub> gas generator and specialized air-tight exposure chamber can be costly and impractical in some situations. The goal of this study was to create and validate an effective, small-scale, decontamination method utilizing ClO<sub>2</sub> gas, which is affordable, efficient, safe, and reproducible. First, we identified a product where 2 dry reagents react to produce ClO<sub>2</sub> gas. To find an affordable exposure chamber, we evaluated the ability of 4 household totes with gasket-seal lid systems to retain ClO<sub>2</sub> gas and relative humidity (RH). Validation of the efficacy of decontamination was assessed by using 2 different biological indicators (BI) concurrently, *Bacillus atrophaeus* (*B.a.*) and *Geobacillus stearothermophilus* (*G.s.*). ClO<sub>2</sub> gas concentration, total exposure dose, and RH were measured using a commercially available photometer and data logger. Our results confirm that ClO<sub>2</sub> gas production is reliable and scalable. All household totes evaluated held sufficient gas and RH for a 15-h cycle (overnight), providing adequate exposure to inactivate both BI evaluated. Our results suggest that a total exposure dose of  $71 \pm 42$  ppm-h and  $1.17 \pm 0.14$  mg of ClO<sub>2</sub> gas over 15 h at  $> 90\%$  RH is adequate to inactivate both *B.a.* and *G.s.* While there was no statistical difference in the 2 BI as indicators for decontamination ( $n=460$  BI,  $P = 0.362$ ), for the end user, *B.a.* was more consistently inactivated. In conclusion, we have successfully brought together a variety of low-cost materials to establish an effective, small-scale method to decontaminate small laboratory equipment. Depending on the size of the tote and whether BI are used, the upfront cost is approximately \$75-\$750. This method is roughly 100 times less expensive than large-scale ClO<sub>2</sub> gas generators used with specialized air-tight exposure chambers.

#### PS60 Evaluating the Physiological Effects of a Novel Gutloading Cricket Diet on Leopard Geckos

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Leopard geckos (*Eublepharis macularius*) are one of the most popular captive insectivorous reptiles. Commercially available insects fed to insectivores frequently have an inverse calcium (Ca) to phosphorus (P) ratio, along with low levels of Ca and vitamin D (VitD). Feeding these nutritionally deficient insects contributes to and may lead to nutritional secondary hyperparathyroidism. To circumvent this problem, insects typically require dietary supplementation (gutloading) to correct these deficiencies and correct Ca to P ratios prior to being fed to insectivores. The effects of gutloaded insects, as compared with fasted insects, on VitD, Ca, P, and bone density in leopard geckos has not been reported. This study examined an experimental gutloading diet fed to crickets (*Acheta domesticus*), who were subsequently fed to leopard geckos. Our hypotheses included the following: 1) the leopard geckos fed the supplemented crickets will maintain higher calcium concentrations than the geckos fed the non-supplemented crickets; 2) the leopard geckos fed the supplemented crickets will have increased bone mineral density in comparison to the geckos fed the non-supplemented crickets; and 3) the leopard geckos fed the supplemented crickets will have increased VitD concentrations compared to the geckos fed the non-supplemented crickets. Leopard geckos were matched by weight and sex, then randomly divided into treatment ( $n=12$ ) and control ( $n=12$ ) groups. Geckos assigned to the treatment group were provided crickets that were fed the experimental diet for 4-6 h prior to being fed to the geckos, while geckos in the control group were provided non-supplemented crickets over the course of 90 d. Blood was collected at days 0, 45, and 90 to measure Ca, P, and VitD. Computed tomography (CT) images were taken at baseline and 90 d for bone (mandible and vertebrae) mineral density measured in Hounsfield units. The crickets fed the experimental diet for 4-6 h maintained a more appropriate calcium to phosphorus ratio (1:1) than those that are fasted (1:7). Ca and P levels within the blood at 45 d were not different compared to baseline values.

#### PS61 Comparison of Sedatives in Combination with Isoflurane for Pupillary Light Reflex Imaging in Mice

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The *Guide* indicates that pharmaceutical-grade chemicals "should...be used, when available, for all animal-related procedures" and "[t]he use of non-pharmaceutical grade chemicals or substances should be described and justified in the animal use protocol..." For vision research in isoflurane-anesthetized mice, the nonpharmaceutical grade sedative chlorprothixene has been widely used to reduce spontaneous eye movement in pupillary light reflex imaging, and to lower the isoflurane to a level that does not substantially weaken light-evoked responses in electrophysiological recordings. However, data is lacking to justify the use of chlorprothixene. This study evaluated whether pharmaceutical-grade sedatives would be appropriate alternatives. Male 15-wk-old B6129SF2/J mice were IP-injected with 1 mg/kg chlorprothixene ( $n=5$ ), 5 mg/kg acepromazine ( $n=5$ ), 10 mg/kg chlorpromazine ( $n=4$ ), or saline ( $n=5$ ), then induced with isoflurane after 1 min of sedation. Anesthesia maintenance was 0.5% and 1% isoflurane for mice administered sedatives and saline, respectively. A 16.0 photons  $\text{cm}^{-2} \text{s}^{-1}$  470 nm light stimulus was applied to the right eye, and the left eye was imaged for consensual pupillary constriction and involuntary pupil movement. Induction parameters measured were time to immobilization and loss of righting reflex, and physiologic parameters were assessed during anesthesia. ANOVA analyses showed no significant differences in baseline pupil diameter, pupillary light reflex response, and induction parameters, while mean heart rate in the saline group was significantly lower. Substantial involuntary pupillary movement was observed in 2/5 mice in the saline group, moderate movement in 1/4 mice in the chlorpromazine group, and slight movement in 1/5 mice in the acepromazine group. Full recovery, as defined by purposeful movement, response to tactile



stimuli and full alertness, was not achieved in any sedative group during the 1.5-3 hour postanesthetic period due to continued substantial sedation. In conclusion, acepromazine is likely a suitable pharmaceutical-grade alternative to chlorprothixene, but lower dosages may need to be further investigated for use in survival procedures given the lack of full recovery in the experimental groups.

### PS62 Bringing Yin and Yang to the Vivarium: Adapting Acupuncture to Diverse Animal Models

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Acupuncture is a nondrug therapy modality that can be useful in laboratory animal medicine. Acupuncture training is classically taught using dogs and horses. Five clinical cases are presented (4 successes, 1 failure) to illustrate potential usefulness/limitations of acupuncture in lab animal care. The first is a 10-y-old male rabbit (*Oryctolagus* sp.) that presented with hindlimb paresis. Radiographs revealed thoracolumbar narrowing of vertebral disk spaces. Acupuncture was performed twice weekly at 8 points, with gradual improvement of hindlimb function. The second case was a 39-y-old chimpanzee (*Pan* sp.) that presented with ventricular premature contractions (VPC). The animal had multifocal VPCs that were refractory to sotalol, with an adverse reaction to amiodorone. It was treated bilaterally for 10-min sessions at 2 cardiotherapeutic acupuncture points: pericardium 6 (PC6) and heart 7. There was an initial increase, then a gradual decrease in VPCs over the next 12 mo. In the third case, neonatal apnea was observed in 2 squirrel monkeys (*Saimiri* sp.). After cesarean section delivery, single-point acupuncture at governing vessel 26 was used in one case of respiratory arrest and 1 case of respiratory depression with immediate improvement of respiratory status in both cases. Owl monkey (*Aotus* sp.) wasting disease (OMWD) is characterized by gradual weight loss, leukopenia, hypoproteinemia, brain lesions, normal appetite, and stools. Eight owl monkeys with OMWD received twice weekly vitamin B12 aquapuncture at 4 points: PC6, stomach 36 (ST36), gallbladder 34, and spleen 6. Animals began to gain weight in 1-2 wk and were essentially normal in 3 mo when treatment was terminated. Lastly, a 13-y-old male *Aotus* had intermittent melena/diarrhea refractory to Western medical treatment. Repeated bacterial culture and fecal parasitology were negative for pathogens. An ultrasound exam was negative for neoplasia. Three-point acupuncture was performed at large intestine 4 and 10 and ST36 twice weekly for 3 wk but there was no change in the animal's condition. Adapting acupuncture to research animals can be facilitated by adjusting traditional methodology to research requirements, diagnosis, animal temperament, anatomy, and size.

### PS63 MRI-guided Convection-Enhanced Delivery into the Striatum in Cynomolgus Macaques (*Macaca fascicularis*)

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Gene therapy is a promising area of drug development for a number of diseases, including neurological disorders. Cynomolgus macaques (*Macaca fascicularis*) show high nucleotide sequence homology with humans and need to be used where test article cross-reactivity occurs. An IACUC-approved regulatory toxicity study required bilateral administration of a gadolinium-labeled viral vector into 4 locations into the striatum (caudate nucleus and putamen) with temporarily implanted catheters. Magnetic resonance imaging (MRI) was used for calculation of trajectory and for surveillance of test article delivery during convection-enhanced delivery. Twenty-four cynomolgus macaques (4 groups, 12 M/12F) were prepared for a 6-8 h anesthesia including presurgical analgesia, induction of anesthesia with ketamine/medetomidine, intubation, clipping, disinfection, and placement in a stereotactic frame. Inhalation anesthesia was conducted with isoflurane. Animals were transferred to the MRI to obtain data for

calculation of position and angle and depth for catheter placement, assuring that the trajectory will not cross blood vessels or ventricles. Following transfer to the surgical suite, the stereotactic frame was used to ensure correct placement of the catheters based on the coordinates determined by MRI. The skin was reclined from the skull and holes were carefully drilled. Catheters were cut to specific length and inserted along the calculated trajectories to administer the viral vector to 4 different locations into the striatum. Catheters were connected to infusion lines containing the gadolinium labeled test article. Infusion (rate 0.3mL/h) was monitored in the MRI for 80 m to verify correct administration into the striatum. Thereafter, animals were transferred back to the surgical suite and catheters were explanted. Animals recovered from the 6-8 h anesthesia within 15-30 min and were treated with analgesics and antibiotics. Two animals presented laryngeal swelling were treated with corticosteroids successfully. No animal showed neurological abnormalities. Histopathology results will be presented. Using convection-enhanced delivery, 96 catheters were successfully implanted with fast and uneventful recovery from a 6-8 h isoflurane anesthesia. There was successful intracranial administration into the striatum.

### PS64 Meloxicam Sustained-Release Injection Site Skin Reaction in Sprague Dawley Rats (*Rattus norvegicus*)

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Pain management in laboratory rodents is a critical component of animal welfare. Meloxicam is a nonsteroidal antiinflammatory drug (NSAID) commonly used to manage pain in rodents and other species. An extended release formulation, MSR, has been developed to provide 72 h of continuous analgesia. While standard formulations of meloxicam are frequently used with no observable injection site reactions, the potential adverse effects from MSR have not been sufficiently characterized and clinical cases in rodents have been observed. According to the manufacturer, the full formulation of MSR was not tested for safety and published studies have not observed animals long enough to detect reactions developing beyond 3-5 d. We evaluated injection site reactions following a single subcutaneous administration of MSR in 22 age and sex-matched Sprague Dawley rats. Mass score, erythema score, and mass dimensions were measured daily for 2 wk and the injection sites were collected postmortem for histopathology. Animals were euthanized at 7 (n=12) and 14 d (n=10) postadministration to capture the subacute and chronic phases. No rats in the saline control group developed lesions, while all 16 rats in the MSR treatment group developed lesions ( $P < 0.001$ ). The median time to first lesion in the MSR treatment group was 3 d (95% confidence interval 2-3 d), showing a very consistent pattern, again highly significantly different from the control group ( $P < 0.001$ ). A more detailed examination of the trajectories of lesion severity showed rapid progression from onset around d 2-3, at stage 1 lesions characterized by palpable thickening with undefined borders, mild alopecia and/or mild erythema, to stage 2 lesions characterized by a measurable, defined mass with moderate alopecia and/or moderate erythema for almost all animals by d 5 or 6. Histologic evaluation of lesions were characterized by localized inflammation with central necrosis and peripheral fibrosis, with some developing draining tracts. The MSR treatment rats, in contrast to saline controls, uniformly developed observable lesions early in the observation period, continuing with rapid growth through the first week, stable after that, and very few reversions. Given the high prevalence and severity of localized skin reactions, careful consideration should be given when potentially including MSR in analgesic regimens.

**PS65 Use of a Novel Scoring System to Evaluate a Polyunsaturated Fatty Acid Product for Treating Atopic Dermatitis in Rhesus Macaques (*Macaca mulatta*)**

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Atopic dermatitis is a chronic condition that is notoriously difficult to evaluate and treat in various animal species. Although glucocorticoids and calcineurin inhibitors have been efficacious in many of these cases, their use in laboratory macaques can present a myriad of challenges. Recently, a topical, bio-diffusible, polyunsaturated fatty acid (PUFA) product has shown promise in ameliorating the clinical signs of atopic dermatitis in dogs and cats. In this study, 3 rhesus macaques (*Macaca mulatta*) affected with chronic dermatitis, along with age and sex-matched, unaffected controls, were assessed using a novel Macaque Atopic Dermatitis Extent and Severity Index (MADESI). This scoring system was adapted from one widely used in dogs. Full-thickness skin biopsies, photographs, and routine bloodwork were also obtained prior to treatment. A spot-on product was then applied cageside to the dorsum of all animals at weekly intervals for 12 wk, followed by a repeat of the MADESI scoring, biopsies, photographs, and bloodwork. Thereafter, treatments were continued at monthly intervals with MADESI scoring every 3 mo. Prior to treatment, high MADESI scores in the affected macaques mirrored the histopathological abnormalities of their skin biopsies. Following the initial 3 mo of treatment, the atopic animals exhibited a marked reduction in clinical lesion severity, as evidenced by lower MADESI scores, along with decreased acanthosis, hyperkeratosis, and inflammation. In contrast, control animals that underwent treatment had minimal changes in their MADESI scores or histopathology over time. Animals and their cage mates demonstrated no adverse response to the treatments. These results indicate that the MADESI scoring system is a valuable tool for tracking chronic dermatitis in macaque colonies. Additionally, a spot-on PUFA treatment reduced chronic dermatitis and improved the quality of life in affected macaques, causing minimal stress to the animals or effect on research paradigms.

**PS66 Adverse Effects of a Standard Multimodal Analgesic Regimen in a Rat Model of Spinal Cord Injury**

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A high incidence of adverse treatment effects were observed during a series of studies evaluating the use of a multimodal buprenorphine and bupivacaine analgesic regimen in a rat thoracic spinal contusion model. Adult female Sprague Dawley rats (*Rattus norvegicus*, Hsd:SD, n=46) underwent dorsal laminectomy and T8 spinal contusion. Treatment groups received a preoperative subcutaneous line block of bupivacaine (8 mg/kg) and varying regimens of buprenorphine treatment: buprenorphine HCl (0.05 mg/kg) every 8h for 24h or 72h, or a single dose of sustained-release buprenorphine (1.2 mg/kg). Control animals received volume- and site-matched injections of sterile saline. Over-grooming and self-injurious behaviors directed at the forelimbs or the site of buprenorphine injection were observed in 42% of rats receiving analgesics during study-related behavioral observations. These behaviors occurred during the 6-24h postoperative period in a subset of treated rats across all of the tested analgesic regimens. Associated clinical signs were observed, including alopecia with normal underlying skin, superficial cutaneous wounds and, less commonly, deep tissue damage resulting in removal from the study. No control animals exhibited these behaviors. Although self-trauma is reported to occur sporadically in spinal cord injury models, it is expected to affect the hind limbs or tail in thoracic spinal contusion models. In fact, self-injurious behaviors directed at these sites were observed in equal proportions between treatment and control groups ( $P = 0.72$ ) and rarely resulted in clinical signs. The buprenorphine regimens used in these studies are widely used for postsurgical

analgesia in laboratory rats. Adverse effects of buprenorphine such as pica and injection site reactions associated with sustained-release formulations have been previously described in rats. Pruritus associated with buprenorphine administration has been reported in primates, and self-injurious behavior has been described rarely in rats receiving high-dose buprenorphine. To our knowledge, this is the first report of self-injurious behavior associated with standard buprenorphine dosing in rats, and the potential for these effects to occur should be considered during study design and postoperative monitoring.

**PS67 Pharmacokinetics of a Long-acting, Highly Concentrated Buprenorphine Solution in Rhesus Macaques (*Macaca mulatta*)**

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Opioids are essential for rhesus macaques (*Macaca mulatta*) requiring multimodal analgesia or those unable to receive NSAIDs as part of their pain management plan. The current opioid epidemic has universally limited the availability of these vital analgesics, compelling clinicians to investigate other options including new opioid formulations or different dosing regimens. A long-acting, highly concentrated formulation of buprenorphine is available as a single dose, injectable solution that provides therapeutic plasma concentrations lasting 24 h in cats (*Felis catus*). We hypothesized that this highly concentrated buprenorphine solution (HCBS) would achieve therapeutic concentrations ( $\geq 0.1$  ng/mL) for at least 24 h in rhesus macaques similar to the cat. The objective of this study was to evaluate the pharmacokinetic profile of a single subcutaneous dose of HCBS in rhesus macaques at 0.24 and 0.72 mg/kg and then compare them to each other and the cat. Six healthy, adult rhesus macaques (3 male and 3 female) were included in a randomized, 2-period, 2-treatment crossover study. Plasma buprenorphine metabolite concentrations were determined prior to and for a maximum of 120 h after administration, measured using liquid chromatography-tandem mass spectrometry and pharmacokinetic analysis was performed. The low dose achieved a maximum plasma concentration of  $19.1 \pm 5.68$  ng/mL at  $19.6 \pm 4.02$  h with an AUC of  $236.4 \pm 22.5$  h/ng/mL and a terminal elimination half-life of  $19.6 \pm 4.02$  h; for the high dose, these parameters were  $65.2 \pm 14.7$  ng/mL at  $0.034 \pm 0.004$  h,  $641.3 \pm 79.4$  h/ng/mL, and  $20.6 \pm 2.30$  h, respectively. The mean concentration at 24 h post-injection was significantly ( $P < 0.01$ ) above the therapeutic threshold for both dosages in macaques. One animal showed mild pruritus at both doses and another animal showed mild somnolence at both doses. These findings support the use of HCBS in rhesus macaques for once daily dosing without problematic adverse effects and represent a potential new alternative.

**PS68 Enucleation and Temporary Tarsorrhaphy for the Treatment of Unilateral Exophthalmia in Cotton Rats (*Sigmodon hispidus*)**

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Twenty-five cotton rats (*Sigmodon hispidus*) 8-13-mo-old presented with acute, unilateral exophthalmos over a 6-mo period. The cotton rats otherwise appeared healthy and had no previous clinical signs of disease. The majority of affected cotton rats were retired breeders and were not exposed to experimental manipulations such as retroorbital blood collection or anesthesia. Differentials for exophthalmia in cotton rats included traumatic proptosis, infectious agents such as adenovirus, cardiomyopathy with secondary retroorbital thrombi, or a

retroorbital mass. Diagnostic samples collected from these animals included histopathology (n=14), corneal bacterial culture (n=9), and human adenovirus PCR panel (n=2). The bacterial cultures grew multiple strains of opportunistic bacteria and the adenovirus PCR panel was negative. Interestingly, ocular histopathology identified marked keratitis and conjunctivitis along with occasional retrobulbar fibrin thrombi in some of the samples. Additionally, histologic evidence of cardiomyopathy and pulmonary thromboemboli was observed in some of the rats. Together these findings suggest that exophthalmos was likely caused by retroorbital thrombi secondary to cardiomyopathy. This case study demonstrates the potential for high incidence of ocular issues in older colonies of cotton rats. Clinical cases were managed with systemic analgesics and prompt enucleation due to rapid clinical progression to severe keratitis and secondary infections. In order to reduce the number of enucleations performed, a simple temporary tarsorrhaphy procedure was designed for mild cases detected early in malady. All animals thrived postoperatively; therefore, based on severity of the lesion, enucleation and temporary tarsorrhaphy are potential surgical interventions for cotton rats presenting with exophthalmia.

### PS69 Anesthetic Management of a Dog (*Canis lupus familiaris*) with X-linked Muscular Dystrophy and Cardiac Compromise

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Canine X-linked muscular dystrophy is a well-established model for Duchenne muscular dystrophy in humans. Anesthetic risks associated with the canine X-linked muscular dystrophy phenotype include regurgitation and aspiration associated with megaesophagus, intubation difficulties due to macroglossia and trismus, acute rhabdomyolysis, and cardiovascular collapse secondary to contractile deficiencies and fatal arrhythmias. We report an anesthetic technique for a 15kg, 8-y-old dog, with X-linked muscular dystrophy and functional cardiac compromise. Pre-anesthetic cardiac evaluation revealed left-sided cardiomegaly with severely decreased left ventricular systolic function, and mild mitral insufficiency with a left-sided murmur. Serum chemistry changes were consistent with the X-linked muscular dystrophy phenotype. The goal was to maintain a stable surgical anesthetic plane, with minimal changes to cardiac contractility and systemic blood pressure. This was accomplished with sufentanil, administered as a continuous rate infusion (1.5µg/kg/hr), initiated prior to induction and continued throughout the procedure. The patient was induced with intravenous boluses of sufentanil (1.5µg/kg), midazolam (0.2mg/kg), and etomidate (2mg/kg to effect). Anesthesia was maintained with a sufentanil continuous rate infusion to reduce the alveolar concentration of isoflurane needed to maintain a stable anesthetic plane and facilitate rapid anesthetic recovery. Complications observed during anesthesia consisted of normotensive bradycardia, and apnea which was addressed with mechanical ventilation. Anesthetic recovery was rapid with minimal dysphoria. In the first 36-h postsurgery, the patient developed gastric dilatation without volvulus, which was corrected with orogastric intubation and suction to decompress. Potential causes for the gastric dilatation include pharmaceutical and phenotypic associated alterations in gastrointestinal motility, and aerophagia associated with phenotypic megaesophagus. In conclusion, we describe a cardioprotective anesthetic protocol that permits precise control of anesthetic depth and rapid recovery, used in a canine X-linked muscular dystrophy patient. This protocol could also be applied to other canine Duchenne muscular dystrophy models.

### PS70 Ethanol Overdose as a Refinement to CO<sub>2</sub> for Euthanasia of Chickens (*Gallus gallus domesticus*)

glos

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According to the AVMA Guidelines for Euthanasia of Animals, injectable pentobarbital and inhalant CO<sub>2</sub> are 'acceptable' and 'acceptable with conditions' methods of euthanasia for avian species. However, barbiturates are controlled substances and challenging to use in the field and laboratory setting. Additionally, there is limited literature on the use of CO<sub>2</sub> in avian species. CO<sub>2</sub> also has been reported to induce anesthesia and euthanasia at inconsistent time intervals and is cited by users to be visibly distressful to birds. Recent studies demonstrated that intraperitoneal ethanol overdose is a novel euthanasia agent in mice, but is inconsistent in rats. Ethanol is easily accessible and its pharmacological properties suggest that it could induce a humane death. Thus, we sought to determine if intracoelomic (IPc) ethanol could be used as an alternative euthanasia agent in chickens. To evaluate this, we compared IPc ethanol to IPc pentobarbital. Chickens weighing approximately 2.0kg were fitted with ECG, capnography, and doppler. Chickens were randomized into 3 groups: 20ml of 100% ethanol, 20ml saline, or 0.5ml of pentobarbital IPc. Loss of consciousness was assessed by intubation time, capnography was used to confirm respiratory arrest, and ECG to confirm cessation of cardiovascular function. Time to intubation was significantly different in chickens receiving ethanol (n=6, 494.56s +/- 330.11s) and pentobarbital (n=9, 250s +/- 96.70s) by 2-sample t-test (P = 0.049). The time to loss of respiration was markedly different between the ethanol (n=5, 964.8s +/- 200.2s) and pentobarbital (n=5, 342.2s +/- 11.14s) groups by 2-sample t-test (P = 0.0005). Time to reach asystole in chickens was significantly different between the ethanol (n=4, 1052.60s +/- 200.01s) and pentobarbital (n=5, 772.2s +/- 54.67s) groups by 2-sample t-test (P = 0.03). There was a total of 8 chickens (4 per group), who failed to reach asystole. No overt signs of pain or distress were observed. There were no significant histological changes and there was no degradative effect on RNA extraction. Further, there were no significant aberrations on ECG recordings between groups. We conclude that 20mL IPc ethanol and 0.5mL IPc pentobarbital induces euthanasia inconsistently; demonstrating that ethanol as an alternative euthanasia method in chickens requires further investigation.

### PS71 One-hundred Percent Ethanol Injected Intracoelomically as a Novel Method of Euthanasia in Zebra Finches (*Taeniopygia guttata*)

glos

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Euthanasia methods for avian species outlined in the 2013 AVMA Guidelines for Euthanasia of Animals are extrapolated from methods used in mammals, with injectable pentobarbital and inhalant CO<sub>2</sub> as the 'acceptable' and 'acceptable with conditions' methods for euthanasia of avian species. The objective of this study was to determine if intracoelomic (IPc) injection of 0.5mL of 100% ethanol is an efficacious means of euthanasia in zebra finches (*Taeniopygia guttata*). Adult cull zebra finches were block randomized and received an IPc injection of 0.5mL of saline (n=21), 0.5mL of 100% ethanol (n=22), or 0.05mL sodium pentobarbital (n=21). Finches were placed in an observation box and video recorded. Time to loss of the righting reflex (LORR) and cessation of all movement (CAM) was recorded.

Tissues were submitted for histopathology scoring and postmortem blood glucose was performed. Videos were blinded and randomized for retrospective analysis of pain and distress. LORR following IP<sub>C</sub> ethanol (72.73s +/- 55.17s) was not significantly different from the IP<sub>C</sub> pentobarbital (48.38s +/- 20.73s) or the IP<sub>C</sub> saline (59.05s +/- 19.68s) groups ( $P = 0.09$ ). There was no significant difference in CAM following ethanol (183.45 s +/- 161.37), pentobarbital (183.86 s +/- 146.07), or saline + CO<sub>2</sub> (100.05 s +/- 21.11) groups using 1-way ANOVA ( $P = 0.13$ ). Retrospective behavioral scoring was not significantly different between groups. There was a significant difference in the blood glucose levels with birds euthanized with 100% ethanol exhibiting significantly lower blood glucose levels (369.55 mg/dL +/- 78.63) than birds euthanized with CO<sub>2</sub> following saline injection (n=21, 445.57 mg/dL +/- 97.16) using 1-way ANOVA ( $P = 0.02$ ). Histopathologic scoring showed no significant difference between the groups. Another group of cull zebra finches were randomized and used to determine an effective dose 50% (ED<sub>50</sub>) of 100% ethanol via the Dixon up-and-down method. This information was then used to develop a linear regression model. The ED<sub>50</sub> of 100% ethanol IP<sub>C</sub> was 0.023 mL/g (standard error = 0.004 mL/g). We conclude that 100% ethanol, dosed at 4 x the ED<sub>50</sub> (0.09mL/g), is an efficacious and novel method of euthanasia in zebra finches.

### PS72 Enrichment for *Xenopus laevis*: A Novel Approach to Feeding

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Enrichment for *Xenopus laevis* is often limited to social housing with or without shelters. The predatory nature of *X. laevis* provides an opportunity to explore novel feeding strategies. A pilot study using 4 adult female *X. laevis* was conducted to assess the safety of a feeder and its effects on behavior. We hypothesized that the use of a feeder would increase the percentage of time spent exhibiting active species-specific behaviors in the hour after provision of feed. Experimental frogs were provided frog brittle inside a feeder. Control frogs were provided frog brittle directly in the tank water. For each feeding day (D1, D3, D5), frogs were recorded for 1 hr after feeding and behaviors defined by an ethogram were scored during 2 observation periods: immediate (first 6 min after provision of food) and intermediate (the remainder of the hour). During the immediate period, experimental frogs were less active than controls (53% vs 40% of the time). During the intermediate period, experimental frogs spent more time feeding (7% vs 1%), less time swimming (7% vs 25%) and less time clumped together (11% vs 38%) than controls. Experimental frogs also spent less time displaying social conflict behaviors than control frogs in the intermediate period (0.34% vs 1.5%). In addition, stereotypic behavior was seen in a control frog 14% of the time across feeding days whereas stereotypic behavior in other frogs was observed <10% of the time. Contrary to the hypothesis, the evaluation showed that the feeder was associated with decreased activity during the immediate period but increased activity during the intermediate period. These observations along with the observations of stereotypic behavior add to the limited literature on *X. laevis* behavior and supports the need for further studies on the impacts of feeding enrichment on behavior and welfare in this species.

### PS73 Enrichment Preferences of Singly Housed Zebrafish (*Danio rerio*)

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Despite the increasing popularity of zebrafish (*Danio rerio*) as an animal model, environmental enrichment preferences of this species have been largely unexplored. We sought to determine enrichment preferences of mature female zebrafish that were singly housed with or

without access to 1 of 10 inanimate forms of enrichment that recapitulated species-specific behaviors. Enrichment items were rotated every 7 d until the completion of all 10 conditions. Place-preference, as indicated by fish location within the tank, was observed by video recording. All subjects showed a preference for the front of the tank when caretakers entered the room, demonstrating an effect of human presence on tank location ( $P < 0.00001$ ). Out of the 10 conditions tested, subjects showed the strongest preference for the back of the tank when housed with mirrored paper on the side of the tank when compared to the barren half of the tank ( $P < 0.0005$ ). Fish were also observed interacting with 3 of the other items in species-specific behaviors. These included PVC pipe, marbles, and tulle. Given that enrichment imitating social interaction had such a prominent effect, we set up a second study to assess the value of visual exposure of conspecifics in adjacent tanks. The subjects were then provided 1 of 3 conditions: a singly housed neighbor fish, group-housed neighbor fish, or an empty neighbor tank. All zebrafish housed next to neighboring fish showed a preference to be on the side of the tank nearest to the other fish ( $P < 0.05$ ). Overall, our data indicate that singly housed zebrafish prefer enrichment items which promote social behaviors, either in the form of self-visualization or neighboring fish. Thus items such as mirrored paper or housing next to conspecifics should be strongly considered as enrichment strategies for singly housed zebrafish. Items such as PVC pipes, marbles, and tulle should be considered as alternative enrichment strategies if opportunities for visual imagery of fish are not available.

### PS74 Evolution of a System for Video-based Automated Assessment of Activity Measures and Behavior of Mice in Ventilated Racks

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In recent years there has been increased interest within the laboratory animal science community in the usefulness of automated profiling of rodent activity in their home cages. Activity data may reflect health, welfare, phenotype, and/or effects of toxicity. Video-based systems offer distinct advantages in being able to recognize discreet behaviors, and some video-monitoring functions have been designed into specialized mouse racks. Wide-spread use of this technology may be achieved by retrofitting existing high capacity racks with video-monitoring capability and developing data management tools. We demonstrate a mechanical design that uses 3-D printing technology and inexpensive electronic components. Custom-designed printed circuit boards are used to provide constant near-infrared illumination, allowing consistent image acquisition with no inference with photoperiod. Our newest system utilizes commercially available IVC racks, and we have developed software to assess activity and behavior measures during prolonged experiments. Algorithms are being developed to provide detailed analysis of rodent activity within their home cages on ventilated racks.

### PS75 Real-time Monitoring of Animal Welfare through a Smart Animal Alert System

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A primary goal in animal research is respectful and responsible care aimed toward minimizing stress and discomfort while enhancing collection of accurate and reproducible scientific data. A major challenge in monitoring animal welfare is that most cage-side observations are attempted during the light cycle when rodents are predominately asleep, making it difficult to discern potential health conditions. Alternatively, animals are handled to uncover health

conditions but handling can induce stress which may increase the inter- and intrasubject variability. We investigated how technology can complement existing means of monitoring animal health by capitalizing on noninvasive, cage-level video monitoring, and computer vision-derived metrics of animal motion and breathing rate. We analyzed historical data collected from rodents that represent a diverse range of therapeutic models including inflammation, respiratory, oncology, metabolism, and aging disorders, and identified abnormal patterns that were associated with sick animals, but absent in healthier controls. Currently, this alerting system can detect abnormal physiological and behavioral changes associated with decreases in motion, changes in breathing rate, and abnormal circadian rhythms. The performance of the alert system was evaluated using the results of a survey taken by 5 scientists with a combined 10+ years of using the metrics and measured the value of proposed alerts at specific time points. The alert system accurately identified 83.3% of desired alerts, while only falsely identifying 6.6% of alerts. An animal alerting system improves animal welfare by enhancing the ability of vivarium staff and researchers to automatically identify animals that 1) require more careful observation, 2) need medical attention, or 3) should be euthanized, without the need to handle the animal. This alert system effectively utilizes near real-time physiological metrics tied to vital life functions and normal animal behavior and represents a critical advancement toward merging traditional animal housing with technology such that critical health conditions are not missed and the utility of every subject in a study is maximized to drive responsible scientific advancement.

#### **PS76 Assessing Technical Proficiency in a Germ-free Facility**

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A university started a 4,533-sq.-ft. a germ-free facility that consists of 80 flexible film isolators that house approximately 300 mice and 100 rats. Six technicians carry out the day-to-day husbandry and technical procedures within the facility. Since research staff is not permitted to perform technical procedures for ongoing studies, it is imperative to have an effective and successful proficiency training and assessment program. This is essential so that we can provide documentation to researchers and contractors requesting our services, demonstrating that the staff has been deemed proficient to perform the requested technical procedures needed for their studies. We have set up a training plan covering technical procedures for proper restraint, injections (ID, IP, IV and SQ) and blood collection (maxillary vein) in mice and rats. Checklists are used to assess the 6 different technical skills for mice and rats. Trainees deemed as proficient are capable of consistently and accurately performing the specific techniques with an 80 to 100% pass rate. Each year the technicians are reassessed and deemed proficient. Reassessment is vital in verifying that the technician's technical skills are sufficient and helps in evaluating the current training needs.

#### **PS77 Using 3D Measurement and Machine Learning to Quantify Subcutaneous Tumors and Improve Animal Welfare and the 3rs in Cancer Research**

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The standard method of using handheld callipers to measure tumor growth and response to drug therapy in preclinical oncology trials results in operator and interoperator bias. Length and width measurements are used to estimate volume under the assumption that tumors are spherical, leading to further inaccuracies for nonuniformly shaped, flat, and small tumors. Overall this can lead to repeat studies, resulting in more mice being used and longer and more costly trials. We developed a novel 3D scanning system to capture and

automatically detect and quantify the length, width, and volume of tumors in mice. 3D surface images are captured using stereo and photometric imaging and are analyzed, segmented, and characterized. Machine learning has been used to train and independently validate a dataset of 2197 3D mice scans consisting of 19 tumor types and 6 mouse strains which were compared against measurements from callipers. We demonstrated that it is possible to record tumor measurements in a rapid, minimally invasive, morphology-independent, and human bias-free way, removing interoperator variability while providing full reproducibility, transparency, and traceability of data used in studies. With callipers only 9% of measurements are within 25% of the measured excised tumor mass compared with 60% using our 3D scanning method. Machine learning will drive further improvements. Animal handling is minimized as tumors can be measured in under 5 s and sedation is not required, an advantage over alternative calliper replacement technologies. Our results show much promise for reducing the handling and use of mice and decreasing the cost and duration of cancer drug development. This digital method demonstrates better welfare in adherence with the 3Rs and provides greater confidence in when to stop testing and could enable systematic topical symptoms to be recalled and exploited as surrogate endpoints for early diagnosis of undesired effects.

#### **PS78 Epigenetics and the Inheritance of Acquired Characteristics in Laboratory Animals**

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Much has been written recently regarding the lack of reproducibility between different experiments involving rodents in the laboratory setting. In order to produce consistently meaningful results, animal characteristics must be kept as consistent as possible. However, anyone who has worked with these manipulated species for any length of time can recount instances of purportedly "identical" animals producing widely varying results. We discuss 1 of the chief culprits responsible for this conundrum, epigenetics. In short, while genotype may be the same from 1 animal to another (as in monozygotic human twins or inbred isogenic mouse strains), recent findings have clearly demonstrated the disproportional impact that nongenetic variables, such as environment (both pre- and postpartum), diet (both of the animal and of the animal's ancestors), and social structure can have on their ultimate phenotype, and thus, their performance in a given experiment. We provide a general primer on epigenetics, introducing those new to the field to its basic concepts, as well as providing further information relevant to the laboratory animal community regarding epigenetic effects on animal research outcomes and the medical, husbandry, and welfare issues associated with these animals.

#### **PS79 An International Crowdsourcing Data Project to Investigate Aggression in Group-housed Laboratory Male Mice**

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The UK's National Centre for the 3Rs (NC3Rs) led a crowdsourcing data project for animal technicians to collect data on the prevalence and potential triggers for aggression in laboratory mice. Aggression in group-housed mice is a serious welfare concern, but so too is housing mice singly. Further understanding of the causes of mouse aggression could have a significant positive welfare impact on a large number of animals worldwide. The crowdsourcing approach allowed data to be collected from multiple institutions and, to our knowledge, is the first time such an approach has been applied to a laboratory animal welfare problem. During a consecutive 4-w period over October and November 2017, technicians observed group-housed, male mice during daily routine cage checks and recorded incidents of aggression-related injury and the actions taken to minimize further aggression. Each facility also completed a questionnaire about their standard husbandry practices

and submitted an injury log. Data gathered included mouse characteristics, weaning ages, stocking densities, enrichments, and experimental procedures. Participants continued to observe the cage over a 7-d period to record if the remedial action taken prevented further injury to mice in the cage. In total, 44 facilities from 9 countries participated in the study. Data was collected by 143 animal technicians who developed their skills in data collection and recording of behavior. A total of 788 incidents of aggression-related injuries were reported across a sample population of over 130,000 mice. The mean prevalence of aggression-related incidents reported across facilities was 2.66% of total mice held during the collection period (ranging from 0-48%). The additional information provided was collated and used to identify patterns and potential triggers of mouse aggression. The results will be used to generate a published evidence base to inform and support best practice to minimize aggressive behavior in group-housed, male mice.

#### PS80 A Comparison of Laboratory Rat Behavior and Welfare in Standard Enriched and Larger Enriched Tower Cage Housing Conditions

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The design of any captive animal housing system should aim to promote welfare by encouraging species-specific behaviors, however, many laboratory animal cages fail to meet the needs of the animals, providing either insufficient space and/or complexity to allow the expression of full behavioral repertoire. Over a 10-wk period we studied 12 groups of 4 to 12-wk-old male Sprague-Dawley rats housed in either standard laboratory rat cages (58cmL, 38cmW, 22cmH) containing a shelter, tunnel, nesting material and aspen chew block, or larger ("tower") cages (73cmL, 58cmW, 46cmH) with multiple shelters, perches, nesting material and locations, tunnels, and the aspen chew block. Behavioral data were collected from video during periods of relatively high activity in dark phase, with activity, posture, and location compared between the 2 housing systems. Rats in tower cages groomed less ( $F_{1,50}=55.80$ ,  $P < 0.001$ ) than rats in standard cages and were more likely to show tactile social behaviors ( $t=2.60$ ,  $P = 0.026$ ) such as placing a paw on another rat. Rats in tower cages also performed fewer antagonistic behaviors such as a prolonged pin-down of a subordinate rat, without allowing escape, ( $t=-2.86$ ,  $P = 0.017$ ), and fewer bouts of sleep ( $F_{1,50}=27.83$ ,  $P < 0.001$ ) than those in standard cages. The increase in positive affiliative behavior, reduced aggression, and reduced self-directed behavior suggests that rats housed in tower cages have improved welfare compared to rats in standard cages even when both systems feature extensive cage enrichment. All animal studies were ethically reviewed and carried out in accordance with Lincoln University Ethical Review Procedure, and the GSK Policy on the Care, Welfare and Treatment of Animals, which determined that the work did not constitute a procedure under A(SP)A 1986.

#### PS81 Hyperemic Interdigital Webbing in a Group of *Xenopus laevis*

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Three cohoused adult female South African clawed frogs (*Xenopus laevis*) used for egg harvesting developed acute onset of redness of the interdigital webbing. One wk prior to presentation, a faulty gasket was noted in the recirculating water system and was replaced 2 d later with no evidence of clinical abnormalities observed in the colony. The ammonia, pH, conductivity, and temperature parameters were all within normal range. Physical examination revealed hyperemia and necrosis of the hind limb, interdigital webbing, erythema of all 4 limbs, depigmentation of the skin, and buoyancy compromise. One frog also had discoloration of the right front limb and had difficulty extending the affected limb. Inspection of the other tanks connected to the recirculating water system, revealed 6 other frogs with minor

erythema of their hind limbs. Necropsy of the 3 clinically affected frogs revealed micro and macro bubbles around the webbing of the feet and subcutaneous emphysema in the hind limbs. Culture of the skin was positive for *Aeromonas hydrophilia* for all 3 frogs and *Bacteroides uniformis* was cultured from one of the frogs. The history of compromised recirculating water system and the pathognomonic lesions of gas bubbles in the interdigital webbing and subepidermal regions of the hind limbs in the frogs are diagnostic for gas bubble disease. All other frogs recovered with no additional morbidity or mortality noted in the colony. Gas bubble disease is an acute disease that occurs due to oversaturation of dissolved gases, such as nitrogen and argon into the water, commonly caused by defects in the piping of the aquatic system. The animals in this case presented 5 d after issues in the recirculating water system were repaired suggesting that gas bubble disease can present even after restoration of the facility recirculating water system.

#### PS82 Scaly Skin and Lethargy in a Nude Mouse

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A 13-wk-old male athymic nude mouse (Crl:NU(NCr)-Foxn1<sup>tm</sup>) enrolled in a subcutaneous heterotopic human pancreatic tumor study was placed on report for hyperemia and a severe generalized hyperkeratotic dermatitis. The mouse was otherwise bright, alert, and responsive and in good body condition, but by the next day had become lethargic. Due to the clinical presentation and lethargy, the mouse was euthanized for colony health surveillance. A gross necropsy was performed and was within normal limits except for the diffuse severe hyperkeratosis and mild splenomegaly. The differential diagnosis list included *Corynebacterium bovis*, *Staphylococcus xylosum*, and chemical exposure. Three samples (1 skin swab, 1 oral swab, and feces) were submitted to a commercial laboratory for culture and PCR of *C. bovis*, *S. xylosum*, and *Corynebacterium* spp. (HAC2); all test results were negative. The mouse was then submitted for full histopathologic evaluation. Skin changes included marked acanthosis, hyperkeratosis, and parakeratosis with mild dermal fibrosis and lymphocytic/neutrophilic infiltrate. Few scattered gram-positive coccobacilli were identified within layers of the thickened stratum corneum with more diffuse bacterial colonization on the head. Histopathology suggested infection with a *Corynebacterium* spp. so dry swabs (head, body) of 3 cage mates and 1 other animal in the experimental cohort were collected and submitted for culture. *Corynebacterium mastitidis* was isolated from all 4 submitted samples. Although *C. mastitidis* was not isolated directly from the affected mouse, it is the most likely etiologic agent based on the histopathology in addition to the high sensitivity of the PCR tests ruling out infection with *C. bovis*, *S. xylosum*, and *Corynebacterium* spp. (HAC2). *C. mastitidis*, which can be found in cell lines and has been isolated from preputial gland abscesses in mice, should also be considered as a differential diagnosis for generalized hyperkeratotic dermatitis in nude mice.

#### PS83 Acute Neurologic Presentation of a Rhesus Macaque (*Macaca mulatta*)

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A 13-y-old, 12.8 kg Chinese-origin rhesus macaque (*Macaca mulatta*) individually housed indoors since import at 1 y of age presented with acute onset left-sided hemiparesis. The animal had exhibited self-injurious behavior at 7 y of age following experimental amphetamine treatment between 2.5-4 years of age. Cognitive testing batteries during his juvenile and sub-adult periods revealed consistently poor performance, with an average percentile rank of 37.0% across several cognitive tasks and below median performance in each task. Awake

physical exam at acute presentation revealed a mentally appropriate attitude, normal visual tracking and menace responses, intact limb withdrawal reflexes, and deep pain present. He exhibited difficulty manipulating and chewing food on the right side of his mouth. Sedated physical exam revealed normal vital signs and mild muscular atrophy of the left limbs. Differential diagnoses at this time included a vascular infarction, neoplasia, trauma, intervertebral disk herniation, fibrocartilaginous embolism, and infection. Cerebrospinal fluid cytology, thoracic and skull radiographs, complete blood count, blood chemistry, coagulation panel, and D-dimers were unremarkable; euthanasia was elected. Necropsy revealed generalized hydrocephalus and microcephaly, a right-sided focal cortical depression with corresponding subdural mineralization, and signs of chronic diffuse cortical degeneration (such as gliosis, Rosenthal fibers, hemosiderin-laden macrophages). Chronic cortical changes secondary to an early-in-life insult, such as bacterial meningitis or trauma, are suspected to be the proximate causes of subnormal intelligence. No clear cause of acute paresis was identified via pre- or postmortem diagnostic procedures; it is possible that the chronic changes noted acutely exceeded the ability of the animal to compensate.

#### **PS84 A Suspicious Mass in a Lab Rat**

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A 1-y-old, male CD rat exposed to an EEG/EMG telemeterized, experimental nerve agent presented with an approximately 5 x 4 cm mass with focal necrosis on the left distal thorax under the subcuticular telemetry device. The animal appeared normal otherwise. Surgery was performed to relocate the implant and excise the mass. The firm, infiltrative mass had multifocal cysts containing serosanguinous fluid. The surgeon elected to relocate the implant and sought advice before excising the mass. One wk later, a focal area of necrosis occurred over the new implant site. A second surgery involved relocating the implant and fully excising the mass. The mass appeared 2 times larger and engulfed the wires and the telemetry device. The rat recovered without complications and survived to the study endpoint. Grossly, the excised mass was 5.5 x 4.0 x 2.5 cm, firm, pale, and multilobulated. Histologically, the mass was densely cellular and composed of neoplastic spindle to strap-like cells forming streams and bundles on a fibrovascular stroma. Neoplastic cells had pale eosinophilic cytoplasm and elongate nuclei with granular chromatin and often blunt, cigar-shaped ends. There was marked anisokaryosis and occasional multinucleated cells. Mitoses averaged 5/HPF. Multifocally, there were extensive areas of necrosis and hemorrhage. With Masson's trichrome stain, the neoplastic cells stained red. These histologic findings are consistent with leiomyosarcoma. Leiomyosarcomas are malignant neoplasms that belong to a group of soft tissue sarcomas and derive from smooth muscle tissue. They most commonly occur in hollow organs with smooth muscle but can form in any place with smooth muscle including skin (arrector pilae). Foreign-body carcinogenesis from the local inflammation and extensive fibrosis associated with the subcuticular telemetry device is the most likely factor involved in the formation of this rat's sarcoma. Interestingly, several other implanted rats in this study had extensive fibrosis and occasionally other types of implant-associated sarcomas.

#### **PS85 Abdominal Mass in a Female Baboon**

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A 15-y-old female baboon on an IACUC-approved protocol was examined for a firm swelling in the left lower quadrant of the abdomen. The animal had no abnormalities noted on prestudy physical examination 7 mo prior, or during study-related procedures 2 mo prior to the mass being observed. Technical staff performing observations did not note any signs of pain or distress and the animal moved around normally in her enclosure. The palpable mass

measured 13cm in diameter. Radiographs demonstrated irregularity of the pelvic bones from the pubic symphysis to the obturator foramen, a flattened left femoral head, and acetabular irregularity. Ultrasound showed a well-defined, firm, echogenic soft tissue mass. On bloodwork the animal was anemic, and urinalysis was normal. A fine needle aspirate was nondiagnostic. Due to the poor prognosis, euthanasia was elected and a complete necropsy was performed. On gross examination, the mass extended from the pelvic symphysis, infiltrated the pelvic bones, and extended along the abdominal wall displacing the abdominal organs laterally with regional adhesion to the urinary bladder. The mass was firm, solid, mottled yellow-tan to dark red, and bulged on cut section. The cortex and medulla of the pelvic bones and the abdominal wall tissues were effaced and replaced by dense sheets of large polygonal cells with interspersed deposits of osteoid and cartilage alternating with areas with dense collagen with streams and bundles of spindle cells and minimal supporting stroma. Areas with large multinucleated polygonal cells had frequent mitotic activity ranging from 0-6 per high powered field. Histologic features were consistent with an osteosarcoma, most likely originating from the pelvic bones. This is an unusual presentation of osteosarcoma.

#### **PS86 Hypothermia in an Immunosuppressed Yucatan Pig**

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As part of a xenotransplant study, a 7-mo-old castrated male, 33 kg, Yucatan miniature pig received a myocardial infarction, jugular catheter, ECG telemetry implant, and daily amiodarone, an anti-arrhythmic. Two wk after infarction, stem cells were injected at the infarct site, and daily immunosuppressive medications including cyclosporine and methylprednisolone were administered. The pig also received antibiotic prophylaxis with cephalexin and trimethoprim sulfamethoxazole. Two wk after cell injection, routine blood chemistry screening revealed elevated GGT (93 U/L) and AST (100 U/L), and hypercholesterolemia (378 mg/dL). Three wk after cell injection, free T4 levels (1.2 ng/dL) and total T4 (2.1 µg/dL) were elevated with suppression of TSH (0.08 ng/mL), and elevated BUN (61 mg/dL). Known side effects of amiodarone include hepatopathy and hyperthyroidism. After a 50% reduction of the amiodarone dose, AST and thyroid values returned to baseline. Five wk after cell injection the pig was examined for hypothermia, and presented as bright, alert and responsive, euhydrated, with normal respiration, yet was hypothermic (rectal temperature 99.7°F) and mildly tachycardic (130 bpm). Heart failure secondary to myocardial infarction was a primary differential. However, clinical pathology supported an acute inflammatory insult of infectious etiology, with neutropenia (448/µL) with a left shift and moderate toxic changes, and reactive lymphocytes. Thrombocytopenia (67,000/µL), hypoproteinemia (4.5g/dL) and marked hyperglycemia (artifact of glucose-containing catheter lock solution) were also noted. Due to progressive hypothermia and inappetance, euthanasia and necropsy were elected. Pleural effusion, pulmonary edema, and hemorrhages on the serosal surface of the small intestine were noted grossly. Histopathologically, there were cytomegalic cells with intranuclear inclusion bodies in the lungs and liver, consistent with cytomegalovirus. Additional findings included bacterial pneumonia, severe hemorrhagic nephritis, serosal hemorrhagic enteritis, and systemic intravascular thrombi. Tapering of immunosuppressive drugs as well as antiviral prophylaxis were successful in preventing opportunistic CMV infection in the remaining pigs from this experimental cohort.

#### **PS87 Coelomic Distention and Erect Scales in a Zebrafish**

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An adult wildtype zebrafish presented with marked generalized coelomic distention and erect scales. Swimming patterns, buoyancy, and system water quality parameters were within normal limits

(temperature, pH, conductivity, ammonia, nitrite, nitrate, alkalinity, and total hardness). The fish was euthanized in buffered MS-222 and submitted for necropsy and histopathology to help elucidate a cause for the coelomic distention. Differentials included renal damage or failure, coelomic cavity mass/tumor, systemic infection(s), and branchial abnormality. At necropsy, approximately 0.3 ml of clear fluid was removed from the coelomic cavity via syringe and needle. The carcass was fixed in Bouin's fixative. On histopathology, a large, well-demarcated coelomic mass was noted, composed of densely cellular basophilic spindle cells arranged in sheets and whirls within a fibrous stroma. There were also numerous inflammatory cells (lymphocytes and macrophages) present throughout the stroma, and cholesterol clefts were noted within the caudal aspects of the mass. There was local invasion of the mass into surrounding organs, including ovaries, spleen, and liver. Within the spinal cord, a focal microsporidium xenoma with numerous oval shaped spores was observed. Special stains were submitted to further characterize infectious (Fite's acid fast, gram stain) and neoplastic (pancytokeratin, vimentin) etiologies. Positive staining for epithelial cell marker (pancytokeratin) was noted in the mass, and a mild, diffuse, systemic, bacterial infection (gram-negative bacteria) was noted in most tissues outside of the mass, including liver, spleen, and kidney. The histologic appearance, staining, and location of the mass were most consistent with a peripheral nerve tumor. Zebrafish are more predisposed to developing neoplasia of the nerve sheath of the peripheral and cranial nerves compared to mammals. The bacterial infection noted is most likely secondary to the primary neoplastic mass and may be associated with immunosuppression secondary to its chronicity. Microsporidium spores within the spinal cord were consistent with *Pseudoloma neurophilia*.

#### PS88 Rash and Lethargy in Castrated Male Miniature Yucatan Pig

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A 9-mo-old castrated male miniature Yucatan pig presented for acute onset dry, nonpruritic nodular rash over the hind end and dorsum. The pig was on a xenotransplant study, and had received daily oral immunosuppressives (cyclosporine, methylprednisolone) and prophylactic antibiotics (cephalexin) 1 mo prior to presentation. The pig was otherwise normal on initial exam. Over the next 3 d it became progressively lethargic and inappetent, and was administered oxytetracycline IM. Seven d after initial presentation, the pig was euthanized for endpoint. The major gross findings in addition to the dermal lesions included mildly enlarged, pale kidneys with cortical ptechia and multifocal to coalescing; multifocal moderate chronic-active pneumonia; focal mild granulomatous hepatitis; and mild mesenteric lymphadenopathy. Key histopathologic findings included systemic segmental necrotizing vasculitis predominantly in the kidney and skin accompanied by hemorrhage and necrosis. Additional findings included focal lymphoblastic lymphoma in the liver (consistent with reports of cyclosporine-induced lymphoma), and severe mixed bacterial pneumonia with abscessation. The clinical signs and lesions noted in this case are consistent with porcine dermatitis and nephropathy syndrome (PDNS), which is commonly associated with porcine circovirus 2 (PCV2) and other infectious and noninfectious causes, including bacteria and drugs. The underlying pathogenesis of PDNS is still ill-defined, yet there is evidence for an immune-mediated component. Lymphoma has been reported in immunosuppressed mini pigs leading to their proposal as a model of posttransplantation lymphoproliferative disease. This case demonstrates the impact of immunosuppression in mini pigs and has management implications for future studies.

#### PS89 Circulation of Multidrug-resistant *Campylobacter* Species in Nonhuman Rhesus Monkeys (*Macaca mulatta*)

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Antimicrobial resistance (AMR) is an ongoing public health issue as antimicrobial usage for human and in animal production continues. As one of the important zoonotic agents, *Campylobacter* causes Campylobacteriosis, the most common foodborne disease worldwide. Every year over 1.3 million people in the United States are affected with *Campylobacter*. Two *Campylobacter* species, *C. jejuni* subsp. *jejuni* and *C. coli*, are major zoonotic pathogens causing gastroenteritis worldwide. Nonhuman primates are also susceptible to *Campylobacter* infections. A total of 155 rhesus monkeys (*Macaca mulatta*) were screened and 14 bacterial isolates were preliminarily identified as *Campylobacter* species based on morphological characteristics using gram-staining, scanning electron microscopy (SEM), and biochemical assays. Further speciation was performed to differentiate *Campylobacter* isolates at a species level using multiple biochemical and molecular tools. However, because *Campylobacter* isolates are biochemically inert and share a high level of DNA sequence identity, automated biochemical identification system (Vitek2C), fatty-acid profile-based identification system (MIDI), and 16S rDNA sequencing method failed to differentiate *C. jejuni* subsp. *jejuni* and *C. coli*. In contrast, a MALDI-TOF-based identification system was successfully able to differentiate the 14 *Campylobacter* isolates. The subsequent whole genome sequence revealed the Biotyper system correctly identified them at the species level. Of the 14 isolates, 7 isolates (50%) were identified as *C. coli*, 6 (43%) as *C. jejuni* subsp. *jejuni*, and 1 (7%) as *C. fetus* subsp. *vermirealis*, respectively. In addition to intrinsic antimicrobial resistance, 10 (71%) were ciprofloxacin-resistant and/or tetracycline-resistant, 7 (50%) were ciprofloxacin-resistant and tetracycline-resistant, 2 (14%) were ciprofloxacin-resistant, and 1 (7%) was tetracycline-resistant. Our findings indicate the necessity of active surveillance programs to early detect and correctly identify circulating multidrug-resistant *C. jejuni* subsp. *jejuni* and *C. coli* in nonhuman rhesus monkeys to protect research and animal care professionals.

#### PS90 Gentle Handling Benefits Animal Welfare without Disturbing Gut Microbiota in a Rat Model of Alzheimer's Disease

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Gentle handling and socialization of research animals are important for improving animal welfare. Rats, one of the most commonly used research animals in the United States, have been shown to respond positively to gentle handling and "tickling" by humans, which simulates play behavior. This refinement decreases anxiety-like behavior and makes these animals easier to handle. However, handling could affect the gut microbiota (GM), an important factor in research reproducibility that can be influenced by a variety of environmental factors. F344-AD rats, a model of Alzheimer's disease, startle easily and can be difficult to work with. Their GM also differs from their wild-type littermates and may be involved in disease pathogenesis. We set out to determine whether routine gentle handling of this model would 1) increase positive interactions with the handler, and 2) affect the GM composition. Transgenic and wild-type rats were allocated to handling groups that received daily gentle handling for 3 wk, or control groups that were only handled during cage changes. Fecal and cecal samples were collected at the end of the study and bacterial populations were characterized via targeted 16S rRNA sequencing. Video testing of rats' response to handling was conducted at the end of the study. As anticipated, differences in cecal GM were noted between transgenic and wild-type rats ( $P < 0.05$ ). However, no significant differences were seen in GM between handling groups. Rats in handled groups voluntarily spent more time with the handler and exhibited more rearing behavior, indicating increased comfort with their environment ( $P < 0.05$ ). These results support the implementation of a handling program to improve the welfare of rats at our institution without risking major alterations in GM, and the role of GM in the pathogenesis of Alzheimer's disease remains worthy of further study.



### PS91 Effect of Pelleting, Irradiation and Autoclaving on Mouse Parvovirus and Mouse Norovirus Infectivity In Rodent Feed



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Mouse norovirus (MNV) and mouse parvovirus (MPV) are among the most common adventitious viral infections in laboratory mice and emerge in barrier facilities despite rigorous biosecurity programs. Extensive research has been done on pathogenesis, monitoring, and eradication of the virus but very few have evaluated the source of viral entry into facilities. Some have implicated nonirradiated feed as a source of MPV in rodent facilities but none have conclusively documented viral particles in the feed. We hypothesize that both viruses can resist the pelleting process but not subsequent irradiation or autoclaving, thus revealing a potential source of outbreaks in rodent facilities. To test this hypothesis, we contaminated powdered feed with 10-fold increasing concentrations of MNV and MPV and fed it to both Swiss Webster (SW) and C57BL/6 (B6) mice to determine a 'powdered ID<sub>50</sub>' based on seroconversion over a 28-d period. We repeated the experiment using powdered feed contaminated with 10-fold multiples of the powdered ID<sub>50</sub> which was subsequently pelleted and determined a pelleted ID<sub>50</sub>. We finally looked at the effect of irradiation and autoclaving on contaminated pellets using the same experimental design. The powdered ID<sub>50</sub> was relatively low and identical in both mouse strains (2.51X10<sup>2</sup> pfu) for MNV but higher in B6 (3.2X10<sup>6</sup> cp) than SW (2.5X10<sup>4</sup> cp) for MPV. As hypothesized, mice were effectively infected by contaminated rodent feed despite the pelleting process. Indeed, pelleting resulted in a 1-2 log increase in ID<sub>50</sub> in both strains for MNV and MPV. On the other hand, irradiation and autoclaving effectively prevented seroconversion of mice exposed to high doses of MNV contaminated pellets while 1 mouse seroconverted at the highest dose for MPV. These data suggest that conditions reproducing the pelleting process for rodent chow does not inactivate MNV and MPV and that nonirradiated rodent chow might be a source of viral outbreaks. On the other hand, autoclaving and irradiation of the feed mitigate most of the risks of viral contamination.

### PS92 Comparison of Antibody Titer and Seroconversion Kinetics of Outbred Heterozygous Nude and Swiss Dirty Bedding Sentinels to Murine Norovirus

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The production of outbred athymic nude mice requires breeding heterozygous nude females with homozygous nude males. This breeding scheme results in 50% heterozygous nude offspring that do not have a common use in research. An alternative use for these excess mice is as dirty bedding sentinels, as they are outbred mice that are reared in isolators with a similar health status as some outbred Swiss mice such as the CrI:CD1(ICR)-Elite (CD1-E). Heterozygous nude mice have a thymus, but there are reports of decreased thymic size and decreased bone marrow stem cells compared to control background strains suggesting that they might not be immunologically normal. The aim of this study was to compare the antibody titer and seroconversion kinetics of heterozygous nude CrI:NU(Ncr)-*Foxn1*<sup>nu/+</sup> (Het-nude) and CD1-E dirty bedding sentinels to murine norovirus (MNV). Sixteen Het-nude and 16 CD1-E female dirty bedding sentinels were exposed to 100% dirty bedding from MNV positive colonies every 1 or 2 wk (depending on housing location) during the quarter. Blood was collected for serology at 3 and 9 wk post dirty bedding exposure, and at the end of the quarter (14-19 wk post

dirty bedding exposure). There was no significant difference between antibody titers to murine norovirus between Het-nude and CD1-E mice. There was a significant relationship between weeks of exposure and titer levels ( $P < 0.001$ ) with an increase in titer over the testing time period. At 3 wk post exposure, only 21% of mice seroconverted, at 9 wk 75% of mice seroconverted, and by the end of study 100% seroconverted. This study demonstrates the possible utility of Het-nude mice as dirty bedding sentinels as they have an equivalent antibody response to MNV as CD1-E mice. In situations where dirty bedding sentinels may be utilized for 9 wk or less (e.g. quarantine), the addition of fecal PCR or direct colony testing may be necessary to increase MNV detection rates.

### PS93 Comparing Shedding Profiles and Detection of *Corynebacterium bovis* in Immunocompetent and Immunocompromised Mice

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*Corynebacterium bovis* (*C. bovis*) is a gram-positive bacterium that causes hyperkeratotic dermatitis in immunocompromised mice, negatively impacting both animal welfare and research outcomes. Facility outbreaks are difficult to control and eradicate due to environmental persistence and fomite transmission. Although immunocompetent mice do not show clinical signs of *C. bovis* infection, it is unknown whether immunocompetent mice become subclinically infected and shed infectious material. To investigate the bacterial loads of *C. bovis* in immunocompetent and immunocompromised mice, we co-housed C57BL/6NcrI (B6) (n=10), nude (CrI:NU(Ncr)-*Foxn1*<sup>nu</sup>) (n=5), and NOD.Cg-Prkdc<sup>scid</sup>112rg<sup>tm1Wjl</sup>/SzJ (NSG) (n=5) mice with a clinically infected NSG mouse overnight (day 0). Skin was swabbed on days 3, 5, 7, 10, 14, 21, 28, and 35, and samples were processed for quantitative polymerase chain reaction (qPCR) analysis. To compare detection sensitivity of different sampling sites, buccal mucosa was also swabbed in nude and NSG mice. At the observed timepoints, B6 mice did not exhibit quantities detected by qPCR above noise level, similar to negative controls. Nude mice exhibited bacterial shedding at the earliest measured time point (day 3), continued to rise through day 7, and plateaued at day 10. While NSG mice also exhibited bacterial shedding at day 3, bacterial load continued to increase throughout the study. Skin swabs and buccal swabs were comparable in sensitivity in both nude and NSG mice. Our results suggest that B6 mice do not actively amplify *C. bovis*. These data provide information for management decisions and diagnostic testing during a *C. bovis* outbreak.

### PS94 Assessing Genotypic Differences between Isolates of the Opportunistic Pathogen *Corynebacterium bovis* Obtained from Various Hosts

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*Corynebacterium bovis* is an opportunistic bacterial pathogen that has been shown to cause eye and prosthetic joint infection and abscesses in humans, mastitis in dairy cattle, and skin disease in immunocompromised laboratory rodents. At present, little is known

about the genetic characteristics and genomic diversity of *C. bovis* as only 1 draft genome has been sequenced. The aim of this study was to sequence, characterize, and compare the genome of *C. bovis* isolates obtained from different host species and, with respect to murine isolates, different geographical locations and time points. Whole genomic sequencing was conducted for 20 *C. bovis* isolates (6 human, 4 bovine, 1 rat, and 10 mice origin). Sequences were analyzed using various comparative analysis tools. Sequencing generated high-quality scaffolds with an average size of 2.53 Mbp and the number of coding DNA sequences (2,174) was similar among all isolates. A neighbor-joining tree for the *Corynebacterium* genus revealed *C. falsenii* as the genetically closest species to *C. bovis*. Interestingly, genome relatedness indices showed that isolates were grouped according to the pathogen's host with human and bovine isolates clustering together and the rodent isolates forming a separate group. Furthermore, the average number of putative genomic islands and virulence factors were significantly higher in rodent isolates compared to the human/bovine isolates. The *C. bovis* pan-genome (total number of nonredundant genes) contained 3,067 genes and of these 1,354 were core genes (genes shared by all isolates). The core genome showed a large number of genes related to metabolism and information storage and processing. However, the highest proportion of genes were classified as function unknown or unclassified and a large number of virulence factors were only classified as toxins, highlighting the need to characterize proteins with unknown functions to shed light on bacterial pathogenicity. In conclusion, the genomic diversity of *C. bovis* was greater than previously expected with human/bovine isolates and rodent isolates forming 2 distinct clades with different pathogenicity characteristics.

#### PS95 *Helicobacter saguini* Causes Multigenerational Inflammatory Bowel Disease in C57/129 IL-10<sup>-/-</sup> Mice

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Inflammatory bowel disease (IBD) is an idiopathic disease in which family history is a significant risk factor. Cotton-top tamarins (CTTs) develop multigenerational chronic IBD that resembles human IBD. We isolated a novel *Helicobacter* species, *H. saguini*, from CTTs with chronic colitis. As *Helicobacter* species are associated with intestinal inflammatory diseases, we hypothesized *H. saguini* infection has the pathogenic potential to induce IBD. IL-10<sup>-/-</sup> specific-pathogen free (SPF) mice orally inoculated were not colonized with *H. saguini*; however, *H. saguini* mono-infection in germfree IL-10<sup>-/-</sup> mice developed IBD. Using this model, we found mono-infection of *H. saguini* could naturally transmit and colonize 4 sequential generations (F1–F4) and result in significant inflammatory lesions in the large intestine. Fluorescent in situ hybridization confirmed *H. saguini* persistently colonized the mucosal surface after 40 wk of infection. Additionally, immunohistochemistry for  $\gamma$ -H2AX, a histone marker for DNA damage, was significantly higher in the cecum of infected mice than age-matched controls. Representative isolates from F2-4 generations cultured for whole-genome sequencing analysis revealed host- and generational-dependent increases in single nucleotide polymorphisms in genes responsible for environmental signaling, suggesting *H. saguini* underwent genetic adaptations to a murine host during multigenerational infection. SPF IL-10<sup>-/-</sup> mice (9 controls, 10 infected, equal genders) were then orally inoculated with the *H. saguini* F4 isolate to test if a mouse-adapted strain could colonize conventional mice. *H. saguini* was detected by PCR of fecal samples at 4wpi in infected mice, but not sham-dosed controls. By 10wpi, 3/10 infected male mice developed rectal prolapse. Histology of the colon revealed moderately severe colitis with mild epithelial hyperplasia and dysplasia in 5/10 infected mice (4 males, 1 female). Controls appeared clinically and histologically normal. Together, our findings indicated that *H. saguini* mono-infection persistently colonized and induced multigenerational IBD in germfree IL-10<sup>-/-</sup> mice. Overall, this data provides evidence that specific microbial infections can play a role in the etiology and pathogenesis of IBD in CTTs and humans.

#### PS96 Murine Astrovirus 2: A Novel Virus Infecting Laboratory Mice

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We previously reported on the detection of an unknown virus in sentinel mice in a research mouse colony during routine colony health monitoring. Multiple soiled bedding sentinels in 4 adjacent rooms apparently seroconverted to MTLV on a multiplex immunofluorescence assay (MFIA) using a novel antigen produced in a murine AKR T cell line. The MTLV assay antigen had previously been obtained from thymocytes harvested from MTLV-infected neonatal mice. Sero-conversion was also observed in these mice when their sera was used in an IFA employing the uninfected AKR T cell line. The cell line was subsequently found to be positive by Mouse Antibody Production test using the same MFIA. Using metagenomics, we identified a novel murine astrovirus in feces from Swiss Webster mice (Tac:SW), placed as sentinels, which had recently seroconverted in the novel MFIA. The astrovirus was genetically highly divergent from MuAstV1 commonly present in research mice, yet closely related to viruses isolated from feral Norwegian and Sikhram rats in China. Using a PCR assay developed to detect both the Chinese rat and our mouse astroviruses, the T cell line was confirmed to be infected with a closely related astrovirus. Interestingly, an astrovirus similar (89% genome identity) to those found in our colony and the cell line was recently identified in feral mice in New York City. The PCR assay was subsequently used to implement a test and cull eradication plan in our research colony. We focus on 2 key issues related to this novel astrovirus: how a virus contaminated cell line and research mice serendipitously led to the identification of a new virus and how the virus was eradicated from the research colony.

#### PS97 MagPlex MFIA: A Next Generation Multiplexed Fluorometric Immunoassay for Serodiagnosis of Rodent Infectious Diseases

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Multiplex immunoassays including multiplexed fluorometric immunoassay (MFIA) based on Luminex polystyrene (PS) beads have been in use for more than 15 y for routine serosurveillance of laboratory rodents. A new MFIA using the next generation magnetic MagPlex microspheres was developed. MagPlex beads have several advantages over PS beads, including no pre-filtration of samples, no leaky expensive filter plates, and improved washing efficiency. MagPlex is quick and easy to separate from solution using a magnetic separator. Antigens for several common infectious agents in lab mice and rats, including mouse parvovirus, mouse hepatitis virus, adenovirus, CAR bacillus, *C. piliforme*, *E. cuniculi*, lymphocytic choriomeningitis virus, pneumonia virus of mice, reovirus-3, rotavirus-A, and Sendai virus were part of the 33- and 28-member mouse and rat MFIA bead panels. Whole virus or purified recombinant antigens were individually coupled to different color-coded bead sets. In addition, several system and sample suitability controls including tissue control beads to determine the sample related nonspecific antibody binding, species-specific IgG and anti-IgG beads, were added to respective panels to validate individual runs of the MFIA. Efficacy of this next generation MagPlex MFIA was compared to PS MFIA in a validation study using 16 known positive sera from naturally or experimentally infected rodents (mice and rats each) for 1 or more of the above mentioned infectious agents. A similar number, 16 known negative sera for each species were used from specific pathogen free colonies. All samples were tested by 2 different technicians on 3 different days for a total of 6 runs. A total of more than 6,000 assays

were performed and analytical performance of the rodent MagPlex MFIA assay including selectivity and limit of detection was found to be comparable to or better than those obtained by PS MFIA. Overall diagnostic sensitivity of rodent MagPlex MFIA was >97% compared to >98% for PS MFIA. Diagnostic specificity of both MagPlex and PS MFIA were nearly 100%, suggesting that MagPlex MFIA is an acceptable alternative assay for serodiagnosis of adventitious infectious agents of laboratory rodents.

#### PS98 Evaluation of Exhaust Air Dust Testing Using an Inline Collection Device on an Individually Ventilated Cage Rack without Cage-level Air Filtration

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Exhaust air dust (EAD) PCR testing is a sensitive tool in screening for rodent infectious agents on individually ventilated cage (IVC) racks. Previous reports for EAD testing using an inline filter for this rack type proved the detection of MNV. Our study demonstrates the ability of this inline filter, as well as pooled swabs IVC rack and air handling unit (AHU), to detect many agents including viruses, bacteria, parasites, and protozoa. We compared detection by EAD sampling using the inline filter and pooled IVC/AHU swabs to traditional soiled bedding sentinel testing. Inline filters were placed in the exhaust plenum just prior to the AHU and in front of the prefilter within the AHU. Pooled swabs were collected from plenum, exhaust hose, prefilter, and stainless steel drawer in the AHU. Baseline swab testing by PCR prior to the start of the study demonstrated the absence of rodent pathogen nucleic acid on the rack and AHU. Pet shop mice simulating a 15% prevalence were used to provide pathogen nucleic acid. Testing was performed at monthly intervals for 3 mo. EAD samples were tested by PCR only while soiled bedding sentinels, contact sentinels, and control mice were evaluated by PCR, serology, pathology, parasitology, and bacteriology. A total of 23 agents, including viruses, bacteria, protozoa, fur mites, and pinworms were detected in the pet shop mice upon arrival using PCR testing of antemortem sample types. K virus, MCMV, *Giardia*, *Campylobacter*, and *CAR bacillus* were identified in the pet shop mice, but were not transmitted to sentinels nor were they detected by EAD sampling. In general, more infectious agents were detected by EAD than by soiled bedding sentinel testing. Agent detection rates for bedding sentinels were 33% for traditional testing and 35% for PCR testing. Combined pooled swabs of the rack and AHU detected 74% of the infectious agents, while overall the inline collection devices detected 70% of the agents. Mouse adenovirus, Rotavirus, *M. pulmonis*, *P. pneumotropica*, *S. moniliformis*, *Cryptosporidium*, *S. muris*, and *Tritrichomonas* were detected by EAD only. This study supports that EAD testing using either an inline collection device or combined pooled rack/AHU swabs is a viable alternative to traditional sentinel monitoring.

#### PS99 Effectiveness of Aerosolized Hydrogen Peroxide in Simultaneous Decontamination of a Laboratory and a Biological Safety Cabinet

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The use of aerosolized hydrogen peroxide (AHP) in laboratory decontamination is becoming prevalent due to a need for safe, complete surface disinfection. AHP is a proven method of spore inactivation within sealed rooms by combining liquid and vapor phases. Achieving similar outcomes within the plenums and filters of a biological safety cabinet while simultaneously treating the laboratory would provide a safe means of comprehensive decontamination. Operational biosafety cabinets filter out 99.99% of aerosols suggesting any type of aerosol decontamination may be compromised, thus the efficacy of AHP in simultaneous treatment of a cabinet and laboratory was studied. A 3000-cubic foot lab was sealed and equipped with an

AHP generation system and biosafety cabinet. The generator controlled the injection of 7% hydrogen peroxide and pulse phases. Three decontamination times and 6 repeatable treatments were tested. The cabinet operated at normal, reduced, and no flow conditions. In each test, vapor monitors and a minimum of 30 *Geobacillus stearothermophilus* biological indicators (BIs)  $\geq 1 \times 10^6$  were placed in critical locations in the laboratory and cabinet. A gaseous phase resulted from the cabinet's internally re-circulated airstream that exhausted back into the laboratory. Gaseous concentration depended on the evaporation rate of the lab aerosol and liquid phase collected on the cabinet's filters. Photographs demonstrated a reduced aerosol concentration in the lab when the cabinet was on. While the biosafety cabinet was operational, all 184 BIs were successfully inactivated, signifying spore sterilization. The only exceptions across all 6 tests were 9 BIs in the cabinet's internal plenums when it was off. In conclusion, perceived efficacy challenges with filters engaged were proven unfounded. Biosafety cabinet operation, while reducing aerosol concentration, had no significant effect on gaseous concentration and did not compromise decontamination. Outcomes demonstrated AHP is a viable solution to simultaneous decontamination of a laboratory and its contents, including typically challenging areas within the internal plenums and filters of a biosafety cabinet, provided the cabinet is operational.

#### PS109 Assessing the Efficacy of Intranasal Midazolam in Sedation Of Young Pigs (*Sus Scrofa*)

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Due to their energetic nature, young pigs in research often require anesthesia for noninvasive procedures such as echocardiography and bandage changes. However, drugs currently available for anesthesia in swine are often detrimental to the cardiovascular system and are not ideal for use in animals involved in cardiopulmonary research. Midazolam is a fast-acting benzodiazepine frequently used in veterinary medicine that causes muscle relaxation, hypnosis, and has little cardiovascular effects. The intranasal (IN) administration of midazolam is often used to sedate human pediatric patients for noninvasive procedures, eliminating injection-associated stress in the recipients. The purpose of this study was to evaluate the efficacy of IN midazolam in young pigs for noninvasive procedures. Transgenic domestic piglets (*Sus scrofa*) with targeted disruptions of the RBM20 gene were selected for this study. Thirteen piglets, between 7 and 9 d of age, were separated into 3 IN midazolam dose groups, 0.25 mg/kg (n=4), 0.50 mg/kg (n=5), 1.0 mg/kg (n=4). To assess sedation, we adapted a sedation scoring system previously used in dogs. Adequate sedation (AS) was defined as tolerance of a nonnoxious stimulus (application of moderate pressure to the thorax for 30 s to mimic an echocardiogram) while in a sling at any time during the study. Using a 1-sided trend test, we found that 10/13 pigs reached AS (50%, 80%, and 100% in the 0.25 mg/kg, 0.5 mg/kg, and 1.0 mg/kg groups, respectively). Regardless of dose, AS was affected by ease of IN administration, with AS reached in 100%, 60%, and 50% of pigs that, following administration, did not sneeze, sneezed slightly, or sneezed significantly, respectively. These results suggest that IN midazolam at 1.0 mg/kg can be reliably used to sedate young pigs for nonnoxious procedures, and ease of administration affects adequate sedation in pigs of this age group.

#### PS110 Ultrasonographic Anatomy of the African Clawed Frog (*Xenopus laevis*) and Sexing of Juveniles

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The African clawed frog (*Xenopus laevis*) has been an invaluable research tool for developmental biology, electrophysiology, biochemistry, and neurobiology for almost a century, yet literature on

amphibian medicine and diagnostics is underrepresented. Because of this, disease in laboratory frogs is not well understood, nor are clinicians prepared to utilize and interpret diagnostics, such as imaging. Additionally, *X. laevis* is a sexually dimorphic species, however, phenotypic signs of sexual maturation can take 1 to 2 y to develop. The ability to sex juvenile frogs has the potential to improve colony management and reduce the number of animals used in research. Ultrasonography provides an easily accessible, non-invasive platform useful for both research and diagnostics, and has recently become more popular in aquatic medicine. Traveling easily through water and the external slime coat of the frog, ultrasound waves allow for easy identification of the majority of *X. laevis* coelomic organs. Needing only light anesthesia with tricaine methanesulfonate (MS-222), or gentle manual restraint, the normal size, echogenicity, and echotexture of heart, lungs, major arteries and veins, liver, gallbladder, stomach, gastrointestinal tract, kidneys, urinary bladder, and gonads were recorded in 4 adult males and 4 adult females. Half of the females were also imaged before and after hormonally induced ovulation, a non-invasive technique commonly used for egg harvesting in biomedical research. Electronic calipers were used to measure oocyte diameter and results reflected the asynchrony of oogenesis in this species. Furthermore, juvenile animals (15-22g) not yet old enough to be sexed by phenotypic characteristics, were imaged with ultrasound before being euthanized and necropsied to correlate the images with sex. This is the first report documenting baseline ultrasonographic anatomy of adult male and female *X. laevis*, with assessment of oocytes before and after ovulation, providing clinically relevant data for veterinarians and *X. laevis* investigators. These results also demonstrate the potential use of ultrasonography as an early and non-invasive way to sex juvenile *X. laevis*.

**PS111 Ocular Pharmacokinetics of Insulin-loaded, Thermoresponsive, Biodegradable Nanogels for the Treatment of Diabetic Retinopathy**

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Diabetic retinopathy (DR) affects approximately one-third of the estimated 422 million people with diabetes mellitus and is a leading cause of vision loss worldwide. DR is associated with retinal neurovascular degeneration, and studies indicate that systemic, subconjunctival, or intravitreal injection of insulin may reduce the risk of DR onset and progression. However, insulin has a short half-life, and the risk of hypoglycemia limits patients' ability to take enough insulin systemically to prevent and treat DR. The purpose of this project is to develop thermoresponsive and biodegradable nanogels for sustained release of insulin to the retina after subconjunctival injection to treat DR. Thermoresponsive and biodegradable nanogels containing N-isopropylacrylamide, Dextran-lactate-2-hydroxyethyl methacrylate, and acrylic acid were synthesized by surfactant-free emulsion polymerization. Insulin at 15 wt% was loaded into the nanogels during nanogel synthesis. The nanoparticles were characterized with respect to size, zeta potential, yield, and insulin loading efficiency and capacity. The in vitro release kinetics of insulin from the nanogels over two weeks were studied by using dialysis method with the released insulin quantified by ultra performance liquid chromatography. Fluorescent-labeled nanogels alone and insulin-loaded nanogels were subconjunctivally injected in the left eyes of Sprague Dawley (SD) rats (5/group) at 10 mg/ml and 20 mg/mL. The ocular pharmacokinetics of the nanogels and insulin released from the nanogels at 1 and 7 d post-injection were investigated by using fluorescent reader and liquid chromatography-mass spectrometry, respectively. The results showed that the yield of the nanogel synthesis was >68%, and the sizes of the nanogels were 100-200 nm. The nanogels could load insulin with extremely high loading efficiency of >98%. The nanogels were able to sustain the release of insulin in vitro for at least 7 d and cross the sclera, choroid, and retinal pigment epithelium to reach the retina after subconjunctival injection in SD rats. The insulin-loaded nanogels

showed promise for the future of effective therapies for the prevention and treatment of DR.

**PS112 Genotoxic *E. coli* Strains Encoding Colibactin, Cytolethal Distending Toxin, and Cytotoxic Necrotizing Factor Colonize Laboratory Rats**

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While many *E. coli* strains are considered commensals in mammals, strains encoding the cyclomodulin genotoxins are associated with clinical and subclinical disease in the urogenital and gastrointestinal tracts, meningitis, and inflammatory disorders. These genotoxins include the polyketide synthase (pks) pathogenicity island, cytolethal distending toxin (cdt), and hemolysin-associated cytotoxic necrotizing factor (cnf). These *E. coli* strains are not excluded from rodents housed under specific-pathogen free (SPF) conditions in academic or vendor facilities. Our recent publications have noted a high incidence of pks+ *E. coli* colonization in mice and clinical disease associated with pks+ *E. coli* in immunocompromised mice. The aim of this study was to isolate and characterize genotoxin-encoding *E. coli* from laboratory rats obtained from 4 different academic institutions and 3 different vendors. Sixty-nine distinct *E. coli* were cultured from fecal, rectal swab, or extraintestinal regions of 52 different rats and biochemically characterized. PCR for pks genes, cdt genes, cnf genes, and phylogenetic group was performed on all 69 isolates. Forty-five of 69 isolates (65%) were positive for pks, 20/69 (29%) were positive for cdt, and 4/69 (6%) were positive for cnf. Pks was the sole genotoxin identified in 21 of 45 pks+ isolates (47%), whereas cdt or cnf was also present in the remaining 24 isolates (55%). Cdt or cnf was never present together or without pks. All genotoxin-associated strains were members of pathogen-associated phylogroup B2. Select *E. coli* isolates were characterized by HeLa cell in vitro cytotoxicity assays, serotyped, and whole genome sequenced by Illumina MiSeq. All clb, cdt, and cnf-encoding isolates induced megalocytosis in HeLa cells. Serotypes corresponded with vendor origin and cyclomodulin composition, with the cnf+ serotype representing a known human uropathogen. Whole genome sequencing confirmed the presence of complete pks, cdt, and hemolysin-cnf pathogenicity islands. These findings indicate that genotoxin-encoding *E. coli* colonize laboratory rats from multiple commercial vendors and academic institutions and suggest the potential to contribute to clinical disease and introduce confounding variables into experimental rat models.

**PS113 Mammary Tumor and Mastectomy Synergistically Promote Chronic Neuroinflammation in a Breast Cancer Survivor Model**

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Even after treatment for breast cancer ends, many women experience mental sequelae including depression and anxiety that can last for years. Understanding the cause of these cognitive deficits is essential for developing targeted treatment plans and improving quality of life for breast cancer survivors. Microglial priming results in heightened responses to homeostatic disturbances thereby exacerbating neuroinflammation and neurodegeneration, and offers a potential mechanism for this cognitive dysfunction. This study examined whether mammary gland tumors prime microglia and augment the inflammatory profile and behavior of mice. To test this, we injected nonmetastatic mammary tumor cells (67NR) orthotopically into BALB/c mice, allowed them to grow for 16 d, and then removed the tumors via mastectomy. Following a 14-d surgical recovery, we challenged the mice with LPS, and then evaluated central and peripheral inflammation, anxiety, and depressive-like behavior (n=10-15/group). Open field test assessed anxiety, whereas forced swim and

sucrose anhedonia tests evaluated depressive-like behavior. Here we show that after 16 d of tumor growth (the time of mastectomy surgery), there were no differences in inflammatory markers in the hippocampus or serum apart from increased serum CXCL1 concentrations in tumor-bearing compared to non-tumor-bearing mice. Similarly, mastectomy surgery alone did not significantly affect major central or peripheral inflammatory markers. Interestingly, hippocampal mRNA expression of major proinflammatory cytokines IL-1 $\beta$  and TNF $\alpha$  along with microglial scavenger receptor CD68 was increased following surgical recovery in mastectomy tumor removal animals relative to controls. This means that tumor growth nor surgery alone resulted in a proinflammatory response, but together they led to neuroinflammation persisting past surgical recovery. Nonetheless, after LPS administration there were no exaggerated immune responses or behavioral changes to substantiate microglial priming in the tumor removal group. In summary, these data demonstrate that tumor-bearing together with mastectomy surgery promotes neuroinflammation and microglial activation; however, immune challenge with LPS did not elucidate this inflammation as maladaptive for the host.

#### **PS114 Recurrent Laryngeal Nerve Transection in Mice Results in Translational Upper Airway Dysfunction**

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The recurrent laryngeal nerve (RLN) is responsible for normal vocal fold (VF) movement. The RLN is at risk for iatrogenic injury during anterior neck surgical procedures in human patients, resulting in subsequent VF paralysis that may contribute to swallow, voice, and respiratory dysfunction. Unfortunately, treatment for RLN injury does little to restore function, as no treatments can truly promote regeneration of the injured nerve. Thus, we sought to create a mouse model with translational functional outcomes to further understand spontaneous RLN regeneration and investigate therapeutic interventions. To do so, we performed a ventral neck surgical procedure in 21 C57BL/6J male mice. Mice were divided into 2 groups: unilateral RLN transection (n=11) and sham injury (n=10). Furthermore, mice underwent the following assays to determine upper airway function at multiple time points prior to and following surgical manipulations. Transoral endoscopy was utilized to assess VF motion. Videofluoroscopic Swallow Studies were used to quantify swallow function. Vocal function was assessed using ultrasonic vocalization assays, while whole-body plethysmography was used to assess respiratory function. Results revealed that RLN transection created ipsilateral VF paralysis that did not recover by 12 wk after surgery. Furthermore, there was evidence of significant vocal and respiratory dysfunction in the RLN transection group, but not the sham injury group. However, no significant differences in swallow function were found between the 2 groups. In conclusion, our mouse model of RLN injury provides several outcome measures to increase the translational potential of findings in preclinical animal studies. We aim to utilize this mouse model and our regimen of behavioral assays to assess various treatment options to promote RLN nerve healing.

#### **PS115 The Role of Type II Alveolar Epithelial Cell Injury in Development of Idiopathic Pulmonary Fibrosis in Murine Models**

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Idiopathic pulmonary fibrosis (IPF) is a debilitating, progressive, and fatal lung disease. It is characterized by excessive extracellular matrix

deposition in the pulmonary interstitium which blocks gas exchange. IPF affects greater than 5 million people in the world and can be as high as 400 cases per 100,000 people over 65 years old. Lung transplantation remains the only therapeutic option that can prolong survival in patients with IPF. A better understanding of the pathogenesis of IPF in animal models will hasten the development of antifibrotic drugs. Numerous lines of evidence, from our group and others, support the idea that alveolar epithelial cell (AEC) injury and apoptosis is important for the development of fibrosis, but the mechanism by which AEC injury/death promotes fibrosis is unknown. We hypothesize that progressive pulmonary fibrosis is driven by a phenotypic change in alveolar macrophages that is induced after their engulfment of apoptotic AECs, a process termed efferocytosis. Murine AECs were isolated from both wild-type (WT) and SPC-GFP mice, and treated with UV light to induce cellular apoptosis. Coculture of apoptotic AECs with alveolar macrophages showed that macrophages could uptake apoptotic AECs, and then upregulate expression of profibrotic genes such as arginase and TGF- $\beta$ . In order to understand if fibrosis is mediated by efferocytosis, we repeatedly administered apoptotic bodies derived from type II AECs or a mouse lung epithelial tumor cell line (MLE-12) to the lungs of WT mice and mice which lack CD36, an important efferocytosis receptor. CD36 null mice demonstrated less fibrosis on histology and by hydroxyproline assay of lung tissue. Bronchoalveolar lavage fluid in CD36 null mice contained significantly lower amounts of TGF- $\beta$  than in WT mice by ELISA. Overall, these studies support a novel mechanism of how AEC injury initiates progressive fibrosis, and reveal CD36 as a potential therapeutic target against fibrosis.

#### **PS116 Modeling Crohn's Disease: Identifying Environmental Triggers in a Genetically Susceptible *Atg16l1* Rat Strain**

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Crohn's disease (CD) is 1 of 2 chronic inflammatory bowel diseases (IBD) that affect the lining of the gastrointestinal (GI) system. CD is a multifactorial disorder caused by a combination of genetic susceptibility and environmental factors. Numerous susceptibility loci have been linked to CD, 1 of which lies in the autophagy-related 16-like 1 (ATG16L1) gene. The mechanism by which the ATG16L1 variant (T300A) causes increased susceptibility to CD is still incompletely understood, and current mouse models harboring this variant do not properly express the clinical inflammation noted in human Crohn's patients. Our laboratory generated a knock-in rat using CRISPR-Cas9 technology in a Fischer 344 background strain to determine whether the rat could serve as a more characteristic model of the human Crohn's phenotype. To validate this rat strain as a model for CD, it was necessary to identify appropriate environmental triggers of disease. Two experimental groups of heterozygous (HET) rats carrying the T300A susceptibility allele and their wildtype (WT) littermates were chronically exposed to 1 of 2 different known environmental triggers of CD: either a low-dose nonsteroidal anti-inflammatory (NSAID; diclofenac, 1.25 mg/kg/PO) or ad libitum Western diet formulated rodent feed. Each group, including a control group, contained 24 animals cohoused by sex and experimental group (mixed WT/HET housing) with equal numbers of sex and genotype per group. We found that HET rats in both the oral NSAID and Western diet groups had increased inflammation and changes to the mucosal epithelium of the ileum and colon like that seen in patients with CD as determined by blinded histopathologic assessment. It was also found that HET rats given oral NSAID had a significantly altered microbiome profile compared to WT rats and heterozygous controls when serial fecal samples were collected under sterile conditions and analyzed by 16S rRNA sequencing against the SILVA database at the OTU level. These results show that, unlike in current mouse models of CD, known environmental triggers of CD can cause histopathology like human CD patients in rats harboring the human ATG16L1 T300A variant.

**PS117 Lytic Enzymes: A Novel Antimicrobial Treatment for Methicillin-resistant *Staphylococcus aureus* in Instrumented Nonhuman Primates**

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Methicillin-resistant *Staphylococcus aureus* (MRSA) can cause fatal human and animal infections. Alternative therapies are needed due to antibiotic resistance. Lytic enzymes encoded by bacteriophages (lysins) and bacteria (bacteriocins) are the source of novel antimicrobial agents as they can degrade bacterial cell wall resulting in hypotonic lysis. A colony of nonhuman primates (*Macaca mulatta*) with permanent intracranial implants showed persistent cutaneous MRSA carriage. We considered whether lytic enzymes are an effective treatment method for topical MRSA colonization. Treatment animals (n=2) received 5 doses every other day of the bacteriocin lysostaphin (5 mg/mL) active against our MRSA isolate in vitro. Control animals (n=2) received buffer. Treated areas included the cranial implant margin (3mL/animal), nostrils, and perirectal area (both as ointment formulation), shown to be MRSA positive. All animals and their environment underwent a 5-d decontamination to decrease cross-contamination. During animal decontamination, the implant margin was cleaned/debrided with sterile saline and the head and face were wiped with a 2% chlorhexidine solution followed by saline and then dried. Mouth was rinsed with 3 ml dilute chlorhexidine oral rinse. The animals were bathed with a 2% chlorhexidine shampoo and dried. The environment was decontaminated with ACCEL TB. On day1, day 3, and day 5, the animals were placed in new decontaminated cages (mechanical cage wash with exposure to 180°F and detergent) after bathing. Based on pilot studies, all animals received clindamycin 10 mg/kg IM to treat MRSA systemically. Lysostaphin treated areas were swabbed before, during, and after treatment to determine MRSA colony forming units (CFUs). The CFU count decreased below detectable levels (100 CFU/mL) in the nostrils and peri-rectal area in all animals after decontamination and antibiotic treatment. The CFU count from the cranial implant margin decreased (4-5 log) after decontamination and antibiotic treatment in all animals. A week after local lysostaphin treatment, MRSA decreased to below detectable levels (25-125 CFU/mL) in the treatment but not the control group. Three wk after lysostaphin treatment CFU counts in the cranial implants of all animals showed an increasing trend suggesting that topical lysostaphin treatment coupled with decontamination and systemic antibiotics may be effective at decreasing MRSA colonization but failed to eradicate MRSA leading to increased bacterial colonization. We suspect this failure was a result of the cranial implant restricting the ability to administer the bacteriocin into all colonized areas. Nevertheless, lysostaphin shows promise for treating colonized monkeys topically as long as the sites of colonization are accessible.

**PS118 Water-soluble Fenbendazole: A Possible Alternative to Fenbendazole Medicated Feed for Treatment of Pinworm Infections (Oxyuriasis) in Laboratory Rodents**

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Fenbendazole (FBZ) has been extensively studied and used as an anthelmintic medication for several species of animals, including rodents. Fenbendazole has demonstrated activity against pinworms, but FBZ has low water solubility (0.9 ug/ml), and to our knowledge, there are no commercially available water-soluble formulations that might be used in drinking water for treatment of pinworms. Fenbendazole has been formulated as a medicated rodent feed with demonstrated success treating pinworms. More recently, a FBZ oral

suspension became available for delivery in water to use in swine and chickens. Unfortunately, this new product must be mixed fresh daily or requires agitation to prevent precipitation of the active ingredients. This study's new water-soluble formulation of fenbendazole (FBZ-QS, 1 mg/ml) may provide a cost effective, non-precipitating, liquid alternative to medicated feeds. Our current hypotheses tested FBZ-QS's palatability in common strains of laboratory rodents. FBZ-QS was tested at several concentrations in tap water: 0 ug/ml (control tap water), 0.00075 ug/ml, 0.0075 ug/ml, 0.075 ug/ml, and 0.75 ug/ml (n = 16 per treatment group) in a cross over design over the course of eight weeks. Four boxes of four mice each were rotated one week at a time through the treatments above, with a washout week in between each FBZ-QS concentration. Body weight and hydration status were monitored daily. Fluid consumption was recorded weekly, including wash out weeks. All other husbandry guidelines followed the institution's standards for rodent care. As hypothesized, FBZ-QS was readily consumed at all concentrations tested, by C3H/HeN mice. No significant differences were detected between control and any other medicated water treatment (214.62 ml/kg/day ± SEM 5.59). At the highest FBZ-QS treatment, mice consumed 158.64 ug/kg/day ± SEM 7.70. No evidence of dehydration was detected and mice gained weight as expected throughout the study. Results for Balb/c mice (Balb/cAnNHsd), Wistar (Hsd:WI), and Sprague-Dawley rats (Hsd:Sprague Dawley) are pending. A positive outcome for this study would provide an alternative and possibly a more cost effective method for treatment of pinworms in rodents.

**PS100 Clinical Management and Pathologic Findings in Immunosuppressed Yucatan Swine**

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We report common pathologic findings and subsequent medical management of a cohort of immunosuppressed Yucatan swine. The 4-8 mo-old, castrated male pigs, were on longterm immunosuppressive regimes (greater than 2 wk) as part of a xenotransplant study. Animals were obtained from a vendor that routinely vaccinates and test negative for the most common porcine pathogens. Pigs were immunosuppressed with an oral regimen of methylprednisone and cyclosporine, as well as a twice monthly IV dose of Abatacept, a T cell modulator, up to endpoint (2-8 wk). Animals were administered IV antibiotics during surgical procedures and were on daily oral prophylactic cephalixin postoperatively. Throughout the study, several pigs presented clinically with vomiting, inappetence, and diarrhea. These animals were usually managed with fluid therapy, oral rehydration solutions, gastroprotectants, and a variety of antibiotics; however, most animals were poorly responsive to therapy and had progression of clinical signs, so early euthanasia was required. Consistent necropsy findings included wet congested lungs, gastric ulcerations, and renal hemorrhages. Histologically, multiple animals had rampant pneumocystis pneumonia, evidence of bacterial infections, and severe renal hemorrhagic medullary nephritis with variable fibrinohemorrhagic glomerulonephritis and arteriolar lesions. One animal had early histologic changes to the liver consistent with lymphoma. Additionally, at least 1 animal had evidence of porcine circovirus-associated porcine dermatitis and nephropathy syndrome and 2 had severe disseminated porcine cytomegalovirus infection. After histopathologic review of approximately 20 necropsied animals, the prophylactic drug regimen was modified to include an oral antiviral, sulfamethoxazole/trimethoprim, and probiotics. Once these medications were started, reduced pneumocystis burdens were noted histologically, and fewer animals presented with clinical gastrointestinal disease, and to date, all animals have made it to the planned endpoint. The clinical and necropsy findings in this cohort illustrate the power of clinical care informed by pathology and subsequent prophylactic treatment for the management of opportunistic infections in profoundly immunosuppressed pigs.

### PS101 Respiratory Signs in Swine Vaccinated against Porcine Reproductive and Respiratory Syndrome Virus

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Ten of 14 newly arrived Yorkshire-cross pigs (*Sus scrofa*) (38–80 kg) presented with upper respiratory signs. Clinical signs ranged from sneezing alone to crackles on thoracic auscultation with accompanying lethargy, decreased appetite, and fever. Pigs were experimentally naïve, and had received a modified-live porcine reproductive and respiratory syndrome virus (PRRSV) vaccine administered by the vendor. Differential diagnoses included *Mycoplasma hyopneumoniae*, porcine circovirus-2, swine influenza virus, *Pasteurella multocida*, *Bordetella bronchiseptica*, *Actinobacillus pleuropneumoniae*, porcine respiratory coronavirus, *Salmonella enterica* ser. Choleraesuis, *Haemophilus parasuis*, and *Streptococcus suis*. Pigs presenting with decreased appetite and increased expiratory effort received an injection of long-acting tulathromycin 2.5 mg/kg as empiric therapy for primary or secondary bacterial respiratory pathogens. Within a week of arrival at our facility, pigs underwent terminal surgical procedures. Following euthanasia, a postmortem examination was performed by the veterinary staff for 3 pigs, 2 of which had received tulathromycin. The lungs of all pigs were grossly unremarkable, and lung tissue was sent to a diagnostic laboratory for further testing. The pig who had not received tulathromycin tested PCR positive for *Mycoplasma* spp. All pigs were PCR positive for PRRSV North America strain and PCR negative for swine influenza, porcine circovirus-2, and PRRSV Europe strain. In an attempt to differentiate a positive result due to PRRSV vaccination versus natural infection, histopathology of lung tissue from 2 pigs was performed. Histologic findings were suggestive of natural PRRSV infection, so lung tissue was submitted for DNA sequencing, which revealed PRRSV wild-type virus. PRRSV is an economically devastating virus, causing reproductive disease in gestating gilts and sows and causing respiratory disease in growing pigs, as seen here. This case series underscores the importance of a careful workup and that even when animals are vaccinated against a pathogen, that pathogen should remain a differential until proven otherwise.

### PS102 Multiple Cases of Otitis Media in Immunocompromised Mice Linked to *Burkholderia gladioli*

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Over a 9-mo period, a marked increase in the number of mice presenting with a head tilt was observed in numerous holding rooms within 1 of our 3 barrier vivaria. Mice affected represented several immunocompromised strains, including CB17.Cg-Prkdc<sup>scid</sup>Lys<sup>tg-1</sup>/Cr1.NOD.Cg-Prkdc<sup>scid</sup>Il2rg<sup>tm1Wjl</sup>/SzJ, C57Bl/6J-Rag<sup>1tm1Mom</sup>, and B6.129S6-Rag2<sup>tm1Foa</sup>N12, and were housed in rooms shared with immunocompetent mice. Nine affected mice were submitted for complete necropsy. Grossly, all mice had tympanic bulla empyema, which was evident histologically as marked suppurative bacterial otitis media. Mice also had hepatitis (7/9) and encephalitis (3/9). Bacteria isolated from the bullae via aerobic culture were identified as *Burkholderia* spp. in 7/9 mice. In the remaining 2/9 mice, intralosomal and intracytoplasmic gram-negative rods were seen in the bullae, with *Burkholderia* spp. isolated from hepatic and brain lesions in 1 mouse. A subset of these isolates (6/9) were further speciated by MALDI-TOF as *Burkholderia gladioli*. *B. gladioli* is historically known as a plant pathogen, but is of growing concern as a cause of severe respiratory tract infections in cystic fibrosis patients, particularly

following lung transplantation. To date, only one other account of *B. gladioli* infection in laboratory mice has been reported, when an outbreak of otitis media in a facility housing immunocompromised mice was caused by *Burkholderia gladioli* in 2004. The pathogen was found in oropharyngeal swabs of affected mice and believed to cause otitis via colonization of the Eustachian tube. Following identification of *B. gladioli*, increased surveillance for this bacterium was undertaken in our colonies. *Burkholderia* spp. was subsequently isolated in another barrier from the gastrointestinal tract of a cohort of NOD.Cg-Prkdc<sup>scid</sup>Il2rg<sup>tm1Wjl</sup> mice presenting with *Clostridiales* *difficile*-associated diarrhea. This result suggests this bacterium may be more prevalent in laboratory mice than previously known and may be poised to become an emerging opportunistic pathogen in research colonies using immunocompromised mice.

### PS103 Methods of Microbial Surveillance and Aseptic Maintenance to Reduce Contamination of Germ-free Mouse Derivations

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Embryo transfer (ET) for derivation of germ-free (GF) mice is performed in biosafety cabinets using standard surgical gowning, gloving, and aseptic technique. We discovered that many projects exhibited bacterial growth postderivation during routine weekly microbial surveillance of flexible film isolators used to house the ET recipients. To investigate the stage in the process at which the ET recipients were becoming contaminated, a microbial surveillance program was implemented using daily RODAC plating of gloved hands and various surfaces touched by surgeons and assistants during the ET procedure, as well as ATP sanitation monitoring of surfaces inside the biosafety cabinets. Results of the monitoring showed growth of specific organisms on gloves and surfaces which often matched those identified later as contaminants in animals that underwent surgery the day of the environmental monitoring. Surfaces at high risk for contamination were identified and procedures to optimize asepsis were implemented, such as use of disposable covers for microscope knobs, 70% isopropyl alcohol wipes for disinfection of surfaces, and thorough disinfection of the room, equipment, and biosafety cabinet the week before GF derivations are performed as well as supplemental training of all support staff and surgeons. After implementation of the daily monitoring and procedural changes, the rate of contamination in GF derivations postprocedure was significantly reduced.

### PS104 Immune Mediated Hemolytic Anemia in Humanized NOG Mice

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Sixteen 4-mo-old female hGM-CSF/hIL3-NOG mice were shipped from a vendor to a heightened biosecurity facility after receiving irradiation and bone marrow transplant of human myeloid cells. The mice were kept in sterile housing while participating in a vaccine study. At the age of 7 mo all mice exhibited varying degrees of pale extremities, hypothermia, weight loss, and hunched posture. After a physical exam, supportive care was initiated in the form of a high-calorie diet gel. Empiric medical intervention was avoided to reduce confounding experimental factors. Mouse weights were tracked for 2 wk. At that time 2 mice reached weight loss endpoints, 1 of which received multiple experimental vaccinations and the other received sham vaccinations. These animals were euthanized and submitted for further diagnostics. Differential diagnoses included infection, graft vs host disease (GvHD), anemia secondary to xenographic bone marrow displacement, and graft failure. Noninvasive biologic samples were collected from all mice for a sentinel PCR panel. All 44 murine pathogens tested were negative. Gross necropsy revealed pale livers with multifocal pinpoint hemorrhages and pale kidneys. PCV and CBC revealed a severe macrocytic anemia and lymphopenia.

Chemistry was unremarkable with mild hyperalbuminemia, hyperglycemia, and electrolyte imbalances consistent with hemolysis. Tissues were submitted for histopathology which revealed diffuse collections of iron-positive, hemosiderin-laden macrophages and multinucleated giant cells in the lungs and liver. Collections of lymphocytes were noted in lungs, liver, pancreas, and salivary gland interstitium. These findings are characteristic of immune-mediated hemolytic anemia (IMHA) in which macrophages react against MHC1 mismatched hematopoietic tissues. IMHA is a major contributing factor of morbidity in both GvHD and graft rejection. Humanized mice are at risk for IMHA despite extensive measures taken to prevent adverse graft/host reactions and these conditions should always be considered differential diagnoses despite advances in xenograft technologies.

#### PS105 Novel Focused Ultrasound Technique to Identify Duodenal Ulceration in Common Marmosets (*Callithrix jacchus*)

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Common marmosets (*Callithrix jacchus*) are a new world primate species commonly used in biomedical research with a documented predisposition to a spectrum of gastrointestinal diseases. At our facility, non-invasive methods to evaluate the gastrointestinal tract are limited to radiography with or without the use of intra-oral barium. Recently, several group-housed marmosets developed vomiting and weight loss. Following necropsy confirmation of duodenal ulceration and perforation in several of these animals, a more sensitive noninvasive monitoring technique was desired. In collaboration with veterinary radiologists, a protocol for focused ultrasound assessment of the cranial abdomen of marmosets was developed. The result was a simplified technique that could be easily taught to staff veterinarians and a 6-item checklist for assessment of the peritoneal space, hepatobiliary system, and proximal gastrointestinal tract. This technique was incorporated into biannual health screenings of the marmoset colony, with particular attention paid to any symptomatic animals. A total of 38 animals was evaluated using this technique. Ultrasound findings of proximal duodenal abnormalities ranging from mucosal irregularity to deep ulceration were observed in 6 animals. Of those animals with duodenal ulceration, 1 also had evidence of duodenal perforation at the time of ultrasound examination. Affected animals were treated with antiulcer and antimicrobial therapy or euthanized as appropriate for each individual case. Necropsy confirmed duodenal ulceration and/or perforation in the 2 animals euthanized to date with sonographic evidence of these lesions. Noninvasive diagnosis and early detection are essential for proper management and long-term follow-up of duodenal ulceration and perforation in common marmosets. This focused ultrasound technique is easy to incorporate with routine health assessments of a colony and normal ranges generated in this study will be clinically useful for further studies of gastrointestinal disease in this species. We expect the technique of focused cranial abdominal ultrasonography may be adapted for use in other nonhuman primate species.

#### PS106 *Clostridioides difficile* Typhlocolitis Resulting from Amoxicillin Treatment in Highly Immunocompromised Mice

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*Clostridioides difficile* is an important opportunistic pathogen affecting

both humans and animals. Although its ability to cause naturally occurring disease in mice is extremely rare, *C. difficile* has been described in a few case reports. Little is known about the clinical significance of this bacteria in highly immunocompromised mice. We investigated an outbreak of diarrhea in NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup>tm1Wjl</sup>/SzJ (NSG) and related strains. Affected mice were previously treated with a 14-d course of 0.12% amoxicillin impregnated feed to control a spike in *Corynebacterium bovis* infection. The vast majority of cases were detected, on average, 20 d after the provision of amoxicillin-compounded feed ceased. Most mice (~93%) had been implanted with human xenografts while the remaining (~7%) were naïve. Affected animals exhibited 3 clinical syndromes: 1) peracute death; 2) severe diarrhea leading to death or euthanasia; and, 3) mild to moderate diarrhea followed by recovery. All of these mice could be found within a single cage, occasionally alongside clinically unaffected cage mates. Transfaunation with feces from healthy NSG mice and subcutaneous fluids were administered to some mice with limited efficacy. Fifty-three sick or dead mice were submitted for bacterial culture and histopathology. *C. difficile* was isolated from the cecum or colon in approximately 70% of these cases. The presence of both *C. difficile* toxins A and B were confirmed in 3/4 cases. Antimicrobial sensitivity of 5 isolates revealed 3 different profiles with all isolates having at least intermediate sensitivity to ampicillin, the surrogate for amoxicillin. Histopathological lesions included fibrinonecrotizing and neutrophilic typhlocolitis with characteristic 'volcano' erosions or pseudomembrane formations of varying severity. Four samples from distinct mouse colonies were submitted for whole-genome sequencing to assess strain type, epidemiologic relatedness, and virulence factors. This outbreak was unexpected as we have administered amoxicillin to mice for years without incident.

#### PS107 Hemolytic E. Coli as a Probable Cause of Reproductive Failures in a Specific-Pathogen Free Cat Colony

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A specific-pathogen free (SPF) breeding colony of approximately 25 cats presented with multiple cases of resorbed pregnancies, stillbirths, and pyometras over the course of 6 mo. Litters that did go to term were typically small (1-3 kittens), and these kittens were often of low to low-normal birth weight. The top differential was decreased fertility due to inbreeding of the colony for over 30 y. Other differentials included cystic endometrial hyperplasia and infectious causes such as *E. coli*, *Streptococcus*, or viral infection. One pyometra was successfully managed medically, with Clavamox (62.5 mg BID) and Lutalyse (0.25 mg/kg daily for 5 d). This queen became pregnant and underwent a gravid ovariohysterectomy for experimental reasons, at which point abnormal fetuses were recovered. Two other pyometras were managed surgically by ovariohysterectomy, with uneventful recovery of the queens. A pregnant queen then presented in dystocia after passing 2 stillborn kittens, so a Cesarean section was performed to retrieve the third nonviable fetus. The placenta, fetal tissues, and full-thickness uterine biopsy were submitted for histopathology. There was fibrinosuppurative endometritis in addition to a mild neutrophilic placentitis, consistent with bacterial endometritis. Hemolytic *E. coli* was cultured from the placenta and fetal tissues, implicating this agent as the cause of the stillbirths. Preputial cultures from the 2 intact males in the colony were also positive for hemolytic *E. coli*, as well as light mixed growth consistent with normal preputial flora. The 2 intact males and 2 pregnant females were treated with orbifloxacin (7.5 mg/kg daily) for 2 wk. One wk after completing treatment, follow-up preputial cultures of the intact males revealed no hemolytic *E. coli* growth. The 2 queens that were treated with orbifloxacin had litters of 1 and 2 live kittens. Breeding has been on a temporary hiatus since these litters were born, with no further cases of pyometra in the colony.



### PS108 Opportunistic Infections in 2 Cohorts of New Zealand White Rabbits

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Shortly after shipping from the vendor, 7 rabbits from 2 independent cohorts presented with inappetence and diarrhea. Each cohort consisted of both male and female New Zealand White rabbits that were ordered based on weight (>2.50 kg) and arrived at the facility in February and April 2018. The February cohort had 4 rabbits present with inappetence, and 2 with concurrent diarrhea. Physical examination and blood work revealed mild (5-7%) dehydration, perineal fecal staining, and scant fecal pellets, which were often small and misshapen. Fecal samples were collected for fecal flotation and aerobic and anaerobic culture. Empirical treatment with oral enrofloxacin (10 mg/kg BID) and subcutaneous electrolyte solution (20 mL/kg Q8h) was initiated. Flotation did not reveal evidence of intestinal parasites, but *Clostridium perfringens* was identified on culture. As a result, oral metronidazole benzoate (32 mg/kg BID) was initiated. Despite 3 d of treatment, the rabbits continued to show progressive weight loss and dehydration. Euthanasia was elected. Similarly, the April cohort had 3 rabbits present with inappetence and marked diarrhea. Because these rabbits were intended to undergo infection studies, the possibility of confounding factors led to the decision to euthanize rather than initiate treatment of any kind. Necropsy of the affected rabbits demonstrated both gross and histopathologic findings consistent with severe enterocolitis and bacterial dysbiosis. Cultures and molecular diagnostics from affected rabbits identified *C. perfringens* type A, which the literature supports the role of this bacteria as an opportunistic pathogen secondary to bacterial dysbiosis. Following discussions with the vendor, it was discovered that the rabbits were fed a low-fiber diet at the vendor's facilities prior to shipment, thus predisposing them to bacterial dysbiosis. New practices, such as increasing the fiber content of the diet upon arrival, were set in place to prevent potential future cases of dysbiosis following shipment.

### PS119 Vitamin D Toxicity in a Cohort of *Smad3<sup>tm1Par/J</sup>* (*Smad3<sup>-/-</sup>*) Mice

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A cohort of female *Smad3<sup>tm1Par/J</sup>* (*Smad3<sup>-/-</sup>*) mice (n=9, 5-12-w-old) received subcutaneous slow release vitamin D<sub>3</sub> pellets as part of a study seeking to evaluate the effects of systemic vitamin D supplementation on the gut microbiome. The pellets were purchased from a commercial vendor and were formulated to contain 10 mcg vitamin D<sub>3</sub> per pellet that would be released over a 21-d period. One, 2 or 3 pellets were implanted subcutaneously over the dorsal trunk of each mouse (n=3 per dose) under isoflurane anesthesia using aseptic surgical technique, formulated to release ~0.5 – 1.5 µg (~20 – 60 IU) vitamin D<sub>3</sub>/day/mouse. Mice received a single preoperative dose of buprenorphine (0.05 mg/kg) subcutaneously to provide postoperative analgesia. Two days following surgery, all 3 mice that had received 3 subcutaneous pellets presented as lethargic, hunched, and markedly dehydrated with an unkempt hair coat and poor body condition. They also displayed increased tail tone and intermittent tremors when moving about the cage. The surgical incisions remained clean, dry, and well opposed with no evidence of swelling, erythema, or discharge. Over the next 12 h, similar clinical signs were observed in the rest of the cohort. Animals were euthanized via CO<sub>2</sub> asphyxiation and tissues and blood were collected for clinical and histopathological examination. Differentials for the clinical presentation included sepsis or vitamin D toxicity. Necropsy findings and serum chemistry data were consistent with vitamin D toxicity, revealing moderate multifocal to coalescing acute tubular necrosis of the proximal renal tubules and significant elevations in serum calcium (20.4-23.3 mg/dL) and serum phosphorus (15.2-20.2 mg/dL). Analysis of the pellets by HPLC found

that each pellet contained approximately 2.5 mg of vitamin D<sub>3</sub> resulting in doses ~250 x those indicated by the formulation. This case emphasizes the importance of verification and validation of concentrations for commercially available compounds and describes clinical and pathologic findings associated with vitamin D<sub>3</sub> toxicity in mice.

### PS120 Increased Frequency of Sterile Struvite Urolithiasis in a Colony of Research Dogs

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Struvite urolithiasis is rare in dogs without a concurrent urinary tract infection. However, in 2017, dogs (n=2, males, about 3-y-old) maintained as part of a veterinary research colony on unrelated protocols were diagnosed with sterile struvite urolithiasis. To further investigate if urolithiasis was endemic throughout the colony, free-catch urine samples were assessed from a random selection of dogs across research protocols. Samples with evidence of crystals permitted targeting of dogs (n=22; about 1-6-y-old.; n=9 males, n=13 females, variety of breeds) from whom urine was then collected via ultrasound-guided cystocentesis. Samples were submitted within 2 h of collection for complete urinalysis, aerobic culture, and mycoplasma culture. The average urine pH of the examined animals was 7.65, higher than the pH range of 5.7 to 6.9 reported in clinically healthy dogs. Struvite crystalluria was observed in 36% (8 of 22) of samples. The prevalence of bladder material/stones, combined with high levels of struvite crystalluria seen on urinalysis, was 14%, higher than the reported 0.5% frequency of urolithiasis in the general pet population. Urine cultures were negative in 21 of 22 dogs, and the one positive culture (*Escherichia coli* and *Lactobacillus*) was deemed a likely contaminant by collaborating pathologists. As diet may have contributed to the sterile urolithiasis, feed was changed to a formulation with decreased levels of magnesium, phosphorous, and protein for the 2 dogs that presented with hematuria. Dissolution of uroliths and heavy debris within the bladder was confirmed via ultrasound 5 mo following diet transition, and repeat urinalyses showed improvements in urine pH and crystalluria in 1 affected animal. This study identified unexpected risk factors for struvite urolithiasis in our research colony, including diet, alkalotic urine, and a possible genetic component; future colony management practices will include scheduled urinalyses and continued review of dietary interventions.

### PS121 Clinical Presentation of Spontaneous Cardiomyopathy in a Cynomolgus Monkey (*Macaca fascicularis*)

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A 5-y-old, 9.1kg male cynomolgus macaque (*Macaca fascicularis*) was sedated for an experimental surgical procedure with ketamine (2mg/kg IM) and dexmedetomidine (0.03mg/kg IM). Anesthesia was induced with propofol (0.4mg/kg IV) to facilitate intubation, and isoflurane anesthesia was initiated. Auscultation of the thorax revealed tachycardia and the presence of a gallop rhythm, as well as moderate, coarse bilateral crackles on inspiration. A moderate amount of blood-tinged fluid was observed in the endotracheal tube, and the animal displayed prolonged capillary refill time (2-3 s), but no sign of ascites or other abnormalities. Right lateral and ventrodorsal radiographs were performed, showing an interstitial unstructured lung pattern, bronchial narrowing, and cardiomegaly with right displacement of the heart axis. The animal was presumed to have pulmonary edema due to an underlying heart condition. Treatment was initiated with furosemide (2 mg/kg PO) twice daily until further diagnostics could be performed. After 10 d, the patient was sedated again with the same regimen. On auscultation, heart rate and rhythm were normal, but

occasional wheezing and mild crackles were still present. Upon repeat radiography, the lung fields were clearer and the bronchi appeared normal. The heart still appeared enlarged but less displaced compared to the previous examination. Electrocardiography showed a sinus arrhythmia and marked right axis deviation with normal P waves. Right ventricular enlargement with normal right atrial size was suspected. Due to the absence of a murmur, pathology of the tricuspid or pulmonic valve was not suspected. An echocardiogram confirmed right ventricular enlargement with no evidence of valvular regurgitation. Based on the clinical presentation and the globoid cardiac silhouette, the diagnosis was determined to be congestive cardiomyopathy.

#### PS122 Novel Approach to Optimize the Laying Hen Preclinical Model of Ovarian Cancer

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No appreciable improvements in incidences and mortality rates of women with ovarian cancer have been made over the last 40 y; this fact alone indicates that scientists and clinicians lack the adequate tools to conquer this deadly disease. The American Cancer Society estimates that more than 22,000 women will be diagnosed with ovarian cancer this year, and over 14,000 deaths will be attributed to this disease. This translates to 1 out of every 75 women in the U.S. being diagnosed with ovarian cancer, and of those diagnosed, over 60% will die from the disease. Lack of a more predictive animal model has been an obstacle to progress in ovarian cancer research. It is hypothesized that laying hens, though not fully characterized, could be an optimal animal model for the study of human ovarian cancer initiation, progression, therapy, and relapse. Domestic laying hens (*Gallus gallus domesticus*) spontaneously develop ovarian cancer at a high incidence. In effort to better characterize ovarian cancer in laying hens, a surgically implantable, biocompatible port was created using 3D printing technology, allowing for repeat access to the ovary for laparoscopic serial sampling, observation, and imaging. The ability to follow laying hens via easily accessible ports throughout their lifespan may pave the way for discovery of early diagnostic techniques for this disease. The fact that laying hens are a spontaneous ovarian cancer model with a high incidence of disease suggests their usefulness as a preclinical animal model. Little is known about tolerability and efficacy of chemotherapeutics in the laying hen or in avian species in general. A cohort of 10 4.5-y-old laying hens suspected for ovarian cancer was administered a 6-wk course of paclitaxel to assess chemotherapeutic efficacy. Magnetic resonance imaging (MRI) and positron emission tomography-computed tomography (PET/CT) were used to identify cancerous laying hens as well as to assess changes in tumorigenesis throughout treatment. Results are indicative of chemotherapeutic tolerability and efficacy, as well as the value of using a noninvasive method for diagnosis of cancer within the coelomic cavity, further suggesting the potential of the laying hen as an animal model for preclinical research.

#### PS123 Septicemia and Pneumonia in a Rhesus Macaque with an Indwelling Catheter

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A 16-y-old, singly housed, female rhesus macaque (*Macaca mulatta*) with a clinical history of chronic dysmenorrhea and heavy menses was re-examined due to dyspnea and recurring bruising on the ventral abdomen during menses. She was on a longterm cocaine neuropharmacology study requiring a surgically placed vascular access port and jugular catheter. Physical examination revealed a fever, abdominal bruising, and an enlarged uterus. Diagnostics included a complete blood count (CBC) which revealed mild neutrophilia with a

mild left shift and a moderate thrombocytopenia. A blood chemistry analysis had the most striking abnormality being a moderately elevated BUN and a moderate to severely elevated BUN/creatinine ratio. A coagulation panel demonstrated elevated fibrinogen levels but all other parameters were within normal limits. Septicemia was suspected and blood cultures were drawn. Ultrasound evaluation of the enlarged uterus indicated a large, cystic structure on the serosal surface. Chest x-ray results revealed lobar consolidation of the left cranial lung field, indicating pneumonia. Due to a poor prognosis, the animal was euthanized. Pathological examination detected jugular vascular calcification consistent with an indwelling catheter. A vegetative growth was found on the serosal surface of the uterus and there was a firm mass adjacent to the uterus composed of fibro-fatty tissue that on sectioning contained a cystic space filled with dark brown fluid. Histological evaluation of both these lesions were consistent with endometriosis. There was cranioventral consolidation of the left cranial lung lobe. The left caudal lung lobe had embolic foci, indicative of bacterial showering which was confirmed on histological evaluation. Bacteriology cultures collected from the affected lungs and the blood isolated *Kluyvera cryocrescens*. *Kluyvera* species are gram-negative, rod-shaped nosocomial bacteria occasionally cultured from human bacteremias. To the author's knowledge, this is the first reported case of *Kluyvera cryocrescens* in a rhesus macaque with an indwelling peripheral intravenous catheter.

#### PS124 Developmental Dysplasia of the Hip and Associated Long-Term Malformations in New Zealand White Rabbits: A Model for the Comparison of Late Human Analogies

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Hip instability derived from hip dysplasia is a common finding in human medicine and its genetic role has been widely discussed since infants suffering from this syndrome shows an array of early incapacitating lesions that if untreated leads to late dislocation. The study of this condition has been well documented in several animal species, and recent insights have demonstrated a strong genetic role of candidate genes for congenital splay leg in piglets, which translated into gross physical and radiographic abnormalities. In this study a pair of rabbits (*Oryctolagus cuniculus*) with an unknown genetic load, carriers of splay leg syndrome were mated, resulting in an offspring of 5:2 dysplastic animals studied for a period of 180 d to determine its influence over morphologic and skeletal deviations, further compared with normal rabbit and children hips. Results showed drastically impaired locomotion, limited abduction, and progressive bilateral dysplasia with acetabular involvement including early osteoarthritic changes analogous to human cases, where asymmetric thighs or buttock creases, as well as pronounced shortness of legs, were clinically and radiographically determined. The radiographic study illustrated also that the induced condition in the rabbit shared similar patterns of sex inheritance towards hip dysplasia, interspecies differences, and notorious short-term evolutive maldevelopment such as femoral anteversion and coxa valga, including lesions resembling gross femoro-acetabular impingement (FAI) that resulted in late femoral shaft torsion, making the rabbit a suitable animal for the study of human hip dysplasia. Finally, the clear genetic involvement of this condition needs further research in this species.

#### PS125 Lameness in a Yucatán Mini Pig

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A 1-year-old female spayed Yucatán mini pig presented with nonweight-bearing lameness of the left hindlimb. There was severe swelling proximal to the coronary band on the fourth digit of the left hindlimb. Differential diagnoses include trauma versus infection. Pain and swelling were managed with NSAIDs and opioids. However, these were insufficient and a local liposomal bupivacaine ring block was performed. Radiographs of the limb showed mineral and gas in the soft tissue swelling and moderately widening of the distal interphalangeal joint with focal osteolysis. While awaiting final radiographic interpretation, antibiotics were administered. The final radiology reports supported the high suspicion of septic arthritis. To retain a valuable research animal, digit amputation of the fourth digit was elected. The pig became partially weight bearing on the affected limb immediately upon recovery. NSAIDs, antibiotics, and opioids were continued for the first week postamputation, and bandages were changed thrice weekly until 20 d postamputation. During this time, the pig was comfortable and weight bearing. The amputation site healed completely 5 wk postprocedure and the animal continued to do well. She continued to gain weight until her weight plateaued at week 11 postprocedure. The pig regained full use of her hind limb, was able to be used in a study, and was euthanized at 15 wk post procedure. This case demonstrates that toe amputation can be successfully used to manage pain secondary to septic arthritis of the digits in swine.

#### PS126 Complications in a Stroke Model in Yucatán Mini Pigs

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Stroke was induced in a number of Yucatan mini pigs. As part of the study, reliable vascular access was required to complete study-related blood assays. This was accomplished by surgically implanting vascular access ports (VAPs) and indwelling catheters approximately 2 wk before surgically inducing stroke. A 1.5-year-old female spayed Yucatán mini pig was reported for severe lethargy and lameness on the right hindlimb 4 wk following VAP implantation and 2 wk following stroke. On exam, the pig was laterally recumbent, febrile, and tachycardic. Her right hindlimb distal to the hock was diffusely moderately swollen, warm on palpation, and painful. Differential diagnoses included trauma and infection. NSAIDs were administered, but the pig did not make significant clinical improvement. The next day, physical exam revealed a grade III/VI systolic heart murmur and a delayed capillary refill time. The pig was given intravenous fluid therapy and started on opioids and antibiotics. The animal was euthanized 24 h later. Necropsy revealed severe chronic vegetative endocarditis that grew *Staphylococcus aureus*. Additionally, there was severe cellulitis and tenosynovitis with abscessation in the right tarsus/metatarsus, consistent with septic arthritis. The following week, a second 1.5-year-old barrow Yucatán mini pig presented with lethargy and inappetence. The animal became lame on the left front with a palpably warm carpus. Bloodwork revealed a moderate inflammatory leukogram and NSAIDs, antibiotics, and intravenous fluids were administered. *Staphylococcus aureus* was cultured from the blood. The animal was successfully maintained on NSAIDs, antibiotics, and assisted feedings to reach study endpoint 2 wk after presentation. Endocarditis was confirmed on gross necropsy. Naturally occurring infection with subsequent septicemia and endocarditis in swine is typically caused by *Streptococcus* spp and *Erysipelothrix rhusiopathiae*. In this case, multiple sampling through the VAP likely served to introduce *S. aureus* from the skin directly to the circulatory system resulting in these complications. Retraining staff in aseptic technique and removing the VAP following its use prevented the recurrence of *S. aureus* septicemia in additional animals.

#### PS127 Sexual Maturity Confirmation in Group-housed Female *Macaca Fascicularis*: A Team Collaboration

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Determining sexual maturity of female macaques requires daily manipulation of animals to obtain a vaginal swab. Typically, animals are pair-housed in cages equipped with squeeze-backs to facilitate positioning of the animals for this process. The behavior team at our facility offered portable transport chutes with dividers as a convenient means of swabbing group-housed, adult female macaques to assist the veterinary team without social group disruption. Use of the transport chutes in this way required modifications to the transport chutes and to the process, involving a collaborative effort between behavioral, facility, and veterinary personnel. Modifications to the equipment and process included designing a frame to match the openings of the transport chute to the social housing unit, a cable hoist to open and close the chute door from inside the pen, a ratchet strap to secure the transport chute to the housing unit, desensitization of animals to the process, facilitation of moving the animal's tails away from the divider slots inside the chute, modifying materials used for vaginal swabbing, and the introduction of rods to secure the dividers closed while working animals in the chute. Through communication and these modifications and refinements, our behavior team was able to confirm sexual maturity in group-housed females without disrupting social relationships.

#### PS128 A Novel Approach to Conducting Nonhuman Primate Metabolism Studies that Allows Animals to be Group-housed, Enhancing Welfare and Science

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Currently, metabolism cages for the purposes of conducting absorption, distribution, metabolism, and excretion (ADME) studies, enabling an excretion balance scientific objective to be met, involve single housing of animals. Even though such metabolism cages have limitations for animal welfare, they have been largely unchanged for 25-30 years. We sought to design and build a new metabolism cage to improve welfare and fulfill requirements on enrichments and cage dimensions for standard housing of *Cynomolgus* macaques as described in EU Directive 2010/63/EU. The purpose was to investigate to excretion balance data from group and single housing of nonhuman primates (NHP) in metabolism cages, to demonstrate the suitability of conducting excretion balance studies with a group housing design to improve welfare without compromising the scientific integrity of the study. The assessment on the welfare, in terms of stress and behavior, was also investigated. The excretion balance evaluation has been conducted in metabolism cages with single and group housed NHP, using the radiolabelled test compound, Quetiapine, an anti-psychotic pharmaceutical selected for its suitable excretion profile (including both urine and fecal elimination). Concentrations of radioactivity in blood and plasma were determined by liquid scintillation counting. Cortisol concentrations were determined in serum samples daily. Urine and feces were collected pre- and postdose daily for up to 168 h and the radioactivity was quantified. The overall mean recovery for group-housed animals, 83.2% of the dose, was essentially comparable to that of data from single housed primates,  $87.1 \pm 10.2\%$ . These data are also consistent with the historical data, *ca* 83%, generated during the development of this compound. Group-housed NHP for future metabolism ADME studies does not compromise the scientific integrity of the study, and therefore is a major progression in the design of these studies which enhance welfare. A large degree of inter-animal variation was observed in the serum cortisol concentrations with a general trend of lower cortisol levels from 48 h onwards in the pair-housed animals.

#### PS129 withdrawn

### PS130 Social Housing of Pigtailed Macaques Decreases the Immune Impact of Acute Simian Immunodeficiency Virus Infection



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Macaques may be singly or socially housed depending on the research institution and study parameters. In general, singly housed macaques are thought to have elevated stress compared to their socially housed conspecifics, which may result in immunosuppression. Simian immunodeficiency virus (SIV)-infected macaques are a valuable animal model for the study of HIV pathogenesis, and SIV similarly leads to immunosuppression, marked prominently by the decline in CD4+ T cell counts that is commonly used to monitor disease progression along with viral RNA levels. We hypothesized that socially housed SIV-infected pigtailed macaques would demonstrate less immunosuppression and more control of viral replication compared to singly housed SIV-infected macaques. We compared CD4+ T cell counts and viral loads from 35 singly and 41 socially housed SIV-infected pigtailed macaques (*Macaca nemestrina*); macaques were either singly housed or socially housed with a compatible conspecific, respectively, during both the pre- and postinoculation periods. CD4+ T cell counts and viral loads were monitored at 3 preinoculation baseline time points and on days 7 and 10 during acute infection. Singly housed macaques demonstrated a greater magnitude of decline in the number of circulating CD4+ T cells throughout acute infection compared to socially housed macaques ( $P < 0.001$ ). Singly housed macaques furthermore had significantly higher viral loads in plasma and cerebrospinal fluid throughout acute infection compared to socially housed macaques (linear mixed effects regression;  $P < 0.001$ ), and greater variability in plasma viral load data (linear mixed effects regression;  $P < 0.001$ ). These data suggest that single housing of SIV infected macaques may promote stress-induced immunosuppression with the potential to confound the translational nature and reproducibility of this animal model of HIV infection.

### PS131 Enrichment for Nonhuman Primates: Identifying, Trialing, and Implementing Destructible Enrichment in an Operationally Efficient and Practical Manner

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Destructible enrichment (DE) items that can be manipulated and destroyed are a component of the enrichment program for nonhuman primates (NHPs) at many institutions. We had 6 DE options but all lost their novelty over time, being manipulated only when food treats were present. A larger variety of DE enrichment options was needed. Animal care staff recommended 37 ideas to be evaluated for the weekly intervention rotation, consisting of a wide range of materials including plush toys, paint rollers, coconut discs, manzanita wood, and Greenies. Each item trialed was given "as is" (without treats) to a representative group of rhesus and cynomolgus macaques. Interactions with each item were scored during a 15-20-min observation on Day 1 and the item was checked daily until destroyed which took on average 5 days. Items were assessed for their durability, safety, practicality, animal usage, and engagement with a scale range from 1-3 (1: used enrichment, was challenging, retained attention, destroyed; 2: was not challenging, showed no interest, item was still intact; and 3: could not use, had minimal engagement, animal was likely to throw or hit the object away.) Additionally, species-specific behaviors including chewing, shredding, sniffing, grooming, picking, licking, shredding, and sorting were measured. Items were selected for

intervention rotation based on their ability to meet these criteria while supported by the display of positive species-specific behaviors. The project resulted in the addition of 12 novel destructible options, increasing the total to 18. This facility-based initiative doubled the intervention rotation schedule, allowing a 2-wk Monday through Friday rotation with a different type of DE given each day. With the 200% increase in DE options, staff now have an array of safe, cost-effective supplies to create dynamic interventions and increase animal engagement without adding time to daily husbandry routines.

### PS132 Behavioral Effects of Environmental Enrichment for Laboratory Rabbits

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One of the goals of environmental enrichment is to encourage species-typical behaviors while discouraging abnormal behaviors. Assessing the effectiveness of various enrichment modalities can be a challenging endeavor, particularly for prey species such as rabbits that exhibit freezing responses in the presence of people. We initially constructed an ethogram of laboratory rabbit behaviors. Specifically, we housed New Zealand White rabbits (aged 3-9 mo, ~2-4 kg) in 3 different sized cages and video recorded their behaviors. The 3 housing sizes were our typical rabbit cage (25" x 29.5" x 16"), a recovery cage (28" x 45" x 27"), and a large run (65" x 70" x 96"). Based on analysis of the recordings, ethograms were constructed and behaviors were quantified. The rabbits housed in large runs spent an average of 71.8±12.4% of the time analyzed performing active, exploratory behaviors. By comparison, rabbits housed in the typical rabbit cages spent only 30.7±9.2% on active behaviors. These differences were statistically significant ( $P < 0.05$ ). We hypothesized that rabbits housed in large runs experience a higher degree of well-being than rabbits housed in smaller cages. Unfortunately, space constraints inside research facilities often make it impractical to house rabbits in large runs. Therefore, we decided to explore if enrichment devices could be constructed that would promote the expression of these more active behaviors. We constructed 3 devices: 1) a destructible origami box to stimulate foraging, 2) a wire ball hung high in the cage to encourage rearing, and 3) a bin with substrate to promote digging. All 3 enrichment devices promoted active, exploratory behaviors, paralleling those seen in rabbits housed in large runs (77.1±8.3%, 64.9±8.0%, and 70.0±7.4% of time analyzed, respectively). The origami boxes increased foraging behaviors and the wire balls encouraged rearing behaviors. The bins did not promote digging as expected, but they did encourage other active behaviors. Overall, the addition of enrichment devices or provision of larger caging encouraged a broad spectrum of active, species-typical rabbit behaviors. Our future plans include measuring fecal cortisol levels and scoring animals for ease of handling when given access to various enrichment devices.

### PS133 An Anxious Temperament Predisposes New Zealand White Rabbits to Higher Rates of Intraoperative Breath Holding

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Temperament testing is a behavioral tool used to quantify predispositions in animals. It has a wide range of applications including assessing compatibility for social housing, evaluating personalities, and determining individuals at risk for psychological disorders such as anxiety. Given that anxiety can often impact quality of anesthesia, we sought to determine whether temperament testing (along an anxious to bold continuum) correlated to pre- and intraoperative cardiovascular changes. We examined 31 singly housed, female New Zealand white rabbits scheduled for a brief experimental ophthalmic surgery. One wk prior to surgery, temperaments were assessed using cage-side behavioral observations and a modified human intruder test. Rabbits were induced with ketamine (30 mg/

kg)-dexmedetomidine (40 mcg/kg) i/m, intubated, and maintained on 0.8-1.5% isoflurane. In addition to monitoring standard cardiovascular parameters, we recorded ease of intubation (ranked on a 1-4 scale), and frequency of pre- and intraoperative breath holding. Analysis was limited to the first 15 min of surgical anesthesia to eliminate variables associated with extended surgical time. We found that 10 rabbits possessed an anxious temperament and 21 exhibited boldness. Anxious rabbits performed significantly more breath holding (mean=3.5) as compared to bold ones (mean=0) ( $Z = -2.23, P = 0.02$ ). Temperament had no effect on trends in HR ( $Z = -1.53, P = 0.17$ ) or RR ( $Z = -1.26, P = 0.21$ ), and all rabbits exhibited wide variability in HR (136-254) and RR (0-65). Additionally, there was no correlation between temperament and ease of intubation ( $\rho = 0.10, P = 0.53$ ), age ( $\rho = -0.03, P = 0.89$ ), or duration in single housing ( $\rho = 0.09, P = 0.62$ ). Rabbits were moderately difficult to intubate (mean score=2) regardless of temperament. In sum, our temperament test successfully identified a subpopulation of anxious rabbits that were also prone to intraoperative breath holding. Future research will examine if the addition of a pre-anesthetic anxiolytic will ablate breath holding in anxious rabbits, and investigate further risk factors for the development of anxiety.

### **PS134 Swine Training Program Enhances Animal Welfare and Research Efficiency**

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Swine are commonly used in the biomedical industry to evaluate medical devices. They have similar skin characteristics to humans and large surface area, enabling testing of multiple devices per swine leading to a reduction in animals. Studies require general anesthesia and the ability to safely premedicate, transport, and recover animals efficiently while minimizing stress. Recovery areas require frequent cleaning to minimize risk of infection and meet swine natural behaviors. Animal care staff developed a swine training program to provide environmental enrichment and to avoid stress and time commitment required to successfully complete IACUC approved protocols. Newly arrived swine underwent a 7-d acclimation period during which staff sought to understand individual swine motivators (food reward, human interactions, swine-to-swine interactions). Swine were placed in dedicated play spaces with an experienced 'trainer' swine that readily demonstrated daily routines for handling with animal care staff, injections, physical exams, and transportation in carts for procedures. Desired behaviors were then positively reinforced using individual motivators and clicker training. The training program led to refinements in routine husbandry and research procedures. Swine readily approached the front of their cages for injections and transportation to procedure rooms, reducing animal handling, stress, and hock injuries. Swine were trained to walk back to animal holding rooms post anesthesia to ensure their complete recovery. This refinement decreased postop complications in animals undergoing multiple anesthesia events for study requirements. Researchers and animal staff have observed reduced vocalizations when working in animal rooms, more effective husbandry, and improved animal health leading to quality data and science. The swine training program has contributed to a culture of care as described by outside inspectors and a greater level of employee satisfaction. Vivarium space is more effectively used due to social housing success in swine that initially were aggressive and territorial. Experienced research swine continue to contribute to the research program as 'trainers' building on the principles of the 3Rs.

### **PS135 Targeting to Overshadow Fears and Anxiety in Miniature Swine**

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Targeting refers to social nosing or rooting, which is a natural behavior of miniature swine. We use targeting to replace an unacceptable behavior such as a fear response with an acceptable one that involves a touch in response to the same stimulus. The handler first finds highly prized reinforcers, often a food reward, and gradually teaches the animal that is afraid to interact with people to want to interact with people. In this case, the animal is taught to "touch" a target-stick for a food reward first. It is walked a short way following the target stick and asked to touch the target, then reward. Once the animal can predict a reward is coming when interacting with people, we can condition a miniature swine to cooperate for SOPs such as loading a cart. Gradually over several sessions, the animal will touch closer and closer to the cart. Next, the animal gets in, touches the target, and immediately exits. Eventually, the animal stays in the cart with a target-touch reward interaction coming at about every 3 s. If the animal stays calm and responds to the cue-response reward interaction (CRR) consistently, you can add in distractions such as opening the door or people running around the cart or making distracting noises. Every week, caretakers participate in a performance on cue (POC) tournament with a miniature swine they have trained. Pigs follow targeting cues to navigate through a maze of obstacles leading to a cart loaded with food reward. Fears and anxiety are eliminated because the animal is taught another behavior (touch) that is more enjoyable or pleasant to exhibit in the presence of the stimulus (human interactions, obstacles, and cart) that elicits the abnormal behavior, i.e. a fear response.

### **PS136 Welfare Management of Pigs Undergoing Gastrointestinal Surgery**

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Our institution recently ran its first chronic, translational porcine study. This occurred as a pilot on 3,12-to-18-wk-old gilts that underwent gastrointestinal (GI) surgery, followed by a 2-wk recovery and monitoring period. Various recordings needed to take place over the monitoring period, and cooperation from the pigs was essential. We did not want to give pain relief in an injectable form, and ideally wanted to make use of a transdermal patch. After reviewing literature on analgesic protocols in laboratory pigs (of which very little was available, especially that used transdermal patches) and speaking with experts in human GI surgery, we decided on a multimodal pain relief regime using acetaminophen 30mg/kg PO SID x 5d, meloxicam 0.5 mg/kg PO SID x 5d, a fentanyl transdermal patch 50 mcg/hr/21-24kg x 3d and an incisional block of 2mg/kg bupivacaine. Due to the inherent risk of stomach ulcers in growing pigs and potential stress from the surgery, we also gave omeprazole 20mg PO SID x 7d. A specially tailored post-gastrointestinal feeding regime was instituted and refined over the course of the pilot study. Enrichment and behavioral training were implemented to decrease the chance of stress in the first place. Behavioral training was carried out several times daily, commencing 1 wk prior to the surgery. Behavioral training was accomplished using positive reinforcement techniques. This allowed recordings to occur with minimal restraint and a restraint sling that was purchased did not need to be used. Many enrichment items (both food and non-food items) were trialed, creating a hierarchy of food enrichment and favourite toys for each animal. Training went well, and the pigs learned quickly. Individual pigs expressed personal preferences for certain foods and toys. However, the cohort unanimously enjoyed apples, broccoli, and fruit-flavoured gelatine as food-based enrichment and inflatable balls as toys. The end result was 3 pigs with well-controlled pain who successfully contributed the fields of human and veterinary medicine. Due to the success of the pilot, additional recordings will be able to occur in the main study, further increasing the knowledge gained. Welfare management of the pigs will continue to be refined.