

# Abstracts of Scientific Presentations

## 2023 AALAS National Meeting

### Salt Lake City, Utah

#### Animal Welfare, Training, and 3Rs

##### **P100 Creating a Corporate Compassion Fatigue Awareness Program through a Preventative Medicine Approach**

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Compassion fatigue (CF) is a recognized challenge in the laboratory animal profession, and there is interest in developing tools to respond to employee needs as they surface. Too often, this is a reactive measure, with a focus on managers to identify and develop support mechanisms for employees who may be experiencing aspects of CF. However, CF touches employees at all levels, including managers who may also benefit from support. While laboratory animal people managers should play a critical role in compassion fatigue support and direction, we believe managers should be a complementary component of a CF program rather than the necessary leaders. At AbbVie, we identified an opportunity to approach CF from a more holistic, preventative health perspective, constructed through the guidance of mental health professionals who have expertise in the area of CF and preventative mental health approaches. We began by recruiting leaders from our Employee Assistance Program (EAP) who have impact over the programs at each of our sites, globally. The focus of the enhanced CF program would be holistic – starting with education on the topic at new employee orientation, continuing through ongoing efforts to promote a culture of CF openness, and providing continuity of professional support and resources for staff. Several key aspects of the program were critical to its success and sustainability: 1. Active engagement from our Global EAP teams 2. Active engagement from animal program leadership globally 3. Formal integration of CF program content into new employee orientation 4. Ongoing CF-related programming and 5. Personalized interactions between each site's EAP representative and our animal program and scientific staff. Roll-out of the new Global Compassion Fatigue program occurred at the end of 2022. Receptivity to the new approach has been positive, and a Global CF Working Group has formed to carry concept into practice. Perhaps most appreciated is the corporate-level recognition of the issue and unity of global support for our teams. Not only do animal care and technical staff feel more visible and supported, but a palpable weight has also been lifted off our managers' shoulders.

##### **P101 Learning from Reported Critical Incidents to Develop a Culture of Care for Animal and Human Wellbeing**

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A culture of care includes the focus on the well-being of our employees in animal husbandry and animal research, as well as the benefits of mutual attentiveness and appreciation. To ensure a motivated team structure, it is equally important to give each employee the opportunity and space to deal openly with unexpected incidents or even mistakes in dealing with laboratory animals. In a facility with a well-established culture of care, it is possible to learn from each other and support mutual learning in this way.

Ultimately, this is where the benefits lie - on the one hand, how to ensure the well-being of laboratory animals and, on the other hand, how to achieve scientific progress. The authors will expand on the successful establishment of the web-based database CIRS-LAS, in which incidents in the entire field of laboratory animal science can be reported. We provide an insight into more than 50 specific incident reports that have been published in the database and evaluate the cases in relation to possible risky areas of laboratory animal science. Searching our online database, also possible via mobile application, offers easily accessible and effective learning opportunities to avoid possible risks in animal research. The database is an important resource for staying in touch with the latest 3R prospects and learning from other technical as well as scientific staff who are committed to the culture of care in their facilities. Join us and become part of the online resource used by more than 300 people worldwide to learn about the opportunities to implement 3R measures in the sense of a lived culture of care.

##### **P102 Implementation of online training for the promotion of well-being in laboratory animal personnel**

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There is a continued need for the awareness and support of improving laboratory animal personnel well-being but institutions can find it difficult to consistently dedicate time and resources to providing this training. Previously, Stanford University's Veterinary Service Center (VSC), provided a once yearly in-person training on compassion fatigue (CF) and burnout (BO) for staff, trainees, and faculty. Disadvantages to a once yearly training include variable availability of VSC personnel to attend the training and the training did not include research personnel outside of the VSC. Furthermore, the onset of the COVID-19 pandemic impeded the ability to offer this in-person training in a large group setting. To address these challenges, an online training discussing CF and BO was developed and distributed to VSC staff and research personnel through institutional listservs. Upon completion of the training, participants were asked to complete an anonymous survey. The Likert scale survey addressed participants preferences for online versus in-person training, efficacy of online training, and evaluation of generational differences in responses. One hundred forty-one individuals participated in the online training and survey. Results indicated that participants had a significantly increased awareness of the signs of CF and BO (93% strongly agree/agree [CF]; 88% strongly agree/agree [BO]) as well as the resources available to ameliorate feelings of CF and BO (87% strongly agree/agree). Participants indicated that they preferred an online training module versus in-person training (80% online preference), and in addition, no generational differences between preference were found ( $P = 0.2942$ ). Lastly, the survey provided opportunity for participant suggestions that can now be incorporated into next steps for the wellness program. This online training course remains readily available to all VSC and research personnel to review and can now be offered to newly hired personnel at Stanford University.

##### **P103 The Lesser Known Benefits of Laboratory Animal Enrichment Programs**

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The benefits of enrichment for animals are well documented, however the benefits to humans can often be overlooked. Compassion fatigue has become a more common topic of conversation in recent years. Compassion fatigue is a state of mental and physical distress that occurs when caregivers are chronically exposed to the suffering of others and can worsen over time if the underlying cause is not addressed. At the University of Chicago, we have ensured that we include the entire veterinary, husbandry, and operations staff in our large animal enrichment program to have contact with animals in an entirely positive setting. This has the added benefit of increasing the quality of enrichment for the animals. Thus, we consider our methods an investment in both human and animal welfare. In our facility, we have established standards, such as new toys on cage change day, and a rotation of twice daily documented treat enrichment for non-human primates. We additionally have a rotating monthly special enrichment. This special enrichment time extends the opportunity for all groups to become involved and includes growing and distributing wheatgrass or making papier mâché balloons filled with forage. Because these special enrichments require more time and resources, we invite people from other work groups to make and distribute these special treats. This serves to personalize the enrichment, build connections between working groups and is a great way to improve the daily lives of the animals in interesting ways. Animals at their happiest and healthiest states produce the highest quality data. Participants in the special enrichment have reported that interacting directly with the animals boosts their mood, and usually seek out opportunities to participate again. We have found that the more we include people, the more they want to participate, which can boost morale and retain staff as well.

**P104 How are Apples and Oranges weighed in practice? On the incoherent implementation of the Harm-Benefit-Analysis as a requirement of the EU Directive 2010/63/EU.**

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The use of animals for scientific purposes is regulated in all EU member states (MS) by specific laws. The EU issued the Directive 2010/63/EU (Directive) as a comprehensive legal document, which covers the use of research animals within the EU and it represents the basis for national legislation by EU members. MS were required to transpose the content of the Directive into national legislation and to implement it by January 1, 2013. The intended goal of the Directive is to harmonize laws among MS for the use of animals in research. However, the Directive provides vague explanations for certain requirements, and therefore creates room for interpretation by MS, which consequently leads to varying degrees of harmonization. In this study, we have reviewed the implementation of the harm-benefit-analysis (HBA) among MS, which is explicitly mentioned in the European Directive as a requirement of the project evaluation. The HBA plays a role in the review process of research proposals using animals and its function is to weigh the expected harm to animals against the anticipated benefits and it should ultimately facilitate the decision-making whether the use of animals for the scientific purpose is justified. We are going to present the results of a cross-national review that has shown there are discrepancies among MS in regard to the HBA pertaining to the interpretation of the EU Directive, the transposition into national laws, and the implementation of an analysis in practical terms during the project evaluation process, and these differences ultimately make the expected harmonization in the EU challenging.

**P105 Tail Dosing and Bleeding Revisited: Toppling 35 Years of Dogma**

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It is common practice to use cannulated rats for pharmacokinetic (PK) in-life studies as it yields high-quality PK parameter estimation. While offering many benefits, cannulation requires surgery, post-surgical care, and cannula maintenance. As an alternative approach, the strategy of dosing and bleeding rats via the tail vein in a single experiment is technically feasible and theoretically offers many benefits. Unfortunately, however, as reported by F Tse et al. in 1984, parallel tail dosing and bleeding is scientifically flawed and yields inaccurate estimation of PK parameters following intravenous administration. The underlying causality of poor data quality has not been addressed in over 35 years. To overcome the technical flaws associated with parallel tail dosing and bleeding, we have developed a Tail- Dose-Bleed (TDB) method as a substitute for use of cannulated rats. To demonstrate the proof of principle of TDB, the technique is compared in both stand alone and animal crossover studies employing conventional femoral/jugular dosing and bleeding. Reference compounds dexamethasone, imipramine, propranolol and tolbutamide were used to compare PK parameters of the TDB technique with that of conventional IV dosing and bleeding. A matched pair t-test assessment confirmed that parameters of clearance, volume of distribution and  $t_{1/2}$  results were comparable ( $p = <0.05$ ) for all compounds tested with the exception of the  $t_{1/2}$  result for imipramine. Using this newly described TDB procedure, we demonstrate the ability to overcome documented data quality issues when dosing and bleeding via the tail. The TDB technique has numerous operational advantages, but most importantly, addresses key animal welfare concerns relevant to institutional animal care and use committees (IACUC). The notable advantage here is reduced animal stress and discomfort by eliminating the need for surgery and recovery. And by consequence, allows for animals to be group housed and re-used without concern for loss of cannula patency. The tail dose and bleed method is simple and appears readily transferable to other laboratories.

**P106 Cisterna Magna Ports in Rhesus Monkeys Then and Now**

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The Cisterna Magna Ported (CMP) rhesus monkey model was developed over 20 years ago as a means for chronic collection of "pristine" cerebrospinal fluid (CSF) samples. Major advantages of this model are repeatability and reliability, allowing for an overall reduction in the number animals needed for chronic CSF collection. Over the years, several refinements have been implemented to improve the success of this model, benefiting both the research use and overall welfare of the animals. Between 2004 and 2022, 249 rhesus monkeys had CMP surgery, with 187 surgeries being successful (75% success rate) for long term collection of CSF. Refinements to the catheter design which have been beneficial to the longevity of the model included decreasing the length of catheter that is inserted into the cisterna magna, utilization of a fixed disc and collar placement on the catheter and modification of the catheter diameter have proven to be successful. Reduction in the frequency of port maintenance from 3x/week to 1x/month, for established models, has reduced the number of times a port needs to be accessed thus reducing the chance of introducing contamination and need for repair surgeries to replace deteriorating ports. Another major milestone in the success of this model is the ability to successfully treat bacterial infected ports intra-CMP with antibiotics. Prior to 2010,

ports and catheters were removed from 66 infected models followed by systemic antibiotic treatment and then a CSF tap to confirm there was no infection allowing for animals to be re-implanted. After 2010, 51 infected models were treated intra-CMP with antibiotics and in 80% of the cases the infection was cleared up thus avoiding surgeries for catheter removal and re-implantation. Another refinement to the model was a multimodal analgesic approach to pain management instituted post-surgery to help animals recover more quickly. Finally, paired housing, environmental enrichment, providing foraging material and rotation of fruit and vegetables have also improved the well-being of the animals. In summary, numerous refinements have been applied to the CMP model for chronic collection of CSF in rhesus monkey over the past 20 years, thereby utilizing the 3Rs principles to optimize this valuable research resource.

#### **P107 Where the Honeysuckle Grows...Utilizing Browse in Nonhuman Primate Enrichment Programs**

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There is often a desire among animal care professionals to increase the time NHPs spend on engaging with their enrichment, environments and each other in species appropriate fashions. In introducing and developing a browse program, utilizing non-toxic plant trimmings at an NHP site there has been a documented increase in both engagement and in the behavior repertoire of the NHPs compared to what was typically observed onsite. With an addition of a browse in an enrichment program there are elements to consider such as the sourcing of non-toxic, safe and effective browse, limiting impacts on husbandry routines and drain sensitive settings, and ensuring the program can also be cost effective. In order to ensure the browse program would be set up for success there needed to be a plan in identifying browse sources, identifying animals who may benefit most from using browse and approaches in working alongside husbandry teams trying something new. In addition, program success also depended on developing documentation templates to gather information on NHP behavior observations, the time husbandry spent cleaning up and handing out browse and thoughtful approaches of how to offer browse in drain sensitive settings. Documented observations determined engagement times with browse surpassed engagement times with approved toys and devices by an average of 30 minutes for indoor cage housed NHPs. It was also documented that outdoor housing environments often saw robust observations of species-specific behaviors when offered browse and indirect indicators of hours-long engagement. Impacts that handing out and cleaning up browse had on husbandry efficiency were low, with an average time of 5 minutes for each, with thoughtful drain consideration in browse preparation on some settings going a long way in no drain issues reported. Efficiency for handing out and cleaning up browse was even more evident when comparing time spent preparing, setting up and cleaning toys or devices. While browse cost was determined to be lower than average costs for some toys, it should be noted that browse cannot be re-used. Overall, introducing browse to the NHP enrichment program exceeded expectations and supported a mindset of considering the 24/7 NHP experience while under human care.

#### **P108 Colla-boar-ation: Teaching Chronic Surgical Pigs to Participate in Their Own Care With Use of PRT**

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Post-surgical care of large animals can provide many challenges in the laboratory setting. The type of surgical procedure conducted, and its severity, directly affects an animal's physical needs. Undergoing surgery can incite higher stress levels in the animal. This can be increased further if a technician must perform more intimate care

while the animals are recovering, such as taking vitals or administering medication. Elevated stress can cause the animals to avoid interacting with their caretakers. Having a calm animal that voluntarily participates in its own care allows for accurate data collection, ease of care, and ultimately improved animal welfare. Our facility pursued developing a positive reinforcement training (PRT) program for crossbred swine to prepare the animals for their post-operative care. To aide technicians, detailed training documents were created. The training plan contained small approximations to reach the training goal. Session logs helped visualize progress for each individual animal. Staff were trained on PRT basics, and a high value reinforcer (diluted apple juice) was chosen. Animal training ranged from 6 to 17 days, and the training plan began with positive physical interaction and ended with animals standing still while vitals were collected. By the end of the training plan, the animals were voluntarily participating in all procedures, and receiving medications without physical restraint. In previous years, data was unable to be collected due to the animals being uncooperative. Technicians also noticed that these animals were willing to walk into transport cages for weekly weighing. By providing basic animal training, we were able to reduce stress and build trust between animals and staff.

#### **P109 Reducing the use of live animals in training by building cost efficient and realistic feeling venipuncture training aids.**

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The National Bio and Agro-Defense Facility (NBAF) is a state-of-the-art research facility whose mission is to protect the United States against transboundary, emerging, and zoonotic animal diseases that threaten our food supply, agricultural economy, and public health. While working in ABSL-3, ABSL-3AG, ABSL-4, and ABSL-4AG, it is extremely important that technicians are well trained, confident, and safe during bio-sampling procedures. The Animal Resource Unit (ARU) manages all aspects of training for staff who will be involved in assisting principal investigators (PI) in their research and blood collection on live animals. ARU's training plan intentionally includes various homemade and commercially available simulators at all training levels. This solution decreases risk of exposure and injury while allowing initial hands-on experience for learners in a safe environment. There are no commercially available venipuncture training aids that are both appropriate and cost-effective for NBAF learners, nor any that represent the complexity of species that NBAF will include in our program (mice to livestock). We are not aware of home-made simulators that adequately replicate the tactile sensation (i.e. "feel") of a live animal blood vessel. Drawing on almost 30 years of experience in veterinary venipuncture and staff training, we were able to develop a model that can be easily made in-house, using commonly available products, and can replicate the feel of blood vessels ranging in size from 5mm to 11mm. While the use of live animals during phlebotomy practice will never be replaced completely, the use of a realistic feeling training aid will reduce the number of animals needing to be used, while developing and refining the technical skills of staff.

#### **P110 Moving up: Evaluation of space orientation on the progression of cynomolgus macaques through a training protocol**

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Nonhuman primate (NHP) species commonly housed in research facilities, including cynomolgus macaques, are semi- to primarily arboreal species that rely on the use of species-typical behaviors such as vertical flight to alleviate stress in captive settings. Standard housing configurations for NHPs have historically provided limited access to vertical space. Providing access to opportunities to express

species-typical behaviors is a refinement to NHP housing. We hypothesized that home cage space oriented vertically vs. the same amount of space but oriented horizontally would affect the progression of NHP training after arrival to a new facility. Cynomolgus macaques of both sexes (24 cages; 2/cage) were randomly assigned to stainless-steel quad caging configured to house either 2 horizontal or 2 vertical pairs. NHPs progressed at their own pace through the Monkey and Technician Training (MATT) stages over 4 weeks of acclimation. MATT stages include 1) NHPs shift into new cage 2) Step 1 + desensitize to a falseback 3) Step 1-2 + technician touches NHP's arms. NHPs progressed to the next stage once they scored a 3. The three stages, in addition to human interaction, were scored 2-3 times per week on a 5-point scale based on cooperation (1=0% and 5=75-100% cooperation). Data were analyzed as repeated measures General Linear Models. Over the course of the study human interaction generally increased ( $F_{1,175}=108.1; P<0.001$ ) but vertically housed NHPs had higher scores than those housed horizontally ( $F_{1,175}=30.2; P<0.001$ ). When asked to shift (stage 1), NHPs in horizontal cages had higher scores than those housed vertically ( $F_{1,388}=142.8; P<0.001$ ). In stage 2 (falseback), there were no differences in scores due to space orientation ( $F_{1,388}=0.002; P=0.49$ ). When touching the NHPs' arms, the effect of orientation differed based on where cages were located in the room. Vertical cages near the door had higher scores than horizontal ones. However, when in the middle of the room, orientation treatments were similar. In conclusion, vertical space and cage location impact human interaction and training. Ultimately, vertical space is ethologically relevant and may improve welfare but specific management strategies should be put in place to work with NHPs during training.

#### **P111 Fostering Culture of Care Through Continuous Learning and Improvement**

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AstraZeneca's In Vivo Learning Log was developed as part of a global Culture of Care whereby the in vivo community is enabled and empowered to actively participate in continuous learning and improvement without fear of repercussion. Rather than focusing on who was involved, the Log utilizes Root Cause Analysis (RCA) to understand what happened, why it happened, and how to prevent recurrence. The risk of future occurrences is reduced through understanding the root cause and development of appropriate Corrective And Preventative Actions (CAPA). In addition, by acknowledging that human error can never be fully eliminated we create an environment where employees can identify events or near misses openly and honestly. To make a submission to the Log, an individual scans a QR code or clicks a link to access a template. Once completed, information is uploaded to a database monitored by AstraZeneca's Regulatory Managers. Automated email notifications are immediately distributed to the group and IACUC, prompting timely review. Additionally, the list is accessible to all members of the global in vivo community enabling full transparency and awareness. Submissions to the Log are categorized as events or near misses and are evaluated based on the level of impact to animal welfare and overall scope. Those of medium impact or higher, or of a repetitive nature are progressed to RCA to identify the root cause(s) and develop CAPA. The group that is gathered for input into the RCA is purposely selected to ensure a diversity of roles and experiences. Outcomes are shared with the in vivo community through electronic Learning Cards. In 2022, the first year of implementation, 230 submissions were received. Of those, approximately 60% were identified as events and 40% as near misses. Regardless of categorization, 60% of submissions progressed to RCA. Implementation of and learnings from the Log have benefited our animals and our people. Indicators of success include positive

feedback from RCA participants and in vivo community members who view the Learning Cards. In addition, 87% of submissions are associated with contact information. Because anonymity is the default setting, inclusion of contact details suggests employees feel comfortable being associated with event information.

#### **P112 Training Validation and Staff Incentivization, A Plan to Keep Your Staff Happy and on Track**

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Training validation is important to ensure the effectiveness of a training program, confirming tasks are performed consistently, offering opportunities for coaching and providing opportunities to reward excellent work. Pre-pandemic, the validation process focused on task performance and remediation. The process was revised to delineate training responsibilities and provide positive coaching experiences. Previously, the onboarding process for employees was poorly defined, which resulted in chaotic first days. A checklist was created that named responsibilities for each part of the process. It laid out each step, including admins (policy, uniform, and identity badge), supervisors (locker and facility orientation) and trainers (SOP and specific tasks). A timeline was created of integral check-ins and expectations for the first year. After the first year, employees moved into the yearly validation program. A monthly walkthrough process was created to give supervisors feedback on the status of work areas. That system provided an overview of the area without allocation of responsibility to a single person. Required training is assigned routinely by the institution and must be completed by the individual. Teambuilding exercises were created and distributed each quarter to foster a sense of community. Training validation is often seen by staff as a punitive process, thus, it was important to reframe validation into something positive. The incentivization program combined the monthly walkthroughs and completion of assigned training and team building exercises. Any zone that completed all facets earned free lunch. The goal was to highlight good performance, promote teamwork, and improve job satisfaction. A well-laid-out on-boarding and follow-up process is critical to ensure employees get the support they need to be successful. The walkthroughs have improved team compliance by 18% across all facilities with an increase of 25% for the largest rodent only facility. Staff receive regular feedback and can identify issues before they are seen as a problem. While the incentivization program is in the early stages of implementation, several zones have planned team building activities outside the program and are working together to ensure tasks and training are completed on time.

#### **P113 Thinking Inside the Box: Restraint chair enhancements utilized in transitioning toxicology studies from open to closed restraint chairs.**

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Refinement within animal research methodology and practices is integral to strengthening animal welfare. In an effort to improve both the animal's experience and colleague safety, research institutions globally are prioritizing enhancements with respect to nonhuman primate (NHP) restraint mechanisms. A 2017 survey revealed that pharmaceutical research facilities utilize open restraint chairs despite data showing that closed restraint chairs improve colleague safety and increase cooperative handling opportunities. Open restraint chairs are currently employed at our facility due to animal accessibility and the familiarity of traditional sample collection. To transition study procedures from an open to closed restraint chair, our goal was to increase accessibility and ease in a closed restraint chair for various procedures including blood collection, oral gavage, and regular maintenance of vascular access ports (VAP). Many

commercially available closed restraint chairs are intended for use with larger nonhuman primate species, such as rhesus macaques (*Macaca mulatta*). Therefore, adjustments were made to improve limb access in the relatively smaller cynomolgus species (*Macaca fascicularis*) typically utilized in a toxicology research setting. Through continuous modifications, we developed a closed restraint chair in two different sizes for use in both adult and subadult cynomolgus macaques. These enhancements have allowed our facility to complete in vitro blood sample requests, as well as studies utilizing VAP sample collection and dosing across multiple time points. Furthermore, modifications of guided handling into the closed restraint chair were made to transition animals and colleagues from two-pole handling towards a direct transfer from the animal's home enclosure into the chair. This handling technique lends itself as a transitional step to a fully cooperative handling approach in the future. The modified closed restraint chair provides an operational refinement from open restraint chairs towards safer and cooperative closed restraint chairs.

#### **P114 Surveying IACUC Members on Ethical Deliberation and the Label 'Ethics Committee'**

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Recent discussion within the laboratory animal science community has addressed the role of ethics in IACUC oversight and how best to acknowledge that role. There are clear opportunities for ethics to impact oversight, as reflected in the requirements for the IACUC to evaluate 'harm-benefit' relationships as well as refinement, reduction, and replacement. The perspectives of the IACUC members themselves are important, so this study was designed to survey current and former IACUC members for insights into the contribution of ethical considerations to their deliberative processes. The survey queried current and former IACUC members regarding whether IACUCs perform ethical deliberation and whether they would be comfortable labeling the IACUC as an "ethics committee." Following oversight board approval, a survey invitation was sent to members of AALAS Community Exchange and the NABR IACUC-Admin communities in March 2023. The majority of those surveyed affirmed that the work of an IACUC involves ethical deliberation (178/202; 88%) and, to a lesser extent, indicated support for the statement "The IACUC is an ethics committee" (112/198; 56%). Subgroup means were compared based on the following dimensions: IACUC role, age, race, ethnicity, gender, religion, and political identity. While veterinarians (n=68) affirmed the role of ethics in their considerations on the IACUC, more were hesitant to explicitly support designating the IACUC an "ethics committee" compared with those serving in other roles on the committee. These data suggest that most IACUC members believe that their deliberations on the committee involve ethics, and favor labeling the committee as an ethics committee. Questions of how to support the IACUC's consideration of ethics as well as examination of reasons behind hesitation about the label are presented.

#### **P115 Feasibility of Awake Imaging Utilizing a Fluoroscopy Transport Cart**

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Sedated images are routinely obtained in our facility after a medical device has been implanted. Taking fluoroscopic images requires patients to be placed on a radiolucent table and to do this safely while collecting high quality images, patients are fully sedated. Many times, these anesthetized events are not accompanied by any data collection that would require the patient to be sedated. To decrease the number of sedation events, cost of drugs, animal stress, and technician time, we developed a prototype for a Fluoroscopy

Transport Cart (FTC) to obtain fluoroscopic images while the animal is awake. This development also allows us to capture natural movements such as swallowing, GI motility and diagnostic imaging in emergent cases where our animals aren't clinically safe to sedate. Without the FTC, most facilities have no options for image collection while awake. This leaves a gap in our standard of care for our patients. The FTC needed to be mobile, easily cleanable, accessible for the animals, fit within our mobile C-Arms, and radiolucent in both AP and lateral views. The FTC was designed in a way to fit a large hound, pig (Yorkshire and Yucatan), or a sheep. Imaging procedures using the FTC begin by bringing it to the animal housing, walking the animal into the FTC, while optionally using the accompanying step to decrease the height difference. The cart and animal will then be brought to a procedure room with a mobile c-arm and images can be obtained. The cart can then be brought back to animal housing where the animal can return.

#### **P116 Evaluation of a Novel Anesthesia Apparatus to Perform Breath Holding in Ferrets During Computed Tomography and Magnetic Resonance Imaging**

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Respiratory motion can be an obstacle to obtaining images of acceptable quality during computed tomography (CT) and magnetic resonance (MR) imaging. Artifacts resulting from respiratory motion may be mitigated by respiratory gating or breath holding. Respiratory gating requires specialized equipment/software and typically requires an increase in the time to complete a scan, while breath holding can be performed with equipment that is often already in place in an animal research facility or readily available. A study employing MR imaging in ferrets was designed with a combination of respiratory-gated and breath-hold sequences. The goal of the breath holding was to eliminate respiratory motion in sequences used for quantitative measurements. A breath-holding apparatus for use on intubated ferrets was designed and tested. Specifically, the anesthesia breathing circuit originally included inspiratory tubing connecting a ventilator to one branch of a Y piece, and expiratory tubing connecting the opposite branch of the Y piece to the ventilator. The modification included a four-way fitting on the inspiratory tubing, which allowed connection of a pressure gauge on one side and a breathing bag assembly on the opposite side. Tubing clamps were placed on the inspiratory and expiratory tubes on the ventilator side of the four-way fitting. With this configuration, both lines of the anesthesia circuit could be closed on the ventilator side while simultaneously pausing the ventilator, allowing administration of a positive-pressure breath hold to the anesthetized ferret. The gauge permitted breath holds to be measured and thus limited. Breath holds obtained in anesthetized ferrets using this apparatus were shown to be safe and effective at reducing respiratory-motion artifacts, resulting in reduced scanning and anesthesia time for MR imaging. Higher image quality with improved resolution on CT scans can enhance diagnostic and quantitative evaluation.

#### **P117 Sheep Cuddlers Program Contributes to Animal Welfare and Compassion Satisfaction**

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Sheep are a well-published preclinical research animal model for development and support of medical devices for FDA (Food and Drug Administration) submission. Their herd and predator/prey behavior must be considered in the laboratory animal environment as stress may cause variability in the scientific data. Caring for research animals may also lead to compassion fatigue among animal care teams and investigators. Consideration of the environment starts

with understanding how research animals are housed at the approved vendor. Sheep were hand raised in an indoor and outdoor environment to help develop well socialized sheep for biomedical research. Feed was placed outdoors distant from their home pen area to promote exercise. Once sheep were received in the biomedical research setting, the AV identified a dedicated team of sheep cuddlers to continue to provide routine socialization. The program was developed in less than a month, starting with sheep handling and behavior training based upon direct interaction with vendor veterinarians and publications. Cuddling included sitting at the sheep level, tactile sternal rubbing, hand feeding and exercise in a dedicated enrichment hallway with non-slip flooring. One of 3 group housed sheep jumped the animal holding room internal fence when left alone during a procedure wait time, leading to animal welfare concerns. The presence of a dedicated sheep cuddler assigned to each study day led to reduction in observed escape behaviors, stress and increased sheep seeking interactions with animal care staff. The sheep also appeared healthy with minimal reported health concerns. The sheep cuddlers program provided effective sheep acclimation and training to anticipated routines such as premedication, movement to procedure rooms, and minimal handling for daily health exams. Second, the husbandry team could readily move animals outside the animal holding room during daily cleaning as sheep were easily approachable and followed the human team. Last, the sheep cuddler team felt a sense of purpose and contribution to animal welfare, resulting in employee wellness and decreased compassion fatigue.

#### **P118 Ferret Cranial Implant Management**

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Ferrets are a valuable model for auditory behavioral studies, and custom cranial implants allow for measurement of single-neuron activity during auditory processing. Chronic cranial implants are associated with complications including bacterial contamination, infection, and chronic inflammation. These problems can lead to implant failure, lost research time, animal welfare concerns, and early removal from study. To decrease cranial implant-related complications in ferrets at our facility, refinements to post-surgical implant care and routine management procedures were developed. Post-surgically, implants were managed with daily bandage changes, sparing use of gentle saline lavage, and application of an antimicrobial hydrogel to the wound margin. After animals had healed from surgery, routine implant management focused on 4 pillars: hair removal, lavage of the implant margin, cleaning of the implant wall, and topical antimicrobial hydrogel application. A depilated margin maintained around the implant helped reduce the amount of exudate adhered to the skin-implant margin at bandage removal and amount of exudate produced between bandage changes. We instituted two methods of lavage: a low-volume maintenance lavage, and a high-volume lavage performed weekly. The use of a lower volume maintenance lavage and positive reinforcement during cleaning reduced animal stress associated with lavage of the implant margin. Routine cleaning of the implant wall prevented the build-up of organic material that otherwise may act as nidus for infection. An antimicrobial hydrogel was applied to the skin-implant margin prior to bandage placement. The active ingredient, hypochlorous acid, provides antimicrobial activity without the potential for development of antibiotic resistance. Additionally, hydrogels have been shown to have several beneficial effects in managing chronic skin wounds. In addition to improved cranial implant care, these techniques allowed for management of an animal with chronic multi-bacterial colonization without the use of antibiotics, representing an improvement in antibiotic stewardship.

#### **P119 Using a Culture of Care to Accelerate a 4Rs Approach in Biomedical Research**

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The 3Rs of Replacement, Reduction and Refinement were developed over six decades ago as an ethical framework to incorporate social concerns about animals into the design of experimental work. From the very outset, the 3Rs sought to weave together good science, good care, and socially acceptable practices of animal research. Adding a 4<sup>th</sup> R of Responsibility is needed to hasten adaptation to changing expectations for laboratory animal care and use and to emphasize the active and personal responsibility of everyone working in biomedical research to engage with the 3Rs. Embracing a 4Rs approach to accelerate change in biomedical research is only possible when there is a strong Culture of Care foundation. This concept reflects that research animals and those working with them are treated in caring ways that are ultimately important to the scientific endeavor. Care, in this sense, is built on four basic dimensions of attentiveness (i.e., paying attention to animals and to employees and the needs of both), competence (i.e., being skilled when technical procedures must be performed to minimize adverse stress on animals), responsiveness (i.e., responding to changes in animals on study and employees working with them to better support their needs), and responsibility (one's duty and accountability for excellence in all aspects of daily work). We describe an initiative managed by a newly created Office of Responsible Animal Use, which has developed a series of 4Rs global workstreams created to accelerate 3Rs changes, including Scientific Integrity, Alternative Technologies, Study Design, Historical and Digital Controls, Alternative Large Animal Species, and Animal Housing Standards.

#### **P120 Establishing an Innovative Approach for Documenting Semi-Annual Facility Inspections Deficiencies and Conducting IACUC Member Inspection Training**

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IACUCs are required to conduct Semi-Annual Facility inspections for animal housing and surgery areas. How can IACUCs establish a standardized process for conducting inspections, identifying deficiencies and requiring consistent corrective action plans? The IACUC office at UT Southwestern set out to answer these questions and developed standardized training for newly on-boarded IACUC members and created a robust catalog of commonly found deficiencies to establish consistent corrective action requirements and timelines. Firstly, when a new IACUC member is on-boarded, they undergo comprehensive on-boarding training, including how to conduct Semi-Annual Facility Inspections. A unique aspect of the training is educating inspectors on how to behave and interact with laboratory representatives during inspections. The IACUC office has established standard questions that are asked of laboratory representatives during each inspection, which helps to build consistency on how the IACUC evaluates each space. The IACUC office has also standardized the corrective action requirements and timelines for resolving commonly found deficiencies. To establish these criteria, the IACUC office reviewed the past several years of inspection reports to identify the top deficiencies. Then, standardized language was developed and entered into a database to catalog these deficiencies, the corrective actions required, and timelines for correction. These acceptable resolutions and associated timelines were also codified in an IACUC SOP. This streamlined approach has helped to build consistency in addressing deficiencies, as well as reduce administration burden by having easily accessible template language and deadlines to draw from.

#### **P121 Externships to Employment - Forming Collaboration with Local Veterinary Technology Program to Foster Career Opportunities in Biomedical Research**

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Many institutions employ credentialed veterinary technicians, and it is evident that there is a decline in the number of trained personnel to keep up with programmatic growth and succession. Reviewing our institution's succession plans, taking special consideration to the biomedical research specialty, it is likely that the opportunities will exceed the number of trained veterinary technicians. Key questions about developing future veterinary technicians in biomedical research include: Where can we recruit? How do we educate in our specialty? How do we promote the positive impact that veterinary technicians make in our field? Veterinary Technology programs may be the answer. We sought collaboration with a local community college that administers an AVMA accredited Veterinary Technology Program. This collaboration is novel as it embraces the role of veterinary technicians in biomedical research specifically. Polling experiences of veterinary technicians employed by our institution who completed the same local program, determined that careers in this specialty were minimally incorporated in the curriculum and not credited with the potential they held. Goals were to promote transparency regarding the animals' role in research and through education and experience, recruit more veterinary technicians to the specialty. Our institutions developed a biomedical externship program. We utilized the syllabus from the community college and modified by incorporating content highlighting regulations, occupational health and safety, handling, restraint, and experimental techniques of common research animal species. We merged the course requirements with the day-to-day work performed by biomedical research staff. Throughout the externship, students worked with our veterinary and husbandry teams to experience what a career as a biomedical veterinary technician would consist of. We hosted two students, feedback from both students indicated that their perception of biomedical research was positively changed and that they would likely pursue a career in research. The collaboration shows promise in providing a pathway towards recruitment of veterinary technicians in the field of biomedical research.

#### **P122 Optimizing the impact of habituation for Masked Inhalation Dosing through Positive Reinforcement Training of Laboratory Gottingen Minipigs**

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The use and application of swine as biomedical models has increased significantly over the past 20 years. With consistent advances in research and the decrease in availability of primates and canines, swine have emerged as models for cardiovascular, respiratory, integumentary, digestive and urinary systems. Swine are known to be strong and extremely intelligent with capabilities for memory retention; positive and negative experiences can have a strong impact on their socialization and habituation progress. Working to acclimate and habituate animals minimizes stress and difficulties for technical staff and animals. This allows for ease of restraint and handling for study procedures and animal care, respectively. Lack of cooperation can put both parties at risk of injury and induce stress. Positive reinforcement training (PRT) desensitizes animals to typical stressors such as restraint, moving to a location, or placing a harness or mask for study design and incorporates techniques to reduce the amount of handling needed. A simple, progressive program was developed to train Gottingen minipigs using operant conditioning training, beginning the day after arrival into the facility. This training plan uses PRT to encourage favorable behaviors for a masked inhalation dosing study. The program advances from basic acclimation to the facility with positive human contact, to a series of clicker and target trainings that increase in complexity as individual training goals are met, and finally training towards desirable dosing behavior and study related tasks. The first trial (N=3) was conducted over eight weeks with two sessions for five minutes per day. Within two weeks,

the pigs associated a click with searching for a treat in varying locations. Clicks were progressively replaced with verbal cues and the training transitioned to target, mobile target and fixed target at a desired location. This program allowed for the pigs to be trained to walk into a transport cart, stand and hold position on a scale and to be comfortable wearing an inhalation dosing mask. At the end of this program, the data has shown there is a measurable and significant impact on cooperation and socialization of the minipigs with the use of PRT to promote direct behaviors.

#### **P123 Ferret enrichment toy type preference associated with increased positive species specific behaviors**

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To provide research ferrets with more opportunities to promote species specific behaviors, a short six-week observation study was performed to see which forms of enrichment could be associated with the most positive behavior interactions. We did not remove any enrichment during this study, but rather added in different hanging toys to gauge the interest in manipulable toys outside of a sleeping tunnel/ball and cage-mates. Three different hanging toys were purchased to decide which, if any, were the favorite for manipulation. Twelve pair-housed female Fitch ferrets were observed for interactions with pre-existing enrichment (social-housing and sleeping toys) and new hanging toys. Each cage was observed for 5 minutes, twice a day, three times a week. All cages had matching hanging toys, which were changed out every by animal staff at each cage change of every two weeks. Interactive observations recorded were "locomotory play" (to include sleeping toy and cage mate interactions) with cage-mates (specific behaviors noted), locomotory play with the sleeping toy, "resting" (sleep or otherwise at rest), and "hanging toy" (bell, gem, or jingle). Reliability checks were performed prior to study start for similarity in reporting observations (or lack thereof), with an 89% similarity in observations. Graphpad's Prism software and Excel were used to analyze data. Data Basic Excel analysis showed that ferrets spent less than half of observations sleeping or in a state of rest (41.86% of total observations), while locomotory play made up slightly over half of total observations (51.58%) and hanging toy interactions were measured at 6.53% of the total observations noted. Measured behavioral interactions showed that social housing was a largely positive impact to the daily welfare of ferrets. For the three hanging toys, the gem was the most interacted with, the bell was second place, and the triangle jingle came in last. According to Prism's calculations, Post-hoc Tukey tests showed that the gem (33.3%) and the bell (25%) toys were preferred over the Jingle toy (2.8%,  $p < 0.05$ ). Both methods of statistical analysis results found that ferrets enjoy being able to interact with their cage-mates and sleeping toys, showing little extra attention to hanging toys.

#### **P124 Click It To Trick It: Sheep Training and its Positive Impact on Long Term Residency**

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Animal training aims to condition an animal to cooperate with its trainer to achieve positive behavioral modifications. Clicker training is a well-known technique for animals that are believed to be intelligent, and food motivated. Sheep are mistakenly considered a less intelligent species, resulting in the impression that training is difficult. Their herd mentality and their natural role as a prey species makes them naturally skittish, potentially making routine procedures stressful for all involved. At our facility, a resident blood donor sheep assists with the need of blood transfusions when critically needed. This donor (currently "Dewy") becomes a long-term resident until retirement. This long-term residency offered the opportunity to explore clicker training in this species. The method was to balance

Dewy, a 2-year-old, wether Dorset/Suffolk mix's motivations with his skittish nature, especially when outside the herd. Enticing Dewy to approach to be touched/petted, then given a food reward completed the positive reinforcement cycle. Once trust was established, (n=14 days) the clicker stimulus was added to que the reward (n=11 days). Next, a target item (makeshift ball on rod) was introduced. When touched by Dewy's nose, a click was given followed by treat (n= 6 days). Rewards were then given on different schedules (fixed/variable ratio/ interval), strengthening his memory and determination towards goals. Dewy became confident, curious, exploratory, and comfortable outside his herd. Dewy's accomplishments to date are: targeting, comfortability around staff, turns, circles, reversing, and walking to the scale alone for weigh-ins. Ongoing training includes climbing on stanchions, desensitizing to noise stimuli, and gait training. Overall, Dewy's modified behavior makes everyday tasks easier and less stressful, provides enrichment, and has instilled a newfound trust and confidence. Clicker training in sheep is not only possible, but it can also be easy and rewarding. This method may be considered for future short-term resident sheep at our facility.

#### **P125 Reduction, Refinement, and the FDA Guinea Pig Maximization Sensitization Test**

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The Biological Sciences and Laboratory Animal Research teams support the 4Rs of Animal Research. The Guinea Pig Maximization Sensitization (GPMax) test is an FDA standard safety assessment test that is required in medical device development. Refinements to the GPMax test have led to more reliable data and a reduction in the number of animals used in testing. Historically, additional animals were routinely added to GPMax test cohorts to account for animal removal due to approved study endpoints including severe skin lesions, bandage disruption or loss, random animal deaths, and/or other study losses. The Biological Sciences and lab animal care teams worked collaboratively to evaluate refinements under the approved IACUC Protocols. Refinements included: updating the home cage environment from single housed stainless steel wire bottom cages to larger plastic solid-bottom cages, group housing versus single housing, change in orientation of Induction I injection sites to reduce severe lesions due to artifacts of the site, change in wrapping materials to increase comfort and effectiveness, and addition of certified Timothy Hay cubes following bandage removal for Induction II and Challenge phases. These updates have resulted in improved environmental enrichment and behavior management by positive reward and association. The refinements to each GLP Study Phase (Induction I, Induction II, and Challenge phases) have decreased the severity of Freund's Complete Adjuvant (FCA) induced lesions. The decrease in lesions, the improvements in bandage material and improvements in caging have positively impacted Animal Welfare. In conclusion, the refinements have decreased the number of reported health concerns and the number of veterinary treatments and has reduced the number of research animals used to support GPMax testing by approximately 6% annually. Tracking of this data began in 2018 and shows that the number of animals euthanized due to missing topical patches or severe lesions has decreased from 16 and 9, respectively, to 0. This work supports good Animal Welfare and improved study data quality in support of the 4Rs.

#### **P126 Validating the use of box training as a refinement to rabbit handling**

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In the wild, rabbits are prey animals and so the act of being picked up and handled can be extremely stressful for them. Previous studies in pet rabbits have shown that during the act of lifting, rabbits show signs of struggling and aggression due to fear. This is relevant to the rabbits we house in our facility and can cause unnecessary stress, which potentially will negatively affect the research. In our case, the rabbits are cardiovascular models of Myocardial Infarction and keeping stress levels down where possible is important. We investigated a new method of handling our 12-week-old male New Zealand White rabbits, involving training the rabbits to jump into an animal carrier lined with different vetbed to be transported to their destination – either a playpen or scales multiple times a week. The 2 groups were broken out into groups as follows: Shared vetbed (2 rabbits, vetbed shared between all rabbits and not washed), and own vetbed (3 rabbits - vetbed that was only for the individual rabbit). We trialed several positive incentives but found the vetbed was the most effective. The rabbits were timed on how long they took to jump into the box with a limit of 3 minutes. Out of the two groups, the shared vetbed was preferred due the desire to investigate the different smells left by the other rabbits when it was presented to them. They consistently got quicker jumping in and would jump in very quickly after it was presented, as well as sniffing, scenting and sitting in the box. The own vetbed group however, lost interest in the box after the initial couple of sessions, possibly because it didn't smell interesting. They were not as excited by it and did not jump in quickly like the other group. Since the initial trial, we have now tried this method with 3 female rabbits and have received positive results from them using the shared vetbed. We also measured the effect of surgery on our MI/SHAM model rabbits and found that during post-op monitoring, they were less likely to jump into the box but quickly returned to jumping in as normal afterwards. We have successfully implemented this as the primary method to handle our rabbits, reducing the stress associated with handling. We have seen an improvement in the rabbit's temperament and they are much less fearful during handling.

#### **P127 Welfare and Enrichment of Yucatan Pigs Post Spinal Cord Injury**

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The spinal cord injury (SCI) research model in Yucatan swine is used at our facility to improve guidelines for management of patients in the early stages of an SCI and to provide a more complete picture of pathophysiology of acute human SCI. The injury is surgically induced under general anesthesia at spinal cord levels of T2 or T10, causing partial to complete paralysis of the hind quarters. In our experience, the pig's life post-injury leads to welfare challenges including the inability to express species-typical behavior, discomfort, and distress. Limited resources of standard husbandry practices for post-SCI pigs exist based on our research, so the goal was to develop a program to strive to provide these pigs exemplary welfare. The laboratory technicians collaborated with the research team to create Standard Operating Procedures and recommendations considering the unique needs of the post-injury patients. Special housing is necessary due to the animal's initial inability to right itself, therefore a dwelling was fashioned using the bottom half of a large kennel. Post-injury pigs are not group-housed, so a minimum of two pigs undergo the SCI procedure in the same week and recover in adjacent dwellings to provide same species companionship. The research team performs behavioral clicker training at regular intervals pre-SCI which is continued post-SCI to provide social enrichment and physical activity. An exercise plan for T2-injured pigs was implemented for further physical activity to address their decreased ability to move. Toys and novel treats are provided on a rotating schedule to encourage normal behavior. To assess patient condition, trained technicians perform frequent scheduled checks using facial grimace scoring guidelines and by recognizing abnormal behavior. A Welfare Assessment Guide was developed to evaluate pig well-being based on species-typical behaviors pre- and post-SCI,



and initial analysis found that post-SCI patients score at a similar level of welfare as they did before injury. Through trial-and-error, the care provided to post-SCI Yucatan pigs has been refined, and additional practices will continue to be developed in an attempt to decrease negative stimuli specific to post-SCI pigs and increase their positive welfare.

#### **P128 Development of an enrichment device for Guinea pig in a production room.**

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Guinea pigs are extremely sociable, very energetic, and intelligent. However, since these animals are considered a prey species, they tend to be neophobic, which makes them fearful and cautious, especially with unfamiliar things or surroundings. We wanted to develop a device that provided our animals, in a commercial production setting, a place to shelter while also functioning as enrichment in a fun and innovative manner. A commercial production setting presents unique challenges. The guinea pig housing, as well as cage density is unlike traditional research settings. Over the years, we have tried several types of enrichment including dumbbells, cardboard and metal teepees and plastic tubes. However, to meet the species affinity to structural enrichment, we aimed to develop a dual device used to both shelter the animals and present as additional enrichment. This device hangs on the edge of the enclosure, resting on the bottom. The shape and position of the structure allowed them to shelter and hide beneath it. The front surface of the device is equipped with a stainless steel 2-inch screw and lock nut, allowing other various types of enrichment to be securely affixed to it. The installation of the block is designed to allow it to spin around on its axis, serving as an interactive source of enrichment. The additional piece of enrichment we selected was a hay block made of compressed Timothy hay. The hay block can be eaten and serves as food enrichment. Furthermore, having the hay block presented on the shelter and away from the bottom of the cage limits soiling and makes it easily accessible. We have successfully incorporated the use of this innovative dual-purpose device in our stock and breeding cages. Animal care technicians observed the animals after installation of the novel enrichment device where the animals were observed using the dual-purpose device. They were observed sheltering and the hay blocks were consumed. We subjectively concluded this innovative device is encouraging their natural behaviors by serving a dual purpose and works well in our high throughput, densely populated production setting.

#### **P129 Establishing a New Method of Bone Marrow Isolation to Significantly Improve Donor-Recipient Ratio In A High Throughput Environment**

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Charles River Laboratories (CRL) works on a global scale to deliver high quality data from animal studies. This high throughput requires maximum economy of motion as well as tight consistency in technical procedures. CRL Discovery is dedicated to improving and reducing animal usage for our Graft versus Host-Disease (GvHD) models. These studies involve recipient mice undergoing myeloablative radiation treatment. They then receive a therapeutic intravenous injection of bone marrow and splenocytes isolated from other, healthy mice (donors, with minor or major MHC mismatch). We instituted a new, time-neutral method of bone marrow collection in C57Bl6 mice to reduce the number of donors needed. The standard method involved transection of the diaphysis from the epiphysis in bilateral femurs of euthanized donor mice (n=8). The femurs were then flushed with 1-2 mLs of cold PBS. In the new method (n=8), a single pilot hole was drilled through the proximal epiphyses and tunneled out through the distal epiphyses. The bones were flushed

until translucent or white. This new method was evaluated by use of bilateral femurs and with the addition of bilateral tibias sourced from euthanized donor mice. Fetal Bovine Serum (FBS) was also added to a 2% concentration into the cold PBS to improve cell viability. The standard method took approximately three minutes per animal to perform. The new method took approximately six minutes per animal to perform. The standard method required 3 donors for every 2 recipients. The new method (with the addition of the bilateral tibias) allowed for 1 donor for every 5 recipients. There was a significant increase in the number of bone marrow cells harvested that allowed for an 87% reduction in the number of donors required. This new technique was simple to institute, easily retained by technical staff, required minimal new reagents, and significantly reduced the numbers of animals required for donation when performing GvHD studies in mice. The increase in time spent per animal was offset by the improvement in overall donor to recipient ratio. Future studies will investigate the utility of cryopreserved bone marrow. Additionally, characterization of in-house bone marrow transplants will be correlated to different clinical presentations of GvHD.

#### **P130 Easing Compassion Fatigue**

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Most professionals in the in vivo research community share a common goal of helping people and animals. The ability to feel empathy for others and animals, to care deeply for research animals, and the goal of advancing science are common traits among individuals in this community. When combined, however, they can be a challenging and exhausting endeavor. Compassion fatigue is characterized by physical and emotional exhaustion, a change in the ability to feel empathy, depression and more. To help combat this, AstraZeneca has developed internal compassion fatigue resiliency programs that include a wide range of resources for employees. These resources raise awareness, advocate for employees, and build upon our Culture of Care. Mental, emotional, and physical health and well-being resources are available. In addition, educational webinars, process improvements, periodic surveys, brain breaks and honest sharing are also part of our programs. All animal care staff, managers, trainers, scientists, and IACUC members may participate in whichever components of the program they desire and participation is not mandatory. One main objective of our program is to promote our resources to help and support all people involved, so that they may in turn support each other. Another is simply raising awareness around compassion fatigue and designing activities and resources to promote resiliency. Qualitative and quantitative endpoints were measured through a baseline, 6-month, and 1-year survey on CF resiliency. Survey results showed that several areas of interest are improving. We observed increased staff understanding of the definition of CF, knowledge of strategies to combat CF, and implementation of the strategies. In addition, there is decreased perceived stress and increased resiliency. Survey participants reported positive feedback, such as "I appreciate being able to speak more openly" and "it reassures that the feelings I have are not only for me, but for other people going through similar situations." Our compassion fatigue program is designed to lead to better employee experiences, animal care, scientific quality, and an overall better workplace.

#### **P131 Novel Enrichment Devices for Pregnant Ewes and Lambs**

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Cincinnati Children's Hospital Medical Center is a leader in fetal and

placental research, and pregnant sheep models are commonly used to develop surgical therapies for fetal congenital conditions. Veterinary Services receive timed-pregnant ewes for these studies and houses them in an indoor vivarium for the remainder of their gestation. Sheep are a prey-species and shipment and acclimation to an unfamiliar environment can be a significant period of stress. Observed stress behaviors of ewes includes being unapproachable by animal care technicians and displays of aggression such as headbutting and stomping. Newborn lambs face their own unique stressors, such as social deprivation when a study requires hand-rearing due to attrition or rejection by the ewe. In order to minimize these stressors, we focused on developing and refining novel environmental enrichment items for both pregnant ewes and neonatal lambs. Enrichment for pregnant ewes engages multiple senses, promotes a low-stress environment, and creates more positive social interactions with their care team. Novel enrichment for neonatal lambs is designed to promote social bonding in situations where a foster ewe is not available. Following the implementation of this enrichment, we have seen an increase in proxy indicators of improved animal welfare. For ewes, this includes increased frequency of affiliative behaviors such as voluntary approach to caretakers and duration of caretaker interaction, and a decrease in agonistic behaviors of head-butting and stomping. We have also observed an increase in direct interaction time with enrichment and increased frequency of conspecific play behavior such as frolicking. In offering enrichment to the lambs, we have observed imprinting on caretakers, decreased frequency of vocalizations when housed with a surrogate device, and increased time at rest instead of pacing. Using an enhanced enrichment program designed for these two critical life stages of sheep in our vivarium has improved the complexity of interaction and time spent interacting with environmental enrichment relative to the past. This initiative has resulted in improved wellbeing of both the animals and those who care for them.

#### **P132 Evaluation of abnormal behaviors in two singly housed macaques pre- and post-social introduction: A case study**

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An essential component of psychological wellbeing assessments of macaques in research is the ability to adequately monitor behaviors and properly intervene if abnormalities arise. Among the most commonly observed abnormal behaviors in laboratory macaques are motor stereotypies, defined as repetitive movements of part or all of the body, and hair plucking, defined as removal of hair using teeth or hands, sometimes causing alopecia (Lutz et al 2022, Polanco et al 2021). A literature review confirms socially housed macaques engage in significantly less abnormal behaviors than singly housed macaques. However, previously published studies utilized 5- to 10-minute behavioral scans quarterly to yearly, which may under-report the presence of abnormal behaviors (Lutz et al., 2013, Kroeker et al., 2017). We present a case study of two male rhesus macaques (*M. mulatta*) that were observed to engage in abnormal motor and hair plucking behaviors prior to being socially pair-housed. Our hypothesis was supportive of previous findings but utilized a more rigorous scanning strategy: Introduction of a compatible conspecific should decrease the frequency of abnormal behaviors, and alopecia scores should improve post-pairing. To achieve this, the frequency and duration of self-grooming, hair plucking, and motor stereotypic events were continuously sampled for each monkey's 12-hour light cycle for five days before and five days after the introduction of a social pair. Additionally, a one-year follow-up time point was evaluated for existing compatible pairs using continuous scanning for 5 days. Nonparametric analyses revealed that duration and frequency of self-grooming behavior trended but did not significantly decrease in both animals after pairing. One motor stereotypic behavior, but not all, decreased significantly in one animal. Our results suggest that socialization is only one component of a rigorous, multifaceted program to reduce abnormal behaviors in macaques in

a research setting. An intervention we will evaluate in the future is a schedule choice program to increase animals' ability to make choices about their day. We finally conclude that more frequent observations are needed to confirm the proper interventions are conducted for abnormal behaviors.

#### **P133 Managing Social Contact for Macaques on Infectious Disease Projects**

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At the Washington National Primate Research Center, Behavioral Management Services provide enrichment opportunities vital to the human and ethical care of our nonhuman primates. We place a significant focus on providing social contact to primates because it is dynamic, thus habituation is less likely in comparison to other forms of environmental enrichment. Since the official implementation of our behavioral management program in 1997, support for socialization in infectious disease research has increased as principal investigators recognize the key role tactile social contact plays in preventing or ameliorating stress, which in turn, creates better research models. For some projects, however, valid concerns of inadvertent virus transmission remain. Here, we discuss how we implement and manage socialization options for primates assigned to infectious disease projects without confounding research results. An understanding of the research study and its timeline along with in-depth communication are necessary for success. Infectious disease studies during 2020-2022 were categorized into eleven categories (e.g., Covid, Malaria, SHIV/HIV/SIV, SHIV studies involving tether) and summarized with regard to the percentage of primates assigned, what timepoints in the study allowed tactile social contact, and what level of social contact was permitted. All projects allowed full contact at some point. Projects in two of these categories allowed full contact for the entirety of the study. Animals associated with the SHIV/HIV/SIV and SIV-ZIKA co-infection categories reverted to protected contact during the viral challenge phase. The remaining seven categories had some phases where no social contact was allowed. We are sharing this information because an increase in scientific peer-reviewed publications and presentations will familiarize new scientists with how other researchers are conducting similar studies. This may encourage them to consider incorporating socialization in their research design. As we illustrate here, socialization in infectious disease projects is an attainable goal.

#### **P134 A practical approach to the assessment of mouse enrichment types in a large research program**

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Mouse enrichment is "complex" with many variables that affect how the animals respond, including strain, phenotype, age, gender, as well as housing density, cage size and quality of the space. Many studies have been conducted with varying results on impact and benefit to the mice and to the study outcomes. As the *Guide* notes, "... it is difficult to compare these studies due to the study design and experimental variables that have been measured," and as such the benefits and potential impacts of enrichment in mice "remain complex and should be carefully considered." Our institution houses more than 30K cages of mice, representing diverse models and species demographics. Our standard enrichment for mice includes nesting material and shelters. When stereotypies present, one possible intervention option is to provide additional or different enrichment types. Given the many different choices of enrichment, it is difficult to know what enrichment to choose or how to evaluate it. Therefore, we developed criteria to make practical assessments of

various enrichment types. Criteria for evaluation were simplified into a quantifiable scale so that assessment, involving varying cage demographics, can be assessed in a practical way to produce quick and reliable outcomes. Assessments consisted of video recording, pictures or direct assessment of at least 8 cages for 10 enrichment assessments, evenly spaced at the start, middle and end of the 2-week cage change cycle. Quantitative scales were used (1- least desired, 3- most desired) to assess visibility of mice for health, usage or nest score and proximity of nest in cage or with device. The development of this assessment criteria was made utilizing veterinary student interns, and later used by animal care staff as a routine part of the program. This assessment allows us to effectively highlight areas of concern, reveal disparity in usage between strains and help guide items selection. Initial assessment of enrichment to address stereotypies has mixed results, but we are hopeful that continued assessments will reveal opportunities for positive outcomes. The simplified approach can be adopted by others as a method for assessing enrichment and educating staff about the complexities of enrichment in mice.

### **P135 Implementing Desensitization as Part of Rat Toxicology Studies**

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Techniques to improve laboratory animal welfare, including refinement, have often focused on large animal species. Refinement with rodent species is less common due to large numbers of study animals as well as the prioritized emphasis on high profile animals such as non-human primates and dogs. Pfizer recently developed a Rodent Refinement Committee with a focus on improving rodent handling. A study was conducted to evaluate the effectiveness of pre-study habituation to novel toxicology study activity locations through active desensitization. A total of 48 rats were evaluated; 24 control rats who received no desensitization and 24 experimental rats who received desensitization for either 4 or 10 days. The following measures were evaluated to determine the effectiveness of the desensitization: Animal location in home cages when approached by technician, fecal output while in holding areas, number of treats eaten while in holding areas including latency to begin eating treats, and number of animals per home cage to eat treats from the technician's hand when placed back after completion of study activities. Animals receiving desensitization were in the front half of the home cage when approached by technicians 85% of the time compared to 69% of the time for control animals. Control animals defecated three times more frequently than desensitized animals in holding areas. On average, desensitized animals would begin eating treats at approximately 32 seconds after being placed into novel holding areas while control animals would average 106 seconds. On average, 1.2 control animals per cage would accept treats from technicians' hands more readily when placed back into home caging compared to an average of 2.1 desensitized animals. Active desensitization was similarly effective in both the 4-day and 10-day trial. Habituation to study activities prior to Day 1 of toxicology studies is beneficial to animals and technicians, aligning with Pfizer's commitment to best animal welfare practices.

### **P136 Fully Automated Micromanipulation: Genetic Modification of Mice by Automated Solution Injection**

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Genetically modified mice are essential experimental materials for biologic, medical, and pharmaceutical research and development.

These mice are produced mainly by manual zygote micromanipulation, but reproducible micromanipulation requires specialized techniques performed by a skilled microinjectionist, which impedes the efficiency of the procedure. To improve the efficiency of genetic modification procedures, we developed a fully automated micromanipulation system for injecting solutions into the mouse zygote pronucleus. We first determined the entire sequence of the procedure (determining the start and end of the manipulation, identifying the zygote and pipette positions, clarifying the manipulation, and executing the manipulation). For one zygote, 11 to 13 steps are required for microinjection. We next constructed the motorized hardware. A computer analyzes the information input from a microscope camera and issues commands to the output device, which drives the motorized parts (manipulator, sample stage, pump, injector). We then developed software to control the system (zygote recognition by computer, movement of pipette and zygote, rotation of zygote to move pronucleus to a position where pipette can be inserted, correction of pipette insertion position, and injection of the solution into the pronucleus). By integrating the software with the hardware, we successfully developed a fully automated micromanipulation system. The fully automated micromanipulation system was applied to genetically modify C57BL/6 strain mice. Three types of genetically modified mice were prepared by applying the traditional DNA injection, piggyBac Transposon system, and CRISPR Cas9 system. The genetic modification efficiencies (zygote survival after injection, offspring, and genetically modified mice) of microinjection by a skilled microinjectionist injecting the solution manually were the same as those performed by the newly developed, fully automated micromanipulation system. We believe that this new method is a useful addition to the genetic modification technologies available for experimental animals.

### **P137 Modified Rabbit Sling for Forelimb Orthopedic Procedures**

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Orthopedic procedures are a necessary step in the testing of new surgical techniques, implants, and other devices. Though the experimental surgeries involved are complex, it is often more difficult to prevent post-operative complications. Casts can be particularly challenging to maintain for prolonged periods of time as bone heals. As part of an IACUC-approved protocol, we performed orthopedic surgeries on rabbits where a portion of the radius was removed and replaced with an implant, requiring placement of a post-operative forelimb cast. There are a multitude of approaches to placing a cast in a variety of species but to our knowledge, there are no techniques available to prevent slippage and removal of casts in rabbits. Our first set of surgeries were successful, and casts were placed comfortably, but a few anxious rabbits were able to remove their casts, risking ulnar fracture and subsequent euthanasia. Initially, we attempted pharmaceutical restraint to replace the casts, but we did not feel comfortable repeatedly administering sedatives and anxiolytics to the rabbits. One rabbit removed its cast daily for the first three days in the post-operative period, and even removed its cast immediately upon waking from sedation for a cast replacement. There was discussion about removing this rabbit from study and possible euthanasia because of its repeated need for sedation and risk of self-harm. After several attempts, we developed a modified over-the-shoulder sling, meant for post-operative care and management of rabbit forearm surgeries requiring cast placement. This new technique, along with improvements to the casting procedure, has prevented further cast removal for all 15 post-surgical rabbits since its final revision. Refinement of the casting process with addition of the sling has led to improved stability of the casts, a reduction in sedation to replace removed casts, and significantly less handling stress and restraint of the rabbits. The sling is comprised of simple materials found in most veterinary facilities and has a straightforward design, so this technique can be easily taught to most veterinary staff. The use of this modified sling will help prevent post-operative casting issues, excessive sedation, and even euthanasia in

the case of fracture.

### **P138 A Spotlight on Rodent Enrichments Role in Openness, Transparency and Culture of Care**

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Openness and transparency have become a priority within the lab animal industry. Often the public is misinformed to believe animal research is cruel and unkind, conducted by scientists who don't care about the animals. It is now our turn, as lab animal scientists, to share the truth and passion that goes into caring for our lab animals. Over the last two years the Lab Animal Resources (LAR) group at Alexion, AstraZeneca's Rare Disease Unit have used a virtual technology to offer augmented reality tours of their vivarium and introductions to their research animals. The target audience has been high school and college students. Students have been exposed to purpose breeding of rodent colonies, discussions of phenotypic mice to better understand human disease models, animal handling and enrichment. Feedback gathered from post event surveys of our first virtual tours proved that there was a positive impact on the impression of the students' views on animal research. Each event collected feedback after the events from ~100 participants as well as live feedback during our Q&A sessions. For one event, all participants were required to write an essay. Each essay focused on how the virtual experience changed their view of animals in research. In response to this feedback the LAR team shared a live demonstration of rat tickling and the vocalization software used to determine positive vs. negative rat vocalizations in a virtual presentation. During this presentation the bond between our rats and their handler was obvious to all participants. Much of the feedback received via a post event survey noted the happiness of the rats and the connection they had to humans. These positive experiences in sharing our facility and our techniques have had a large impact on the culture of care within our facility. We have found sharing play and enrichment for animals has created a commonality for the public and can be used to bring greater understanding to why animals are used and how they are cared for in research. In the events we conducted virtually last year we were able to reach over one thousand participants outside of the lab animal field and several hundred in our own global organization.

### **P139 Implementing the 3Rs by Establishing a Training Rabbit Colony**

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Training students, husbandry staff, and investigators in the current best practices for animal-related procedures is critical to the welfare of animals. Prior to 2022, 12-16 rabbits were purchased every year for training use at Purdue University, primarily for training veterinary nursing students. To reduce the number of rabbits utilized for training, and open the resource to others across campus, the Laboratory Animal Program started a training colony of 12 New Zealand White rabbits. These rabbits will be housed for a maximum of 3 years, with 4 new rabbits added to the colony each year, while 4 older rabbits are retired and adopted as pets. The rabbits are pair housed with one neutered male and one spayed female in floor pens to provide additional space for long-term housing. Positive reinforcement training and human interactions are regularly provided during husbandry and veterinary procedures to reduce stress and better acclimate the animals to handling for training classes. Prior to the start of the colony, the veterinary nursing team used rabbits for 4-6 training labs each year. The LAP colony was used for 6 training sessions in 2022. The veterinary college and student clubs also now benefit from the use of the colony. Use is limited to minimally invasive procedures such as injections, blood draws, and anesthesia training. A fee is charged to users per rabbit for each day

of training, which includes a downtime of one week between uses, and ensures that the cost remains significantly less than the cost for the investigator to purchase the animals themselves. The colony embodies the 3Rs by reducing the number of animals needed each year by up to 75% and refining animal welfare via space, enrichment, positive reinforcement training, and socialization.

### **P140 Novel Method for Repairing Female Rabbits After Two Week Separation**

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Social housing in research animals has numerous benefits, including affirming species specific behaviors, providing enrichment, and encouraging activity and should be provided when possible. New Zealand White rabbits (*Oryctolagus cuniculus*) are social species that greatly benefit from being housed in groups/pairs. However, specific research goals may prohibit pair housing animals, including collecting urine and feces, prohibiting contamination of animals in distinct groups, and observing study related effects on individual animals. Although there has been success with pairing rabbits, often rabbits remain singly housed once separated. Disruption of the established social order in this species, such as when animals are separated during studies, can lead to aggressive behaviors when animals are reintroduced. The goal was to determine if previously housed female rabbits could be re-paired after spending two weeks in separate cages for the duration of a study that required singly housed animals. Rabbits were in separate cages throughout the study with a barrier that allowed for visual access. Multiple methods were attempted to re-pair animals – the most favorable pairing method involved pairing animals at time of cage change. Animals were introduced in a large neutral space with enrichment. After multiple affiliative interactions were noted in neutral space, animals were placed together in a clean cage. Animals were monitored to ensure no aggressive or adverse behavior was noted including chasing, thumping, or signs of distress. Feces was collected when animals were initially paired, during pairing process, and after pairs had been established to assess stress response via fecal cortisol tests during these phases. Of 14 individual animals that were attempted to be re-paired, 5 successful pairs were established resulting in 71% of the separated colony in pairs and 74% of animals imported as pairs remaining paired. Four of the pairs were re-paired at the first attempt, and one of the pairs required two attempts before being successfully re-paired. Fecal cortisol assays are pending. Animals are still successfully paired 4 weeks after re-pairing. This success of re-pairing indicates rabbits separated for study needs can be successfully repaired after the study has concluded.

### **P141 Current Demographic Makeup of IACUC Members in the United States**

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Promotion of a diverse, equitable, and inclusive environment is a goal shared by many in laboratory animal science. Progress towards that goal requires benchmarks regarding diversity within the field, including demographics of oversight bodies such as the IACUC. However, data describing the current demographic makeup of IACUCs is limited. This study aims to provide initial data regarding the demographic makeup of current IACUCs in the United States by leveraging information from a dataset created to query committee members on ethics. This dataset was created by inviting members of the AALAS Community Exchange and the NABR IACUC-Admin communities to participate in an anonymous online survey in March 2023. The survey gathered data on current members of IACUCs in the U.S., including age range, gender identity, race, ethnicity, religiosity, and political leaning. These data were compared against

benchmark data on diversity within U.S. academic faculty and the U.S. overall from the U.S. Department of Education and the Census Bureau. Respondents currently serving on U.S. IACUCs (n=167 of an estimated 4940) predominantly identified as white (138/157, 88%, 95% confidence interval [CI] 80-96%), female (98/159, 62%, 95% CI 54-70%), and leaning progressive (107/152, 70%, 95% CI 62-78%). For comparison, data on U.S. college faculty showed 74% identifying as white and 47% as female, while the general population identified as 62% white and 51% as female. Efforts to promote diversity within laboratory animal science, including on IACUCs, should proceed thoughtfully. Evidence suggests committee service can act as a burdensome 'non-promotable task' weighing down rather than lifting up careers. Further research is also needed to address shortcomings in the present study, such as convenience and selection biases. If confirmed, these results suggest that further efforts to improve IACUC diversity are needed, such as incentivizing committee service by explicitly factoring it into faculty promotion.

#### **P142 Institutional Officials Consortium (IOC): An Industry Consortium for Collaboration in Animal Welfare, the 3Rs, and Openness about Biomedical Research**

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Animal care and use programs in industry (pharmaceutical and biotechnology companies, contract research organizations, breeders of animals needed for research) face many of the same challenges and opportunities. Desire for competitive advantage may make industry organizations reticent to share best practices. However, optimizing animal welfare, advancing alternatives and the 3Rs, and communicating to the public why and when animals are still needed for the discovery and development of new therapies is pre-competitive. In this spirit, the Institutional Officials Consortium (IOC) was created as a formal organization where industry peers in animal welfare can work together. IOC supports and synchronizes programs ensuring ethical engagement with animals in pharmaceutical and biomedical research, highlighting alternatives to animal research and supporting implementation of the 3Rs principles, public and factual communication around research, broadening public understanding about the benefits and necessity of animal-based research, and aligning support for pro-research advocacy organizations. Each priority is associated with a working group. IOC holds biannual meetings to assess progress on these working group initiatives, to provide strategic input to research advocacy organizations, and to assess deliverables. Among many deliverables from the working groups in 2016 – 2022 are strategy tools to address challenges to research with animals, guidelines for implementing an Animal Welfare Office, benchmarking and communication platforms, recommendations for internal communication on animal-based research, an annual summit for communications, public relations, government affairs professionals at member organizations, and multiple new public websites on the direct benefits of research to animal and human health. IOC member companies benefit from a network to benchmark with one another, access to exclusive materials to enhance support within a research environment, and participation in recommendation and resource development for organizations and communities on positive, thoughtful, and open sharing within this topic. IOC welcomes the opportunity to engage in open dialog through benchmarking and best practice sharing for all research settings.

#### **P143 Refining Rat Management Programs Through Stakeholder Focus Groups**

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Rats are an important species for biomedical and scientific advancement, and therefore there is an ethical obligation to provide good welfare, housing, and support for every life stage. For the 2020 Lunar 'Year of the Rat,' a global initiative to develop recommendations and best practices for rat care was started through the Rat Welfare Working Group. A list of recommendations was developed through stakeholder focus groups of subject matter experts for topics related to rat behavioral management, procedural refinements, recognition of pain and distress, euthanasia procedures, and welfare assessment. Subcommittees met virtually from 2020-2022, and a virtual Rat 3Rs Workshop was held in 2021 to further discuss refinements for rat care and management. The Rat Welfare Working Group developed 12 recommendations based on discussion and consensus building. The recommendations were then discussed with corporate leadership to achieve buy in and support across business units. The recommendations will be used during regular site visits to monitor rat programs over time as sites work to implement refinements and will ultimately be used to develop a rat welfare assessment tool to allow sites to quantify their rat management programs and identify areas for improvement.

#### **P144 Tunnel Handling Mice Has Minimal Impact on Cage Change and Cage Wash Time**

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Traditionally, mice are handled and moved by grasping the base of the tail. Previous publications have shown that this can elicit an aversive response and invoke anxiety. Published refinements to mouse handling report that moving the animals without the use of the tail can increase voluntary handler interaction and lower anxiety as measured by an elevated plus maze. One refinement (3Rs) is the use of tunnels. While the welfare benefits of this method have been validated by prior work, little information exists on the operational impact of tunnel handling. We completed time in motion studies to assess the workload impact of tunnel handling on cage change out and cage wash operations. We evaluated the time to change individual cages of different strains of mice: BALB/c, NOD SCID, Athymic Nudes, and C57BL/6 (N = 10 cages/strain). In addition, we assessed cage change time for C57BL/6 in both single and group housed settings (3 per cage). Reusable plastic or disposable cardboard tunnels were placed in each home cage and remained for eight weeks with a change out for clean tunnels at the fourth week. Original and replacement tunnels were disposed of or washed, with cleaning and disposal times recorded. Cage change times were also recorded as a separate data set over the eight-week period. During the first week – when the mice and handler were inexperienced with the tunnel – the cage change times were significantly greater than tail handling. By the second week and beyond, change times for 4 of the 5 groups took similar or less time than tail-handled mice. BALB/c were the lone exception that retained longer change out times. Tunnel handling can be successfully incorporated into operational workflows with minimal impact after the initial investment in training staff and acclimating mice.

#### **P145 Comparison of Plastic and Metal Oral Gavage Needles in C57BL/6 Mice**

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Oral administration is one of the most common routes of dosage,

with oral gavage being the most frequently and widely performed technique in biomedical research. In rodents, the use of gavage needles is required for accurate dosing due to their small body size. Metal ball-tipped gavage needles have been historically used and preferred by researchers, but their use has been shown to cause complications including trauma and aspiration pneumonia. Recently, newer polypropylene plastic needles have been introduced as an animal welfare refinement. While these needles have been observed to cause fewer complications, evidence of the benefits compared to metal gavage needles is lacking. In this study, we evaluated the clinical effects of metal ball-tipped gavage needles compared to plastic gavage needles in mice. Thirty, male, C57BL/6NCrl mice (8-9 wks old, 25-35g) were randomized and allocated to ten mice per group (metal gavage needles (20g),  $n=10$ ; plastic gavage needles (20g),  $n=10$ ; no gavage,  $n=10$ ). Animals in the metal and plastic gavage groups were restrained and dosed with 10mL/kg drinking water daily for five days, and animals in the no-gavage group were briefly restrained. Blinded clinical observations were made daily following oral dosing, and body weights were collected prior to dosing and on days five and seven. Animals were euthanized on day seven and tissues (esophagus, stomach, trachea, lung) were collected for histopathology. Microscopic evaluation revealed all mice in the metal gavage group had minimal-moderate degeneration and necrosis of skeletal muscle with mixed cell inflammation. These findings were consistent with a trauma-related injury. This was different from mice in the plastic gavage and no gavage groups (Kruskal-Wallis,  $p<0.001$ ). Body weight was decreased between the no-gavage group and the plastic and metal-gavage groups (Kruskal-Wallis,  $p=0.003$ ). Finally, there were no differences in clinical observation scores (Kruskal-Wallis,  $p=0.2$ ). To our knowledge, this is the first study that directly compares plastic and metal gavage needles in mice. Overall, the results of this study indicated that plastic polypropylene needles offer a refinement to oral gavage dosing in mice and improve animal welfare when compared to metal gavage needles.

#### P146 Refinements in Rabbit Cage Change Procedure

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Refinements in cage change procedures can significantly impact husbandry practices to benefit both animal care staff and animals. Current practices in many facilities housing laboratory rabbits (*Oryctolagus cuniculus*) involve physically removing animals from soiled caging and placing them in sanitized cages, resulting in stress to the animal and potential ergonomic injury to technicians. We sought to reduce stress in New Zealand White rabbits and increase efficiency and husbandry ergonomic safety during the cage change process. Commercially available Techniplast cages enabled the development of a novel method to transfer rabbits during routine cage change. The cages are attached via lateral connections and the divider is immediately removed, allowing the animal to freely transfer to the new cage without manipulation. Once animals move into the new rack the divider is replaced so the rabbits remain in the new cage. This is also a form of enrichment, providing increased space and promoting exploratory behavior. The following variables were measured to assess which method was preferred. The time required for cage change, fecal cortisol levels for three days post cage change, and any differences noted in paired and singly housed animals. A total of 28 (10 single and 18 pair-housed) animals were assessed. The novel stress-free method with no animal manipulation was completed in 60 minutes with a mean time of 44s per pair or singly housed rabbit transferred, compared to 90 minutes with a mean of 51s for animals manually moved to the clean cage. The new method took significantly shorter time than the previously used method ( $p < 0.05$ ). There was no significant difference between paired and unpaired animals. Fecal cortisol assay results are pending. After physically transferring animals, the technicians observed rabbits thumping the rear feet, a defensive behavior, and no

thumping was noted when animals moved without manipulation. Ergonomic injuries while physically transferring animals are alleviated with the new method, which does not require repeatedly bending down, reaching into cages, or picking up animals. This novel stress-free cage change procedure has reduced stress within our colony, enhanced the ergonomic safety of technicians, and increased efficiency.

#### P147 Assessing Stress Impact and Subcutaneous Dosing Effectiveness of a Scruffing Restraint Device in C57BL/6J Female Mice

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Safety, competency, and animal welfare are priorities when training individuals in handling laboratory rodents for experimental use. Our training program routinely searches for opportunities to further the 3R's (Replacement, Refinement, Reduction) to continuously improve animal welfare. As a potential refinement, we evaluated the use of a commercially available mouse scruffing restraint device within an in vivo research environment. The device is meant to assist handling mouse research models by allowing for safer manipulation when restraining or dosing. The goal of the experiment was to evaluate the effects of the scruffing device on mouse stress levels after subcutaneous dosing compared to a general, manual restraint that did not utilize the device, as well as the effectiveness of the aid by monitoring dosing time and accuracy. Our hypothesis was that the group dosed with the scruffing device would have lower levels of circulating serum corticosterone and that the accuracy of dosing would be increased. Two groups ( $n=10$ /group) of C57BL/6J female mice were subcutaneously dosed with and without the device. Dosing time and unsuccessful dosing tallies were recorded to assess speed and accuracy between the two groups. Additionally, blood serum corticosterone levels were tested at the conclusion of the experiment to assess rodent stress. It was discovered that although there was neither an improvement nor worsening of dose success and accuracy, the use of the scruffing device did generally allow for faster dosing without adverse event. Over the course of the study, group one was dosed within a range of 3 minutes, 10s to 4 minutes, 16s, while animals in group two were dosed within a range of 2 minutes, 25s to 3 minutes, 30 s. Furthermore, the experimental results show that serum corticosterone levels were significantly decreased when the scruffing device was used for subcutaneous dosing compared to general restraint methods. Mean corticosterone levels for mice in group one (general restraint) was 60.92 ng/mL, while mean corticosterone levels for mice in group two (scruffing device) was 42.46 ng/mL, indicating that the mice experienced dosing as a less stressful event when the device was utilized.

#### P148 Training Rats to Voluntarily Change Cages Using a Tilt Technique

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Changing cages is a frequent and stressful event for laboratory rats. Training rats to voluntarily move between cages eliminates handling, which makes cage changes a more positive experience. The proposed method uses a cage tilt between the home cage and the training cage to quickly train rats to move over within one week. Long Evans rats were used (8M, 8F) for the training protocol due to their better adaptability. Rats were trained using a 45-degree tilt between the home cage and the training cage ( $n=4$ ), or a 90-degree angle and decreased to 45-degree halfway through the week ( $n=4$ ). The home cage was removed from the rack and placed next to the training cage on the workbench. After 15 seconds, a small pinch of yogurt drops

was placed in the training cage along with the enrichment tube. The time it took for the rats to move to the training cage was recorded. The latency to change cages was highest on the first days, averaging 40s for the rats using a 45-degree cage, and 25s for the rats using the 90-degree cage. The latency to change cages decreased over the 7-day trial with the lowest latency occurring on day 7 for both groups at 1-8s for 45-degree cages and 2-23s for 45-degree cages. We found that, when this method was implemented, rats successfully learned to move cages by the end of the week. This training method can easily be implemented and is a positive alternative to handling rats for cage changes.

#### **P149 Use of Colony Metrics to Reduce Excess Animal Production in a Dynamic Biotechnology Environment**

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Preclinical studies in animals are essential to help discover and develop candidate therapies for human diseases. Most animal needs can be predicted based on the progression of candidate molecules through the drug discovery pipeline, but unpredicted needs for animals often emerge as new obstacles are being discovered along the development path. With such uncertainty, the default strategy is often to produce animals in excess to prevent major delays in advancing molecules. Here, we aim to improve animal usage by developing a colony metrics reporting tool to optimize colony size. First, we ranked colonies based on level of animal waste to usage. Then we identified factors impacting animal waste such as animal age at discard, age at shipment representing usage, and number of breeding trios. We found the ratio of discard age to ship age and the quantity of breeding trios directly impact excess mouse production. Adjusting discard age to align with average age at shipment reduced housing costs. Comparing colony production rate to project needs informs colony owners of whether or not the current number of breeding trios needs adjustment. To measure improvement, we calculated the estimated animal production numbers and housing costs before and after changes were made. Decreasing the number of breeding trios led to 25% reduction in animal production. This, combined with a lowered discard age when applicable, resulted in 15%-20% cost savings. Providing data that supported reasons for implementing changes gave colony owners agency and confidence to make informed decisions that reduced excess mouse production. Of colonies included in our assessment, 75% incorporated at least 1 change that reduced animal production or housing costs. Use of this colony metrics tool allows for improved colony management, ethical use of animals and cost savings.

### **Clinical**

#### **P200 Feeding Patterns of Infant and Juvenile Rhesus Macaques (*Macaca Mulatta*) Living in Large Outdoor Captive Breeding Groups**

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Prior feeding studies of socially housed young rhesus macaques have commonly involved food intake quantification methods with limited accuracy of individual consumption. Few data are also available regarding environmental and biological factors that may influence solid food consumption during infancy through the juvenile period. Here, we present longitudinal calorie intake data of free-feeding, rhesus monkeys (n=26 males, n=29 females) from 1-24mo of age that lived with their mothers in large social groups. Feeding data were generated from a commercially available, automated feeding system that reliably records grams of food pellets obtained in real-time by detecting microchips implanted in each hand of individual animals. The most rapid increase in daily caloric intake occurred during

infancy through 16mo of age, then remained largely unchanged through 24mo of age. Beginning at 4mo of age (peak lactation), males and females born to high-ranking dams consumed significantly more kcals per day than animals born to lower-ranking dams (p's<0.01). By 14mo of age, high-ranking females consume significantly more daily kcals than low- and middle-ranking females (p<0.05). Beginning at 4mo of age through 24mo, high-ranking males consumed significantly more kcals per day than low- and middle-ranking males (p's<0.05), with a distinct peak in kcal intake at 16mo of age. Regression analyses reveal that other factors, including maternal food intake at peak lactation and birth timing (early vs. late in season), account for additional variance in daily calorie consumption of all offspring at 16mo of age (p<0.001). Together, these findings demonstrate that feeding patterns of growing rhesus monkeys are dependent on sex, social rank, and other maternal factors. This is the first study to characterize the feeding patterns of young rhesus macaques across multiple stages of development and to establish expected monthly kcal intake ranges for infant and juvenile rhesus macaques raised in large multi-matrilineal breeding groups, based on sex and familial social rank. Feeding data derived from this study will be used to enhance the clinical health monitoring and management of captive-bred rhesus macaques on an individual basis, representing a paradigm shift from conventional practices.

#### **P201 Jaundice And Hepatic Necrosis in Axolotls (*Ambystoma Mexicanum*)**

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Three of 20 white leucistic axolotls (*Ambystoma mexicanum*), aged 1.5 to 2.5 years, individually housed on a recirculating water system, presented with anorexia and jaundice. Gross examination revealed severe, multifocal to coalescing hepatitis, with necrosis and suspected infarction in the liver. Histopathological evaluation found hepatocellular degeneration and necrosis, variations in hepatocyte size, distinct nuclear changes (karyomegaly, binucleation, and mitotic figures), and the presence of small aggregates of basophilic intracytoplasmic material resembling inclusion bodies. To rule out infectious causes, PCR on liver was negative for ranavirus. A liver culture identified *Aeromonas hydrophilia* and *Aeromonas caviae*, which are opportunistic pathogens associated with aquatic environments. However, this was likely an environmental contaminant as pathology did not identify a bacterial infection. No other overt signs of infectious etiology were observed in these cases or control cases. The housing system further reduces infectious spread as water flows from the filter system into the individual tank, back into the filter system before reaching other tanks. Water quality was suspected to be the cause of the pathology as water assessments revealed a decline in conductivity and increase in nitrates, indicating poor water quality with potential organic enrichment. These values indicate a disturbance in the water conditions, which likely provided a favorable environment for potential opportunistic pathogens to proliferate. Prompt intervention and corrective measures were implemented to address the water abnormalities, including improved filtration, adjusted feeding schedules, and water changes. The successful management of the disease outbreak was attributed to the focus on restoring optimal water quality conditions. These cases highlight the crucial role of water quality in preventing disease outbreaks in axolotls. The association between compromised water conditions, opportunistic pathogen presence, and the observed liver pathology underscores the need for maintaining optimal water quality parameters for the overall health and well-being of axolotl populations.

#### **P202 Canine Distemper Outbreak in Commercial Ferret Population in 2022**

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Ferrets supplied to both the pet trade and lab supply largely come from one source although they are in separate facilities. In September 2022, severe illness and mortality began appearing in pet stores and distributors in Canada and the US. One major store chain sent dead ferrets for necropsies, histopathology and virology to one veterinary laboratory where CDV (canine distemper virus) was identified through PCR and IHC. The breeder began titer testing its colony. The same vaccine had been used, although the location of manufacture may have changed (Distemink; United Vaccines, Inc, Fitchburg, WI, USA). Because no cases were being seen at the facility, the vaccine continued to be used until it was confirmed through genetic sequencing as the likely source of the epidemic.<sup>1</sup> Kits would be vaccinated at approximately 7 weeks of age, and then shipped. Clinical signs and mortality would begin within 3-14 days after shipment. Cases continued through mid-November when the breeder ceased distribution of ferrets to the pet trade. Clinical cases began appearing in the holding facility in the unshipped kits. The CDV from the ferrets was genetically sequenced, and showed 100% identity with the Lederle strain, an established America-1 lineage vaccine strain. Vaccine-induced CD has happened before, and is well documented. The division of the company breeding ferrets for research has not disclosed cases. The author had a discussion with the breeder, and provided the successful treatment regimen for them to use and provide to pet stores, veterinarians, and owners. The breeder did vaccine testing and titers, along with correlating the age of the ferrets and the immune response. Results showed that ferrets must be vaccinated at a later age than previously done, and ferrets will now be shipped at 12-13 weeks of age, 2 CDV vaccinations and rabies. While the ferrets should receive a third CDV vaccine, this is being left to the pet stores and new owners. Limited shipments of ferrets began in April, and the breeder reported successfully treating approximately 80% of the ferrets with clinical CDV. Recovered animals will be suitable for pets as well as non-infectious disease research.

### P203 A Novel Method of Health Monitoring in Laboratory Zebrafish

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Zebrafish (*Danio rerio*) are useful in scientific research due to their close genetic similarity to the human genome and fast reproductive lifecycle. Their increased use in scientific research calls for improved methods of monitoring their health, as current methods involve multiple types of testing including submission of whole fish to identify various pathogens. This study aims to create a novel sampling technique by exposing nitrocellulose filters to sump water over the course of twelve weeks. The filter was compared against other known testing methods of swabbing biofilm from the sump, passing sump water through a vacuum filter, and whole fish PCR. It was hypothesized that the nitrocellulose filter would identify more pathogens over time, reducing the need for multiple testing methods. Weekly PCR testing was conducted to detect *Mycobacterium chelonae*, *Mycobacterium fortuitum*, zebrafish picornavirus, *Myxidium streisingeri*, and *Pseudoloma neurophilia*. *P. neurophilia* was not detected by any of the three environmental sampling techniques. Swabs of the sump biofilm detected the fewest number of pathogens. There was no significant difference between nitrocellulose test filters and water testing methods. Nitrocellulose test filters consistently identified *Mycobacterium* spp and *Z. picornavirus*, and performed similarly to water filtration with less labor in sample collection. Therefore, the nitrocellulose test filters may be a less labor-intensive method for quarterly health monitoring of laboratory zebrafish colonies over time and may eliminate the need for alternative pathogen detection methods.

### P204 Evaluation of Live Insect Feed as a Potential Pathogen Source in an SPF Rodent Facility

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Our vivarium maintains a small colony of grasshopper mice (*Onychomys torridus*), which are provided live insect feed for nutritional and environmental enrichment. Due to the proximity to our SPF rodent colonies, we sought to evaluate the potential for feeder insects to act as fomites for introduction of rodent pathogens. Live Dubia roaches (*Blaptica dubia*), crickets (*Acheta domesticus* and *Gryllobas morio*), mealworms (*Tenebrio molitor*), and superworms (*Zophobas morio*) were obtained from six different vendors and fed to the mice as part of their daily enrichment. Additionally, the colony was evaluated for vivarium-excluded pathogens as part of our rodent quarantine and health surveillance programs. IVC racks were monitored using exhaust air dust (EAD) filters during the 3-month period when the sampled insects were fed to the grasshopper mice. Whole insects, swabs collected from insect shipping containers, and EAD filters were submitted to a commercial diagnostic laboratory for PCR detection of viral, bacterial, fungal, and parasitic agents. Samples from multiple insect species and vendors were PCR-positive for *Klebsiella* spp, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, and *Proteus mirabilis*. Mealworms from one vendor were PCR-positive for mouse parvovirus, which was further speciated as MPV1. Dubia roaches from multiple vendors were PCR-positive for the genus *Campylobacter*; further PCR speciation was negative for *C. coli* and *C. jejuni*. Dubia roaches from multiple vendors were PCR-positive for pinworms; further PCR speciation was negative for *Aspicularis tetraptera*, *Syphacia muris*, and *Syphacia obvelata*. EAD filters tested PCR-negative for the same agents. Further study is needed to determine whether these results represent infectious pathogens or remnant genetic material. Regardless of infectious risk for rodent colonies, the use of live insect feed has the potential to produce positive pathogen results for facilities using environmental PCR testing as part of their rodent health surveillance programs.

### P205 Comparison of Butorphanol-Azaperone-Medetomidine with Tiletamine-Zolazepam-Xylazine For Sedation of Pigs (*Sus Scrofa Domestica*)

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Reliable sedation of pigs for short, non-painful procedures is difficult due to challenges in restraint, injection routes, prolonged recovery times, and insufficient sedation depth. A group of domestic cross pigs required hoof trims under sedation, necessitating a drug combination that could reliably sedate large pigs for 30 minutes to 1 hour without extended recovery times. Pigs weighed 154 to 280 kilograms. Four pigs were sedated with a compounded solution of butorphanol (0.58 mg/kg), azaperone (0.2 mg/kg), and medetomidine (0.23 mg/kg) intramuscular. Two pigs were sedated with a combination of tiletamine-zolazepam (2 mg/kg each) and xylazine (1 mg/kg) intramuscular. Pigs were continuously observed after injection to determine the time to onset of sedation. Heart rate, respiration rate, rectal temperature, and pulse oximetry (spO<sub>2</sub>) were monitored every 5 minutes. Pigs sedated with butorphanol-azaperone-medetomidine were reversed with 2.5 mg naltrexone and 1.1 mg/kg atipamezole intramuscular. Pigs not attempting to stand 10-15 minutes later received an additional 0.55 mg/kg atipamezole. Pigs sedated with tiletamine-zolazepam-xylazine were reversed with 0.1 mg/kg atipamezole intramuscular and if not attempting to stand after 20 minutes received an additional 0.05 mg/kg. Pigs injected with tiletamine-zolazepam-xylazine vocalized and attempted to flee upon



initial injection, whereas pigs receiving butorphanol-azaperone-medetomidine had minimal reaction. Time to onset of sedation and vital parameters during sedation were similar between groups. Pigs sedated with butorphanol-azaperone-medetomidine stood after a mean of 22 minutes, compared to 45 minutes for tiletamine-zolazepam-xylazine. For a 200 kg pig, butorphanol-azaperone-medetomidine requires a 16 ml injection and costs \$101.76, while tiletamine-zolazepam-xylazine requires 10 ml and costs \$346.68. In conclusion, both drug combinations are viable options to achieve sedation for short, non-painful procedures in pigs. Butorphanol-azaperone-medetomidine presents a fully reversible, cost-effective sedation option for large pigs with decreased aversive behavior at time of injection and with shorter recovery time compared to tiletamine-zolazepam-xylazine.

#### **P206 Clinical Management of Complications Secondary to Experimental Administration of Doxorubicin in Swine**

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Doxorubicin is an anthracycline antineoplastic antibiotic used in the treatment of multiple cancer types. Although effective at slowing cancer growth, doxorubicin can cause severe toxicities, such as dose-related cardiotoxicity leading to congestive heart failure. Administration of multiple doses of doxorubicin to swine over several months serves as a model of doxorubicin-induced cardiotoxicity. At our institution, early markers of cardiotoxicity are studied in swine by measuring mitochondrial membrane potential with PET/CT imaging following multiple doxorubicin administrations. This serves as a diagnostic measure for early detection of cardiotoxicity before irreversible damage. Swine are a sensible choice for investigating cardiotoxicity in the laboratory. Yet, animals are also susceptible to myelosuppression, inappetence, weight loss, skin and nail abnormalities, allergic reactions, and extravasation associated with doxorubicin. Through collaboration between the veterinary and investigative teams, a monitoring scheme and strategies for clinical intervention were developed to maintain the health of this swine model, while still achieving study aims. Doxorubicin was administered every 3-4 weeks at 1.95-2.3mg/kg via vascular access port into the jugular vein. Nadir and recovery of blood cells post-administration were monitored through weekly complete blood counts until values stabilized. When leukopenic, animals were monitored for malaise and lethargy, and antibiotics were administered in cases of superficial abrasions. When thrombocytopenic, swine were monitored for petechia, ecchymoses, melena, and fecal occult blood. Suspected gastric ulcers were treated with gastroprotectants. Through close observation, early clinical intervention, and delaying doxorubicin administration until hematologic stabilization, it is possible to achieve study goals in a model with expected hematologic toxicities related to chemotherapeutic administration and their sequelae. Successful management of these animals will support improvements in the safety of doxorubicin use in humans and, by sharing these experiences and strategies, provide other veterinary and investigative teams with the knowledge and tools to maintain animal health during experimental chemotherapeutic administration.

#### **P207 Management of Fur Mites (*Radfordia lemnina*) in California Deer Mice (*Peromyscus californicus*)**

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A closed colony of 2-month to 1-year-old adult male and female *Peromyscus californicus* were imported from an academic research facility and tested positive on a general mite PCR but negative on all species-specific mite PCR assays. Tape tests were performed on 61% (n=54) of the cages, 21% of which were positive for adult mites or viable eggs. Mites were sent for sequencing and identified as *Radfordia lemnina*, for which the natural host is *Microtus pennsylvanicus*. The entire colony was treated with 0.1ml selamectin (5 mg/ml; diluted in 70% ethanol) applied topically to the nape and repeated one month later. A subset of mice, originally identified as positive on tape tests, were targeted for follow-up testing. Tape tests were performed weekly for 15 weeks. Once two consecutive weeks of tape tests were negative, a fur swab PCR was performed, and tape test frequency was reduced to every other week. Offspring generated from the colony were also tested at 12 and 21 weeks via tape tests and PCR. All mice were successfully treated and no gross adverse reactions to selamectin treatment were noted. Following treatment, eggs were identified with decreasing incidence on tape tests and had greatly altered morphology, indicating ovicidal activity of selamectin for this mite. No adult mites were identified after treatment. At 13 weeks post treatment, two negative rounds of tape tests were obtained, although the mite PCR was positive. Scant egg casings (1/week) were intermittently observed after 13 weeks, indicating continued presence of genetic material but not necessarily an active infection. Weaned offspring were negative at both timepoints, providing further support for successful treatment. At 22 weeks, the quarantine was lifted. Adult mice will continue to be tracked until PCR negative.

#### **P208 Presumptive Methyl Methacrylate Toxicity in a Cohort of Northern Tree Shrews (*Tupaia belangeri*) with Cranial Implants**

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Over the course of several months, four tree shrews (3 female and 1 male ranging in age from approximately 2 years to 8 weeks of age) were either euthanized or found dead in the acute post-operative period following a standard cranial pedestal implant surgery using methyl-methacrylate (MMA) routinely performed by the laboratory. For the surgery, animals were sedated with ketamine (100 mg/kg IM) and xylazine (7.5 mg/kg IM), provided pre-operative analgesia (Buprenorphine, 0.02 mg/kg SQ), and maintained on mask isoflurane. Per the laboratory, anesthesia, surgery and recovery for all animals was uneventful. Gross lesions or mortalities were noted 24 hours to 7 days post-operatively and were characterized by erythema and necrosis in the anogenital region. Histologically, animals presented with pneumonitis, and hemorrhagic to necrotizing cystitis. Veterinary staff observed the procedures and evaluated surgical technique, drug dosages, heat support elements, and anesthetic monitoring. All practices were found to be in line with standard recommendations. However, overt fumes were appreciated at that time of observation due to the re-constitution of the MMA occurring on the benchtop in close proximity to the animal under anesthesia and also the recovery area for animals that had already undergone the procedure. Upon investigation, other laboratories on campus were performing this similar procedure in the fume hood. The only pathogenesis that explains the clinical picture (recent cranial implant surgery) and the trio of pathologic lesions (pneumonitis, hematuria/cystitis, and skin irritation/necrosis) is methyl methacrylate toxicity. MMA is rapidly metabolized and is primarily eliminated by the lungs as carbon dioxide through the tricarboxylic acid cycle or as methacrylic acid in the urine. Either directly or via its metabolite (methacrylic acid), MMA functions as a direct irritant to mucosa and skin. While widely regarded as a safe polymer for use in laboratory animals, there is evidence of species-specific variability in sensitivity. Caution should

be utilized when using MMA in a novel species, and appropriate occupational health and safety precautions should be taken at all times.

#### **P209 The Chinchilla Consortium: A Chinchilla Clinical Case Report Gold Mine**

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Chinchillas serve a unique niche in biomedical research as an important laboratory animal model for otologic research. Unlike most of the domesticated species used in biomedical research, many of the resources specific to chinchilla husbandry, care, and medical management lie within the unorganized public domain. A relatively low number of chinchillas are used in research in the U.S. annually which hinders the monitoring of trending clinical cases. With approximately 2,200 chinchillas contributing to research advancements annually in the U.S., few individual institutions have a census that allows veterinarians to see trends in clinical cases. Further, unique cases are frequently siloed due to the lack of applicability to the broader field. The Chinchilla Consortium was founded in 2020 to establish a standard of care and bridge individual institutional knowledge about chinchilla use. The Consortium is currently comprised of 20 institutions contributing to this common goal, including the ability to follow trends of clinical cases with low incidence. Some cases have been noted from multiple institutions and thus considered more common such as dental disease, and subcutaneous abscess formation due to infection with *Streptococcus equi* zooepidemicus. Some common threads are just now starting to be identified between colonies such as idiopathic cardiovascular disease and hormone-related body weight fluctuations in female chinchillas. Finally, some cases are unique within research chinchilla colonies to date and include urolithiasis, umbilical herniation, and neoplasias such as histiocytic sarcoma and lymphosarcomas. The Consortium continues to meet on a quarterly basis to discuss new cases and trends to the benefit of the chinchillas within our care.

#### **P210 Treatment of *Syphacia Obvelata* in a Prairie Vole (*Microtus ochrogaster*) Colony without Breeding Cessation**

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Fenbendazole is a benzimidazole anthelmintic commonly used in biomedical research settings for pinworm elimination in mice, but little data has been reported about its use in the prairie vole. A colony of prairie voles (*Microtus ochrogaster*) tested positive for *Syphacia obvelata* during quarterly sentinel testing of female Crl:CD1(ICR) mice housed on dirty bedding. As a pair-bonded mammal, prairie voles offer a unique model for investigating effects of social hormones. Because maintenance of pair bonds was central to the study team's goals, it was determined that no breeding moratorium

would be imposed during the treatment period. Eradication consisted of a pulse dosing regimen of high-fiber pelleted rabbit chow medicated with fenbendazole (150ppm), along with environmental decontamination and increased biosecurity measures. Treatment efficacy was monitored with weekly in-house tape testing. Confirmatory PCR was performed when all in-house microscopy was negative, which occurred after 9 weeks of pulse dosing. PCR testing was performed twice to ensure the room's negative status, and the outbreak was cleared by the end of a 12-week period. The study team reported no apparent effects on breeding efficiency, litter sizes, or pup weights as compared to baseline values obtained from the same breeding pairs prior to treatment. These data support the efficacy of an approximately 3-month pulse dosing regimen of fenbendazole-medicated feed in the treatment of *Syphacia sp.* in prairie voles while maintaining breeding pairs to preserve experimental aims.

#### **P211 Waterhouse-Friderichsen Syndrome and Acute Death in a Post-Operative Piglet (*Sus Scrofa Domestica*)**

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A singly housed, 7-week-old male Yorkshire/Landrace hybrid piglet (*Sus scrofa domestica*) was noticed to be shaking and warm to the touch. It died minutes later. Three days prior, the piglet underwent a routine cardiopulmonary bypass procedure and was kept overnight on a ventilator. The following morning, the incision was re-opened to control intrathoracic bleeding. The piglet was recovered and profuse diarrhea was noted along with bruising/weakness in the pelvic limbs. A fecal sample was collected for flotation and culture; analysis revealed few *Cystoisospora* organisms and normal fecal flora. Gross necropsy findings included: intrathoracic adhesions, discolored/consolidated cranioventral lung lobes, mottled/pale liver, abundant blood-tinged intrathoracic fluid, approximately 50 mL of yellow peritoneal fluid (cultured positive for *Pseudomonas aeruginosa* and *Enterococcus faecalis*), widespread subcutaneous edema, and enlarged and discolored inguinal/lumbar/thoracic lymph nodes. Histopathology revealed multifocal necrosis and hemorrhage in the liver and adrenal cortices. Multifocal necrosis and mineralization were present in the myocardium and skeletal muscles of the left pelvic limb. Altogether, these findings suggest that death in this piglet was most likely due to a combination of cardiac necrosis and multi-organ dysfunction. The presumed multi-organ dysfunction in light of adrenal cortical hemorrhage is suggestive of Waterhouse-Friderichsen syndrome. This syndrome describes symptoms associated with adrenal insufficiency secondary to adrenal hemorrhage. Multiple etiologies including septicemia are described in human medical literature. While there was no evidence of bacteremia in the examined sections, septicemia would still fit this case presentation. Differentials include procedure-related drugs/methods (notably, heparin), bleeding diathesis, transfusion reactions and fluid overload. This case serves as a reminder to monitor the adrenal glands in patients with sepsis or profound hemorrhage to prevent rapid clinical deterioration.

#### **P212 Sclera-embedded Search Coils: Removal Complication and Successful Treatment**

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The precise monitoring of eye position is an important measure of non-human primate (NHP) gaze and attention for certain primate neuroscience studies. Prior to the development of noninvasive eye-tracking procedures, neuroscientists tracked eye movements using sclera-embedded search coils. These are fine wire coils that are surgically implanted below the surface of the sclera, circumferentially

around each eye. Over time, these coils may become damaged, and must be replaced or removed. With the advent of noninvasive eye-tracking techniques, the use of eye coils has declined significantly. Despite the decline in use, there are many NHPs that still have implanted eye coils that require removal. The current case describes a surgical complication that may result from removing an eye coil, and the subsequent successful management. An 18-year-old male rhesus macaque (*Macaca mulatta*) presented with implanted sclera-search coil wires eroding through the surface of the sclera and skin, at the level of the frontal bone. Surgical removal of bilateral eye coils was performed through a trans-conjunctiva approach. One week post-operatively, a ventral strabismus and adduction elevation deficit developed in the left eye. Through consultation with a human pediatric ophthalmologist, the NHP was diagnosed with acquired Brown's syndrome. Acquired Brown's syndrome (ABS) is described in people and is caused by abnormalities involving the superior oblique tendon-trochlea complex. First-line treatment involves injection of a steroid into the intra trochlea complex. To our knowledge, this is the first documented case and treatment outcome of ABS in an NHP. Two separate injections (triamcinolone, 10mg) performed three weeks apart yielded full resolution of the clinical signs. Resolution was confirmed through non-invasive eye tracking, documenting ability to correctly track visual stimuli. This case presents a potential complication to the removal of sclera-embedded search coils, and a successful non-invasive treatment option for affected rhesus macaques.

#### **P213 Ferret Kit Revival Following Natural Parturition and Hysterectomy**

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Seven time-pregnant jills arrived at a large academic institution to establish a ferret (*Mustela putorius furo*) breeding colony. Three jills exhibited complications, with one case involving maternal rejection and two cases involving dystocia. The jills were approximately 1-year-old, primiparous, and intact. In all three cases, kits required human intervention for revival through manual stimulation and heat support. In the case of maternal rejection, an individual kit was found cyanotic and apneic outside the nest box. Color and respiration improved with manual stimulation and patient warming over 30 minutes. Once revived, the kit was accepted by the jill. One acute (<24 hours) and one chronic (>24 hours) dystocia occurred among the other two jills. The acute case of dystocia failed to respond to medical induction regimens. The chronic case exhibited premature labor over a weekend, and due to length and severity of dystocia, required supportive care prior to surgical intervention. Both cases resulted in hysterectomies; the acute dystocia had no obvious abnormalities and clear fetal movement, while the chronic dystocia exhibited a malodorous, congested, cool-to-the-touch uterine body with no obvious fetal movement. After Caesarean section, kits had their oral and nasal passages cleared, and received manual stimulation for 5-10 minutes, prior to being placed in an incubator. The jill in acute dystocia was recovered from anesthesia and the kits were successfully reintroduced under close supervision. No health issues or instances of cannibalism were observed. The jill in chronic dystocia was euthanized after hysterectomy due to her poor prognosis. The kits retrieved from this case were used in acute studies as per the IACUC-approved protocol. In all cases, kits that received human intervention were revived with a 100% success rate. In conclusion, intervention efforts should be attempted for ferret kits despite what may appear to be a poor prognostic outlook.

#### **P214 Novel Restraint Method for Topical Treatment of California Deer Mice (*Peromyscus californicus*)**

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At our institution, a colony of 109 adult male and female *Peromyscus californicus* required topical treatment for fur mites. This required application to the skin at the nape and access can be difficult given deer mice have a thick fur coat. Although scruffing is an effective and safe restraint method for deer mice, it was not suitable for topical treatment as it would obscure the treatment area. In addition, deer mice can be difficult to handle due to their quickness and jumping ability. Animal care staff may utilize plastic tubs as secondary containment to minimize the opportunity for escape when changing cages. Therefore, we created a novel method of restraint using a modified rat plastic sleeve. Shortening the length of the sleeve and creating a window for treatment proximal to the breathing hole enabled us to access the target treatment location. Utilizing the base of the tail, deer mice were easily moved into the plastic sleeve and the body gently squeezed into the distal end to minimize movement. This technique was quickly learned by all operators with minimal practice. Using a micropipette, the modified sleeve allowed a single user to successfully and accurately apply a topical treatment to deer mice of varying sizes with minimal animal struggling and vocalization. Multiple sleeves were created so that one mouse could remain in the sleeve and be released only after its cage mate was safely contained in a separate sleeve. This ensured each individual mouse was treated when socially housed. Additional benefits included easy sanitation and low cost to manufacture which enabled us to create replacements after multiple uses. The modified plastic sleeve could be easily adjusted for use in other small rodent species for clinical topical treatments or extrapolated to provide restraint for additional procedures.

#### **P215 Polyglucosan Body-Associated Cardiomyopathy in New Zealand White Rabbits: A Case Series**

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Twenty-one New Zealand White rabbits (*Oryctolagus cuniculus*) were acquired from the same vendor for use under anesthesia in terminal or survival procedures (n=12 females), tissue collection (n=7 females), or routine surveillance (n=2 males). Intake physical exams were within normal limits, except for two rabbits presenting with hind limb paresis and severe muscle atrophy. The anesthetized cohort ranged from 5 months – 4 years of age. These rabbits presented as challenging anesthetic patients with variable hypercapnia, dyspnea, and required prolonged postoperative support. The non-anesthetized cohort included the 9 rabbits used for tissue collection and surveillance, which were between 3 months – 3.5 years of age. All rabbits were submitted for postmortem evaluation. On necropsy, 18 (86%) out of 21 rabbits had mild to moderate global cardiac enlargement, 16 (89%) of which were accompanied by serosanguinous pericardial effusion. The same rabbits had moderate to severe pulmonary edema grossly and histologically. Myocardial degeneration and fibrosis, ranging from mild to severe, was noted upon histologic examination. Mild skeletal myofiber degeneration was also noted in 11 (52%) animals. Contained within the cardiomyocytes (17/21, 81%), and rarely the skeletal muscle (3/21, 14%), are scattered basophilic, intracytoplasmic inclusions, which stain Periodic acid-Schiff positive and are resistant to diastase. Samples from the most severely affected rabbits were submitted for electron microscopy (EM). The results of EM confirmed polyglucosan accumulation within the cytoplasm. In this report we describe a novel cardiomyopathy of rabbits with intracytoplasmic inclusions. To the authors' knowledge, this has never been documented in rabbits previously in the primary literature. A total of 44 rabbits were received from this vendor, 21 of which were evaluated postmortem. Though not all animals were necropsied, this suggests a prevalence of at least 48% within the population (21/44). The age range of affected animals, and prevalence within the number of animals

ordered, suggests an early onset, genetic, progressively degenerative disease. Further investigation is required to determine the nature and origin of these inclusions.

#### **P216 Multimodal Anesthetic and Analgesic Regimen for Laparotomy in Pregnant Ferrets**

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A novel study at our institution, involving intra-uterine electroporation (IUE) of embryonic neural tissue, required gestating ferrets (*Mustela putorius furo*) to undergo open laparotomy. Our aim was to develop a multimodal anesthetic regimen that provided appropriate surgical depth without prolonged recovery time, while minimizing clinical and experimental side effects. Jills were 1-year old, primiparous, and weighed approximately 1kg. Four laparotomies were performed mid-gestation for experimental purposes; and one laparotomy with hysterectomy was performed on a naïve, full-term jill in dystocia. Premedication consisted of fentanyl (0.005 mg/kg) and dexmedetomidine (0.03mg/kg) administered subcutaneously in combination. Alfaxalone (5 mg/kg) was administered intramuscularly for induction, with isoflurane and oxygen via mask for maintenance. Analgesia was provided via local anesthetic line blocks (bupivacaine 1%), and subcutaneous extended-release buprenorphine (0.2 mg/kg) and meloxicam (0.2 mg/kg). Supportive measures including subcutaneous fluids and patient warming devices were used. Electrocardiogram (ECG), pulse oximetry (SPO<sub>2</sub>), respiratory rate (RR), heart rate (HR), body temperature, and reflexes were monitored during surgery. Additionally, blood glucose was monitored during the recovery period. Total anesthetic and recovery time averaged 55 and 36 minutes, respectively. HR, RR, SpO<sub>2</sub> and ECG readings remained within reference ranges during surgery and recovery periods. Body temperatures were all within normal limits by recovery. All laparotomies were performed without complication and were followed by short, uneventful recoveries. The jills had good appetites and displayed normal behavior post-operatively, including maternal care and nursing in the hysterectomy case. Seven kits were retrieved via hysterectomy and a total of thirty-seven kits were born after IUE. Twenty-nine kits were used in acute experiments and eight kits were raised beyond weaning with no obvious anatomic or physiologic abnormalities noted. Results suggest that this multimodal approach can be used successfully for anesthesia and analgesia in pregnant ferrets undergoing laparotomy.

#### **P217 Novel Pancreatic Neoplasm in a Female Zebrafish**

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An 8-month-old female un-injected control AB wildtype zebrafish (*Danio rerio*) presented with a severely distended abdomen. The fish had difficulty navigating the vertical water column, remaining near the bottom of the tank, but was otherwise clinically unremarkable as were tankmates. On gross examination, following euthanasia by rapid chilling and formalin fixation, the coelom was markedly enlarged and fluid-filled, 15x30mm in greatest extent. The differential diagnoses included egg-associated inflammation and fibroplasia, neoplasia, and infection. Histopathology identified a partially encapsulated, well-demarcated, multilobular and centrally cystic mass arising from pancreas. The mass was adhered to the viscera and body wall, but was not infiltrative. Lobules were formed by polygonal cells irregularly arranged in dense sheets and occasionally distinct small acini, separated by a fine, lacy fibrovascular stroma.

Cells exhibited moderate anisokaryosis and anisocytosis, and had abundant eosinophilic cytoplasmic granules (zymogen), moderately sized oval nuclei, fine chromatin, small nucleoli, and rare mitoses. Masson's Trichrome and periodic acid Schiff stains highlighted stromal and capsular collagen, cytoplasmic granules, and low numbers of rodlet cells in capsule and mesentery. Mammalian immunohistochemical cytokeratin stains did not perform as expected in normal and neoplastic zebrafish tissues. This pancreatic neoplasm was diagnosed as a likely exocrine adenoma, given only mild atypia and a lack of invasion, falling between hyperplasia and carcinoma. It is considered incidental to the health of the remaining fish cohort. While pancreatic ductal tumors and hyperplasia are common in zebrafish, only a few cases of exocrine pancreatic neoplasia have been reported and only in other teleosts. This case presents a rare neoplasm not previously described in zebrafish.

#### **P218 Prolonged Analgesic Regimen for Intramuscular Injection Associated Myositis in C57BL/6JN Mice**

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On experimental day 0, ten four-month-old male C57BL/6JN mice were anesthetized with isoflurane for bilateral intramuscular (IM) injections in the gastrocnemius. 50uL of mitochondrial isolates (MITO) were given in the right limb and 50uL of vehicle (300 mM sucrose, 10 mM K-HEPES, and 1 mM K-EGTA) in the left limb. Subcutaneous ketoprofen (5mg/kg) was administered. Recovery was uneventful and mice were ambulatory. Day 1, all mice presented with severely swollen right gastrocnemius and resistance to extension of the right limb. The left gastrocnemius was moderately swollen. Five mice in a single cage were lethargic and were euthanized. The second cage of five mice were otherwise active. Day 2, one mouse was dead. Four mice were lethargic to moribund and were euthanized. The differential diagnosis included sepsis, irritation of injectables, or iatrogenic trauma from injection technique. One mouse was necropsied; bacterial culture of the right gastrocnemius grew *Achromobacter xylosoxidans/denitrificans* and *Micrococcus luteus*. The skeletal muscle of the right pelvic limb had locally extensive, moderate, necrosuppurative myositis with intralesional bacteria confirmed by gram stain of the tissue. The left pelvic limb muscle had no detectable bacteria and milder inflammatory infiltration. Bacterial contamination during MITO preparation was the likely cause of decline. A follow up pilot with significant MITO preparation modifications was performed to determine if vehicle administration caused additional irritation beyond expected IM injection inflammation. One group received IM injections with vehicle in the gastrocnemius and MITO in the contralateral limb. A second group received IM injections with saline and vehicle. The mice received ketoprofen daily and were euthanized after 3 days. All mice presented with mild bilateral swelling of the gastrocnemius but were active. One mouse from each group was necropsied. Bacterial blood cultures resulted in no growth. MITO and vehicle injection sites presented with mild histiocytic myositis and more inflammation compared to saline injected tissue. These findings indicated vehicle administration was associated with increased irritation and updates were made for mice under this study to receive NSAID treatment for three days.

#### **P219 Tail Necrosis Following Jugular Cannulation in Rats Associated with Inadequate Thermal Support**

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Five out of eight Sprague Dawley rats (*Rattus norvegicus*) presented

with tail necrosis within 24-48 hours following a surgery consisting of an intracranial injection and jugular vein catheterization. Approximately one-half to two-thirds of the distal portion of the tail was grey/black in color and felt cool to the touch. Two of the rats had self-amputated the necrotic portion prior to discovery and were monitored for healing. Three rats had tail amputations performed proximal to the necrotic area by Veterinary Services. Post-operatively, these rats received meloxicam (2mg/kg, subcutaneous or oral, every 24 hours) for 2 days post-surgery as per the research protocol. To prevent infection rats were provided with 5g, bacon-flavored tablets containing 2mg enrofloxacin for 5 days. One rat also exhibited dark discoloration of two toes on the left hind paw which were monitored until study endpoint, 3 weeks after surgery. Gross examination of the amputated portions of the tails revealed darkened tissues consistent with necrosis. The lateral tail veins were dissected and blood inside was visibly thickened and coagulated. It was determined that while heat support was provided under the body, it was not provided to the tail itself. In addition, the surgeon had been recently trained and was relatively new to the jugular cannulation procedure, which extended the surgery time. Per the surgeon, jugular catheterizations took anywhere from 40 minutes to 85 minutes. Longer surgery times were also associated with the incidence of necrosis. For the next four animals in the study, a new thermal support set-up was employed, and the surgeon was careful to ensure that tails were on the heating pad. These rats had an uneventful recovery and no signs of tail necrosis. Blood vessel constriction and increased blood viscosity have both been associated with hypothermia. Provision of adequate thermal support, including the tail which is a key regulator of body temperature in rats, was found to be critical to prevent this complication and should be considered essential, especially for surgeries of longer duration.

#### **P220 Infiltrative Lipoma in a Zebra Finch (*Taeniopygia guttata*)**

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An aged adult male zebra finch presented with moderate ataxia, bilateral conscious proprioceptive deficits and decreased motor function in the legs. The researcher was concerned that the bird was being bullied and removed it from the common aviary to a cage by itself for monitoring. Other differential diagnoses included: neoplasia, kidney enlargement, other trauma, spinal issues (including disc prolapse and degenerative joint disease) and nutritional disease (high fat diet). However, given the fact there were feathers missing and the animal was on a balanced diet varying in composition of seeds, boiled eggs and fresh produce, bullying was suspected. After a few weeks of monitoring with no improvement, the bird was euthanized and presented for necropsy. The bird was in good body condition and mild postmortem autolysis. There was minimal urate staining around the cloaca. Tail feathers were small, immature and broken. Samples were fixed in 10% NBF for histology. Sagittal sections of the spinal cord at the sacral level revealed well differentiated adipocytes compressing and invading the spinal cord. No mitotic figures were noted in the adipocytes. An infiltrative lipoma was diagnosed as both local compression and infiltration were present. Infiltrative lipomas are uncommon neoplasms but have been reported in the spinal cord of various species. However, documented reports of an infiltrative lipoma of the spinal cord of zebra finches were not found in the literature. This is the first report in a zebra finch. Other histologic lesions included: Moderate, diffuse, hepatocellular hydropic and microvesicular change, invasion of proventricular isthmus koilin and glands with yeast organism whose morphology is consistent with *Macrorhabdus ornithogaster*. This case highlights the necessity of necropsy and histology for accurate diagnosis, even when fighting is suspected.

#### **P221 Topical Fluralaner to Treat Chicken Body Lice (*Menacanthus stramineus*) in a Biomedical Research Setting**

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Infestations of lice and other blood-feeding ectoparasites present clinical risks for poultry flocks, contributing to agricultural production loss and confounding variables in the laboratory setting. The antiparasitic drug fluralaner has been approved for oral administration in agricultural poultry in the European Union and Australia but is limited to companion animals in the United States. Twenty, 41-week-old Buff Orpington hens (*Gallus gallus domesticus*) housed indoors for use on an IACUC-approved biomedical study presented no clinical symptoms from a naturally occurring infestation of chicken body lice (*Menacanthus stramineus*). Initial management with diatomaceous earth dust baths was unsuccessful in parasitic control. Pruritus, indicated by minor pericloacal self-excoriation, and increased infestation prompted medical treatment. Under veterinary guidance in accordance with AMDUCA regulations, investigators opted for an extra-label fluralaner trial to assess efficacy against poultry lice. Topical 250 mg fluralaner solution (Bravecto for Cats) was diluted to 12.5 mg/mL in 100% acetone. Individuals were weighed and administered a one-time, interscapular, topical dose at 1 mg/kg. On Day 1 (dosing), eggs and adult specimens were collected from each pen for taxonomic identification, vent regions were photographed, and clinical presentation was assessed. These steps were repeated at set intervals for 30 days while chickens remained on their primary study. A blinded observer evaluated randomized vent images to assess drug efficacy on parasitic presence. Images were categorized as: 1 (no visible lice), 2 (low to moderate number of lice), and 3 (large number of lice). By Day 5, statistical analysis of the non-normal distribution indicated significant difference in ectoparasite presence. By Day 8, all chickens were placed into Category 1. Fluralaner safely eliminated the infestation on all individuals without detrimental effects or observed recurrence. This treatment allowed for individualized application feasible for a small colony in a controlled research setting. Recurrence in outdoor or open systems should be considered and regularly scheduled preventative dosing should be investigated in the context of egg and meat withdrawal parameters.

#### **P222 An Unusual Outcome Secondary to Indwelling Intrathecal Catheterization in a Rhesus Macaque (*Macaca Mulatta*)**

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An 18-year-old, male rhesus macaque presented for acute onset lethargy, inappetence and depressed mentation. The animal had an intrathecal (IT) catheter and port placed 6 years earlier under fluoroscopic guidance. The day prior to presentation, the animal received IT morphine without complications and was reported to be alert and responsive. Physical exam at presentation was unremarkable. Abdominal radiographs revealed feces in the intestines and spondylosis of the lumbar vertebrae. Gastro-intestinal stasis secondary to opioid administration was suspected, the animal was treated with LRS fluids and Maropitant. Complete blood count (CBC) revealed neutrophilic leukocytosis (WBC 19 K/uL, neutrophils 18.04 K/uL) with toxic changes. The animal continued to be lethargic, so a culture of the IT port was obtained and a course of Enrofloxacin was started due to suspicion of infection. The culture was positive for multidrug resistant *Enterobacter* sp. Through the week, the animal's mentation improved but was persistently hyporexic. A cage side exam revealed bilateral paresis of the hindlimbs, and a sedated exam pain on palpation of the caudo-lumbar spine, most notable at the IT port. A

CT scan showed several areas between L1-L5 where the IT catheter appeared to be traversing the parenchyma of the spinal cord, degenerative changes within the dorsal vertebral column and potential osteomyelitis or mineralized soft tissue in L7. A repeat CBC showed persistent neutrophilic leukocytosis (WBC 15.9 K/uL, neutrophils 11.9 K/uL). Given CT results, neurologic deficits and pain, euthanasia was elected. Gross necropsy and microdissection determined that meningeal fibrosis and reorganization of the spinal cord nerves caused the IT catheter to be drawn inward and entrapped by nerves and fibrotic tissue, making the catheter to appear centralized within the parenchyma. With histology it was established that with the passage of time, chronic inflammation, and fibrosis, along with reorganization surrounding nerve roots caused cord compression and subsequent axonal degeneration. These findings suggest that with the passage of time tissue remodeling due to inflammation could result in relocation of an IT catheter in animals with chronic indwelling catheters, thus these animals should be closely monitored.

#### **P223 Enhanced Surgical Site Infection Prevention Protocols Improve Outcomes in Swine (*sus scrofa domestica*)**

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Two, ~1.5-month-old, 6 kg, male Yucatan pigs housed in raised floor runs underwent survival surgical procedures to test a growth adaptive pediatric pulmonary valve device. Standard peri-operative procedures including administration of ceftiofur and pre-operative incisional site antisepsis (scrub) with chlorhexidine gluconate (CHG) were used however, between 1 and 2 weeks post-op these animals developed surgical site infections (SSI). Wound culture revealed heavy growth of *Trueperella pyogenes*, an environmentally ubiquitous commensal bacteria of the skin and mucous membranes commonly found in the upper respiratory and urogenital tracts of this species thought to cause opportunistic infections. Incisional infections were refractory to systemic broad-spectrum antibiotics and wound management ultimately resulting in early termination of these pigs from study. Considering the organism cultured, we focused on two areas to prevent similar issues (1) peri-operative procedures and (2) post-operative wound management and implemented enhanced protocols with the next two study animals. This multipronged enhanced protocol included (1) presurgical bathing of animals with CHG wipes and packing of hooves with CHG soaked gauze during surgery, (2) use of tulathromycin as the peri-operative antibiotic based on diagnostic lab recommendations, (3) use of improved bandaging and jackets to better protect surgical wounds and indwelling catheters from post-operative contamination and lastly (4) additional CHG bathing at the time of sedation for catheter removal ~3-5 days after surgery. This enhanced, SSI prevention protocol prevented any gross incisional infection in both animals who went on to survive to their intended endpoint. Given the successful outcomes we plan to apply this enhanced SSI prevention protocol moving forward in all swine undergoing higher risk procedures to improve survivability to study endpoint and overall animal health which is a species adaption of procedures used in human cardiothoracic surgery.

#### **P224 Diagnosis & Treatment of Dermatophytosis in a Gottingen Minipig**

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A ~1 year old, intact female Gottingen Minipig (*Sus scrofa domestica*) presented with mild skin irritation in the left periocular region. On physical examination, the skin around the left eye was erythemic and swollen with evidence of pruritus. The pig recently had surgery and was on meloxicam as part of the approved protocol, but systemic

diphenhydramine and topical triple antibiotic ophthalmic ointment were initiated due to signs of inflammation. Despite treatment, the pig's clinical signs progressed and a thick brown discharge began to build up around the affected area along with pustules and the lesions spread to the other side of the face. Due to the lack of improvement, the pig was sedated for examination. The hair on both sides of the face was shaved and the area thoroughly cleaned with chlorhexidine, revealing multifocal to coalescing areas of raised, erythemic skin. Two punch biopsies of the affected areas were taken and sent to a diagnostic laboratory for histopathology. Pathology identified pustular dermatitis along with GMS-positive fungal hyphae and arthrospores in the superficial crusts consistent with dermatophytosis. Although dermatophytosis is considered self-limiting in swine, there is a zoonotic concern for personnel and other animals so treatment was elected. Oral fluconazole was initiated along with injectable ceftiofur crystalline-free acid for dermatophytosis and secondary bacterial infections, respectively. The affected areas were cleaned with chlorhexidine/ketoconazole wipes and topical terbinafine cream as needed. Over the course of 2-3 weeks, the skin began to have less buildup of debris and pruritus had resolved. Treatment lasted for ~6 weeks after which the pig was euthanized at its experimental endpoint. At necropsy, punch biopsies of the previously affected skin were taken to evaluate treatment efficacy. No fungal organisms or other infectious agents were noted. Although uncommon in the laboratory setting, this case highlights the importance of dermatophytosis as a differential diagnosis for erythemic skin lesions with brown crusts in swine.

#### **P225 Zinc Concentrations in Laboratory Society Finches (*Lonchura striata domestica*) and Enrichment Items**

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An institution maintains a colony of society finches (*Lonchura striata domestica*). A bird presented for evaluation of a head tilt. Physical examination revealed a head tilt and horizontal nystagmus. Rule outs included traumatic injury, infection of the middle/inner ear, and intracranial neoplasia. Treatment consisted of 1.0 mg/kg oral Meloxicam SID for three days and 50 mg/kg oral TMS SID for five days. No improvement of clinical signs was observed. A colony history of feather loss and galvanized caging made zinc toxicosis a differential diagnosis. In days following, eleven healthy finches, and the clinically affected bird, were humanely euthanized. Samples were collected for toxicologic and histopathologic screening. Sources of zinc included grit, caging, or toys. Sample items were sent for toxicologic screening. In the clinically affected bird, histopathologic findings included yolk coelomitis, mild rhinitis, and intramedullary hemorrhage of the calvarium. No meningeal or cerebral changes corresponded with this hemorrhage. Histopathologic examination of the pancreas showed no evidence of zinc toxicosis. Toxicology revealed elevated plasma and kidney zinc concentrations (normal plasma range: 0.8-2.5 ppm). The final diagnosis is inconclusive as histopathologic findings could not be associated with clinical signs. Of the eleven healthy birds, seven had elevated plasma zinc levels. Three birds (two with zinc plasma concentrations >2.5 ppm, and one with a concentration of 1.7 ppm), had pancreases sampled for histopathology. No overt evidence of zinc toxicosis was observed. Samples of the grit and bell had zinc levels within an acceptable range (based off parrot feed). The metal clasp on the toy had high levels of zinc at 18.2%. Heavy metal toxicities are more commonly seen in larger birds due to destruction of toys and caging elements, but are less often observed in smaller birds. The samples from the colony gave a unique opportunity to estimate zinc exposure levels and highlights the varying zinc concentrations found in bird toys. Removal of toys with metal clasps and bells was implemented. Due to the varying zinc concentrations present in bird toys, zinc toxicosis

as a differential diagnosis should not be overlooked in cases of neurologic birds with feather loss.

### **P226 *Ophionyssus natricis* Infestation in a Laboratory Hatchling Corn Snake (*Pantherophis guttatus*)**

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A hatchling corn snake (*Pantherophis guttatus*) obtained from a commercial vendor was housed in a biomedical institution. This snake was evaluated for lethargy. On physical examination, the patient had mildly decreased skin turgor. Preceding this clinical presentation, the snake's health had been unremarkable for the three months since arrival. Upon entry into the institution, the snake underwent a 2-week quarantine period where it received prophylactic antiparasitic treatment consisting of 50 mg/kg oral metronidazole (days 1 and 14 of quarantine) and a 5 mg/L ivermectin bath (days 1, 7, and 14 of quarantine). A warm water bath was provided to aid in rehydration which resulted in a substantial number of minute parasites emerging from the scales. A sample of the parasites was examined microscopically. Examination revealed the parasites to be mites. Upon returning to the patient for continued treatment, the snake was found deceased. Necropsy findings revealed an overall pallor of the tissues. Histopathologic findings included skin lesions associated with chronic acariasis, serous atrophy of fat of the heart, and diffuse, chronic hepatocellular atrophy of the liver, indicating a negative energy balance despite the presence of partially digested food in the GI tract. Colleagues at the local zoo aided in speciation of the mite as *Ophionyssus natricis*. All reptiles in the colony were examined and prophylactically treated with topical antiparasitic spray (fipronil). All enclosures and the housing room were sanitized. It is suspected that the mites gained entry into the vivarium through the bedding material. Therefore, institutional practices were revised so that bedding is autoclaved before use, enclosure components are run through cagewash, and prophylactic antiparasitic treatment of the growing snakes was increased from semi-annually to quarterly. This case provides an example of the severe debilitation that can result from infestations of *Ophionyssus natricis*, particularly in a growing animal. It also highlights challenges associated with managing this unique species in a research setting. Although infestations of *Ophionyssus natricis* are seldomly seen in research institutions, it should not be overlooked as a differential diagnosis in lethargic snakes.

### **P227 Malignant Sertoli Cell Tumor with Pulmonary Metastasis Originating from Cryptorchidism in Common Marmoset**

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A 4-year-old, intact male common marmoset (*Callithrix jacchus*) cried strangely when held during a routine physical examination and subsequently presented labored respiration. Thoracic radiographs revealed soft tissue opacity throughout the thoracic cavity and multiple large nodular masses diffused in the lungs. It was humanely euthanized because of severe dyspnea. Gross necropsy findings included a hemorrhagic pleural effusion, white nodular masses of varying sizes throughout the lungs, and mediastinal lymph node swelling. Moreover, unilateral abdominal cryptorchidism with white sclerosis atrophy was observed on the right side of the bladder. Histologically, the hidden testis revealed loss of the structure of the convoluted seminiferous tubules and irregular lobules separated by a well-developed fibrovascular stroma. The irregular lobules consisted of cuboidal to columnar neoplastic cells which formed palisade- or sheet-like structures. Neoplastic cells had round nuclei with one to several distinct nucleoli and relatively abundant acidophilic cytoplasm. Necrosis with mineral deposition was frequently

observed in the center of sheet-like growth areas. Immunohistochemistry of the lungs and hidden testis showed that the neoplastic cells were positive for the Sertoli cell markers, vimentin and WT-1, and negative for Leydig cell marker, calretinin and seminiferous epithelium markers, c-KIT and Oct-3/4. Ultrastructurally, the neoplastic cells showed nuclear atypia with typical, deep nuclear envelope indentations. Based on these features, we diagnosed that dyspnea in this common marmoset was caused by the pulmonary metastasis of a malignant Sertoli cell tumor originating from the right hidden testis. Sertoli cell tumors rarely occur in humans and nonhuman primates, although common in dogs. To our knowledge, this is the first report of a malignant Sertoli cell tumor with metastasis in a nonhuman primate, the common marmoset.

### **P228 Diet-Associated Dermatitis in a Swine Model of Nonalcoholic Steatohepatitis**

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Nonalcoholic steatohepatitis (NASH), an aggressive subtype of nonalcoholic fatty liver disease, affects 5% of Americans. Effective treatment for NASH is limited and affected individuals are prone to developing cirrhosis and liver failure. As a novel model of NASH, the adverse effects of inducing disease in swine have yet to be fully characterized. Twelve, ten-week-old female Gottingen and domestic pigs were started on a high fat, L-amino acid defined, choline-deficient diet to induce NASH. Eleven of the twelve study animals developed multifocal pustular dermatitis after one to six weeks on study diet. Initial lesions were present on the face, chin, and axilla, with dermatitis ultimately spreading to nearly the entire body. Differentials included pyoderma, either spontaneous or secondary to study diet, pityriasis rosea, and dermatophytosis. Daily treatment of affected skin with topical products, including Mal-A-Ket, TrizChlor, and MiconHex + Triz, and empirical antibiotics (ceftiofur crystalline-free acid, 20 mg/kg once, enrofloxacin 7.5 mg/kg daily) was ineffective. Chlorohexidine baths with targeted antibiotic therapy (amoxicillin/clavulanic acid 20 mg/kg twice daily) based on culture and sensitivity for severe cases was effective at resolving existing lesions and preventing dermatitis spread. An increase in number of days to lesion development from diet initiation was noted in pre-emptively bathed animals. An investigation into the underlying etiology included punch biopsies and culture swabs of affected regions, biweekly complete blood counts, and paired blood cultures. Histopathology from multiple affected animals demonstrated perivascular cuffing by eosinophils, suggestive of a hypersensitivity reaction to the diet with secondary bacterial and fungal dermatitis. The diet ingredient that incited the hypersensitivity reaction was not identified. Culture swabs of affected animals demonstrated opportunistic pathogens consistent with normal skin flora and fecal contaminants while blood culture and complete blood counts were unremarkable. Unique study diets can predispose animals to dermatitis secondary to hypersensitivity reactions, bathing and targeted antibiotic therapy is an appropriate treatment for suspected diet-induced hypersensitivity dermatitis.

### **P229 *Cynomolgus* Macaque (*Macaca Fascicularis*) Imported to the United States from Cambodia with Melioidosis**

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Melioidosis, potentially fatal infectious disease that can infect humans and animals, including NHPs, is caused by the high-consequence pathogen, *Burkholderia pseudomallei*. This environmental bacterium is found in soil/water of tropical regions, such as

Southeast Asia, where it is endemic. A 2-year-old, 1.6 kg macaque imported into the U.S. from Cambodia for use in biomedical research presented with a 1x1 cm subdermal mass on dorsal aspect of the lumbosacral region during routine CDC processing. A week later, mass increased in size and visibility. Area around abscess/mass was clipped/cleaned and aseptically prepped, a 2 cm incision made on mass and was flushed with saline. Abscess expelled mucopurulent contents. The incision was then sutured closed. Post surgery, table and all areas were disinfected with Wexcid 128. Samples were collected and sent to VRL lab in San Antonio for culture/sensitivity. Animal's condition deteriorated quickly and presented with quiet behavior, was inappetent, lethargic and dehydrated with left head tilt. Treatments were started – Dexamethasone, Baytril, PPG & LRS IV. Two days post treatments, the animal stabilized. Bacterial isolate reported as *Burkholderia cepacia* sensitive to trimeth/sulfa. Treatment with TMS was initiated. Due to the potential for misidentification of *Bpseudomallei* and because the NHP originated in a melioidosis-endemic region, CDC requested the bacteria isolate be submitted to Texas-DSHS Laboratory in Austin for confirmation. Upon confirmation of *B.pseudomallei*, macaque was euthanized and a full necropsy performed. Gross pathology, multifocal smooth/round, cream-colored, liquid filled lesions on liver w/ omental adhesions. Spleen was solitary, off white-color plaque with an omental adhesion. Cream-colored, lesions were seen in right, middle and caudal lung lobes and spine was 8 mm in diameter lesion between 6/7th thoracic vertebra. Formalin-fixed samples submitted to the CDC for histopathologic analysis. This is the first documented case diagnosed during CDC-mandated post-importation quarantine-period. Euthanasia is recommended, instead of treatment due to concerns about zoonotic transmission, long treatment time, chance of recurrence and in non-endemic areas, or possibility of introducing the agent into the environment.

#### **P230 Pharmacokinetic Study of Subcutaneous Buprenorphine Administration in the Jamaican Fruit Bat (*Artibeus Jamaicensis*)**

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The Jamaican fruit bat (*Artibeus jamaicensis*) is a valuable model for the study of infectious diseases due to their size and ability to harbor viruses without showing clinical signs of disease. Given their important role in research, they may frequently undergo procedures that require analgesia, which is poorly researched in these species. This study aimed to determine the pharmacokinetics of a single subcutaneous buprenorphine injection in the Jamaican fruit bat to guide future analgesia studies. Twenty-one bats were administered 0.5 mg/kg buprenorphine subcutaneously (Buprenorphine 0.3mg/mL). At 1, 2, 4, 8, 12, and 24 hours 0.3 mL of blood was collected non-terminally from their cephalic veins, stored at -80° C, and then analyzed routinely for buprenorphine plasma concentrations. Three of the twenty-one bats were collected at each timepoint and then returned to the colony. Three bats were euthanized for intracardiac sample collection to provide baseline values. The pharmacokinetic analysis via liquid chromatography and mass spectrometry showed that the maximum concentration occurred at 1 hour after injection, with a maximum concentration of 22.6 ng/mL +/- 0.52. The area under the curve last was 94.9 h\*ng/mL +/- 8.80. It dropped below the purported therapeutic plasma concentration of 1.0 ng/mL at approximately 12 hours. This indicates that a 0.5 mg/kg subcutaneous injection of buprenorphine in the Jamaican fruit bat will provide analgesia for 8-12 hours. No mortality was detected in any bats, indicating that this was a safe dose.

#### **P231 Comparison of the Fecal Microbiota of Spiny Mice (*Acomys Spp*) by Sex and Diabetic Status**

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Spiny mice (*Acomys spp.*) are gaining in popularity as a research model, primarily due to their ability to regenerate tissue. To date, they have shown unique regenerative capabilities of muscle, skin, the spinal cord, cardiac myocytes, and kidney structure and function. The housing and care of these animals, however, requires special consideration. They require warm temperatures, special handling due to tail self-amputation when threatened, and readily develop type II diabetes mellitus (T2DM) when maintained on standard rodent chow. At our institution, approximately 30% of our spiny mouse colony have developed T2DM on *ad libitum* standard rodent chow. In refining our practices, we switched them to a diet consisting of 10:3:1 ratio of finch, insectivorous, and grass seeds, respectively, along with fresh produce and meal worms. Breeding pairs are supplemented with a small amount of high protein rodent chow. Prior to the change in diet, we collected fecal samples (n=54) and submitted for whole genome shotgun sequencing of the fecal bacterial microbiota. We compared by sex, diabetic status, and both (MDM/FDM=males/females with T2DM, n=9 and 6, respectively; MN/FN=normal males/females, n= 13, and 20, respectively), as determined by urine glucose results. The greatest strength of differences in alpha diversity (richness, evenness, and diversity) and in number of differences in species, were between MDM and FN, followed closely behind by MN vs FN, then MDM vs FDM. Of the 20 most relatively abundant (RA) species, two species of *Muribaculum* and *Duncanella*, and one of *Turicibacter* were significantly lower in T2DM animals (p<0.05), a finding previously documented in other rodent T2DM models. Females had a higher RA of *Helicobacter ganmani* and a single species of *Prevotella* and *Muribaculum*. Additionally, males had significantly lower richness, evenness, and diversity than females (p<0.005), while there were no differences in alpha diversity between T2DM and non-T2DM animals. Taken together, this suggests that sex may have a stronger influence on the fecal microbiome than diabetic status. Future plans include a comparison after the diet change, as well as examining whether T2DM can be reversed and have an associated change in microbiome with diet change in this species.

#### **P232 Normal Serum Chemistry Reference Ranges for the Jamaican Fruit Bat (*Artibeus jamaicensis*)**

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The Jamaican fruit bat (*Artibeus jamaicensis*) is a natural host for many viral diseases and has been used as an experimental model to study Middle East respiratory syndrome virus, dengue virus, Zika virus, rabies virus, influenza virus, tacaribe virus and most recently SARS-CoV2. There is much interest in their unique immune systems which allow bats to harbor and transmit disease without significant clinical impact on themselves. Many studies evaluate changes in serum chemistry as a measure of disease impact, however there are no established reference values for the Jamaican fruit bat. In this study, we aimed to define baseline parameters from our closed bat colony. Seventy-three adult bats (23 males and 50 females) were randomly selected and euthanized by isoflurane overdose and blood collected by cardiocentesis. Serum samples were routinely processed using a Cobas c501 and commercially available methods. Two samples were excluded as they could not be analyzed due to fibrin clots. Reference intervals for both sexes were established using the Reference Value Advisor 2.1 macro for excel and the nonparametric method, according to current guidelines. When compared against reference values for CD-1 adult mice, most notably bats had much lower cholesterol and total bilirubin, and much higher globulin, ALT, AST and GGT values. Differences between sexes were minimal. This information adds to our knowledge on normal physiological parameters of this species and will serve as a useful guide for future



work performed on Jamaican fruit bats.

### **P233 Suspect Mucoïd Enteropathy Outbreak in New Zealand White Rabbits**

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An outbreak of suspect mucoïd enteropathy occurred in 20 New Zealand White rabbits used in an orthopedic study. All rabbits affected were adult females, between 9 and 15 months. All rabbits were from the same vendor. All were on study prior to presentation with signs, and had not recently arrived. The initial 11 rabbits presented with anorexia, mucoïd diarrhea, and/or dehydration. Eight rabbits were treated with oral trimethoprim sulfa for 1 week (30 mg/kg, q12h), subcutaneous Lactated Ringer Solution, oral metronidazole (35.7 mg/kg, q12h), transdermal mirtazapine (0.1 mg/kg) daily, oral vitamin B12 (300 mcg, q24h), and penicillin G procaine (PPG) intramuscularly (50,000 IU/kg) once. Four did not respond to treatment and were found dead or euthanized due to disease progression. Two rabbits presented acutely and were euthanized due to severity of clinical signs: hypothermia, severe dehydration, and dull mentation. One rabbit was found dead. The housing area was thoroughly decontaminated with disinfectant and cages sanitized. An additional 9 rabbits were affected approximately one month later. They were treated with PPG, subcutaneous maropitant (4 mg/kg) once, and either oral (300 mcg daily) or subcutaneous vitamin B12 (500 mcg, once). Eight out of these nine rabbits recovered, and one was found dead. All affected rabbits were also provided with supplemental fiber once daily, and ab libitum hay daily. Vital signs, food and water consumption, fecal output, and fecal quality were monitored daily. Necropsy was performed on 7 rabbits and intraluminal mucous was seen grossly. Histopathology revealed intestinal goblet cell hyperplasia. Several rabbits had additional histologic findings including multifocal necrotizing bronchopneumonia, typhlitis, and interstitial myocarditis with cardiomyocyte degeneration and necrosis. Three rabbits were diagnosed with mucoïd enteropathy from necropsy reports, the remaining reports were suspected mucoïd enteropathy. Intestinal cultures were performed and heavy growth of *Clostridium perfringens* was found in three rabbits. This case report provides insight about potential treatment options for mucoïd enteropathy. Although prognosis is poor, aggressive treatment with the described treatments can facilitate recovery.

### **P234 Optimization of Intraarticular Administration in Rabbits Using Fluoroscopic Guidance**

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Inflammatory joint conditions are common diseases that affect millions of people. Some of the most common diseases are osteoarthritis, rheumatoid arthritis, and psoriatic arthritis. These diseases are characterized by joint pain, stiffness, and swelling due to damage of the joint. Intraarticular delivery is a route used to deliver potential new therapies for inflammatory joint conditions, which include synovial fluid replacement, stem cells, and viral vectors. When training to dose rabbits via this route, we aimed to reduce the probability of misdosing and find a maximum volume that can be dosed into the joint space without risk of leakage. To accomplish this, we dosed both male and female Dutch Belted rabbits with a weight range of 1.6 kg to 2.1 kg. The animals were anesthetized via Sevoflurane inhalation and placed in a position conducive to the individual's preferred dosing hand. The dose site was shaved and scrubbed with chlorhexidine solution and the hind limb was bent to allow the indents on the sides of the knee to be located. The needle was then gently inserted at a 45-degree angle towards the femur, through the space at either side of the patellar ligament. The needle

dropped into the joint with no resistance until the needle rested against the femur. This dosing angle reduces the probability of misdosing by eliminating the possibility of inserting the needle too far. We used contrast agent and fluoroscopic images to train individuals on this method. This allowed us to confirm proper administration and determine a maximum dose volume for consistently successful dosing. Using these tools, we found that up to 0.75 mls can be dosed with no visible leakage. No side effects, aside from mild stiffness for the first 48 hours following the dose, were noted upon recovery. There is anecdotal evidence that higher dose volumes are possible, but the probability of leakage increases.

### **P235 Clinicopathological Characterizations of Post-Surgical Vascular Access Button (VAB) Sequelae in Rats**

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The surgical implantation of intra-vascular catheters for chronic direct vascular access has produced an essential animal model in rodent preclinical research, with recent refinements leading to the design of subcutaneous vascular access buttons (VABs). Despite their wide availability and use, a published report describing the sequelae and interventional therapies for VAB placement complications is lacking in the current literature. This case series describes the clinical and histopathological post-surgical sequelae in Wistar rats derived from two commercial suppliers with dual vascularized (jugular and femoral vein) VABs. All VABs were commercially available marketed devices. Post-arrival, approximately 80% of surgically implanted rats presented with varying degrees of peri-circumferential button surgical site dehiscence, seromas, and abscess formation under the button platforms. Treatment options and interventional strategies attempted included removal of hiding devices, individual housing, nail trimming, topical antibiotic ointment application, oral gel supplementation, systemic antibiotic therapy, systemic anti-inflammatory therapy and topical hydrogel wound sealant. Despite these varied treatments and interventional strategies, all affected animals required humane euthanasia due to the progression of lesions. Histopathology of the affected button surgical sites was performed for all euthanized animals, with lesions ranging from moderate to full-thickness ulcerative necrosis of the epidermis and dermis subjacent to the buttons, abscessation with mixed populations of bacteria, varying degrees of seroma formation and collagen necrosis within the felt pad used for anchoring. The reported use of surgical glue during the implantation procedure in two groups had no significant effect on the degree of ulceration and necrosis observed histologically.

### **P236 Intestinal Adenocarcinoma in a Rhesus Macaque: A Gut Feeling**

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A 17-year-old, 9.7 kg Rhesus Macaque (*Macaca Mulatta*) housed in a general compound presented multiple times to the vet staff for weight loss and dehydration. The physical exam revealed a large palpable mass located within the abdominal cavity. Possible differentials for an abdominal mass include adenocarcinomas, endometriomas, lipomas or a large cyst. Due to a poor prognosis and a low quality of life the animal was then euthanized and submitted to necropsy for a postmortem examination. This examination confirmed the abdominal mass to be a multinodular mass associated with the ileocecal juncture, the liver was patchy and enlarged with evidence of amyloid as well as the adrenal gland. One cut section of the ileocecal junction was infiltrated by multiple variably sized nodules composed of irregular neoplastic epithelial cells arranged in cords, and acinar forms surrounded by dense fibrous (scirrhous) tissue that affected both the mucosal and serosal layers. In some areas, the mucosal muscular layers were breached and disrupted by the neoplastic proliferation.

The neoplastic foci were often surrounded by and associated with lymphocytic inflammatory cells. Sections of liver were characterized by ample, pale eosinophilic, homogenous material (amyloid) that diffusely separated hepatic cords and filled the hepatic sinusoids. Inflammation was minimal to absent. Both these disease processes intestinal neoplasia and hepatic amyloid are consistent with the animals reported clinical evaluations. In people Adenocarcinomas of the colon and rectum make up 95 percent of all colorectal cancer cases. These cases generally start as a growth of tissue called a polyp. In non-human primates, adenocarcinomas of the ileocecal junction is very common. In this particular case with the evidence of amyloidosis of the liver and adrenal gland would suggest chronic inflammation.

### **P237 Two Cases Of *Rodentibacter*-Associated Pyometra in the White-Footed Mouse (*Peromyscus leucopus*)**

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Within a one-month span, two experimentally naïve, intact, adult female white-footed mice (*Peromyscus leucopus*) originating within an established research colony were euthanized due to poor condition and marked abdominal distension. The first mouse was housed in a breeding group with another adult female and male; the second with a vasectomized male. Differential diagnoses included dystocia, ascites, organomegaly, pyometra, abscess, and neoplasia. Gross examination of the first mouse revealed two 1.5-2 cm yellow masses expanding the left uterine horn and body. The masses contained caseous material with no evidence of fetal tissues. A complete blood cell count and serum chemistry showed severe anemia, marked thrombocytosis, and moderate hyperglobulinemia suggestive of chronic inflammation. Histopathology showed cystic endometrial hyperplasia, suppurative endometritis, and extensive necrotic debris mixed with bacterial colonies. In the second mouse, gross examination identified bilateral distension of the uterine horns with approximately 3 ml of dark red, mucinous fluid. Histopathology showed cystic endometrial hyperplasia, endometritis, myometritis, and serositis with a mixed lymphoplasmacytic and granulocytic infiltrate, multifocal endometrial ulceration and intraluminal bacterial colonies. Cultures of uterine tissue and contents from both mice were positive for *Rodentibacter pneumotropicus* as well as *Streptococcus thoraltensis* and *respiraculi* in the second mouse. Pyometra was diagnosed in both cases. Pyometra has not been well described in *P. leucopus* and these cases suggest *Rodentibacter pneumotropicus* as a candidate etiologic agent in this species.

### **P238 Assessing the Etiology of Dystocia in the Mouse**

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Dystocia is among the most common reproductive conditions in mice. It is classified as obstructive, from fetal factors such as an oversized fetus and abnormal fetal orientation, or functional, from dam factors such as uterine inertia, non-dilated birth canal, nutritional deficiencies and old age. Treatment is based on the dam's condition and the category, but it most often results in euthanasia. A study was conducted to characterize its' etiology to determine if treatment other than euthanasia is warranted. Mice considered to be dystocic, based on specific criteria, were evaluated. The signalment and experimental, clinical and breeding history was obtained and a targeted chemistry panel, radiographs, and a gross necropsy were conducted. A total of 54 mice were evaluated with 45/54 (83%)

diagnosed with dystocia and 9/54 (17%) having other reproductive abnormalities such as neoplasia, metritis and uterine prolapse. Of the dystocia cases, 28/45 (62%) were on a B6 background and the average age at presentation was 181+/-85 days. Hunched posture, lethargy and vaginal discharge were the most common presenting signs. Mechanical dystocia was diagnosed if the pelvic canal width was less than the diameter of the closest fetal head or a fetus was stuck in the canal. Functional dystocia was determined based on clinicopathologic findings or systemic lesions. The number of mice categorized as having a mechanical (n=16) versus a functional (n=11) dystocia was not significantly different than those in which a definitive category could not be ascertained (n=18). Neither clinical signs nor clinical pathology were significantly different between mice categorized as having a mechanical versus functional dystocia. Azotemia (BUN: 69.3±8.1 mg/dL, mean±SE), hypoglycemia (100.7±8.1 mg/dL), and hyperglobulinemia (3.0±0.1 mg/dL) were common. A cage-side method for differentiating mechanical vs. functional dystocia could not be determined. However, based on clinicopathological findings, dams that appear stable without a mechanical dystocia, could benefit from subcutaneous fluids and dextrose. In cases where a neonate was trapped in the pelvic canal, lubrication and gentle traction can be considered. For dams in poor condition, e.g., moribund and/or hypothermic, euthanasia is recommended.

### **P239 Spinal Infiltrative Lipoma in a Zebra Finch (*Taeniopygia guttata*)**

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Spinal infiltrative lipomas are locally invasive, benign tumors composed of mature adipocytes that are rarely documented in avian species. In this case, we describe the clinical presentation and post-mortem findings of a spinal infiltrative lipoma in an adult zebra finch (*Taeniopygia guttata*) within an established research colony. A 23.6-gram 15-month-old male wild-type zebra finch presented for right leg lameness and generalized unthriftiness. Medical management (subcutaneous fluids, NSAID therapy, and supplemental high-protein commercial diet) resulted in initial clinical improvement. However, clinical signs progressed after a week, and unilateral (right-sided) wing trembling was additionally noted. At this time, euthanasia and complete necropsy were elected. Initial differentials included musculoskeletal injury, peripheral neuropathy, hypocalcemia, hypoglycemia, infectious etiology, and neoplasia. Terminal blood collection was performed. Packed cell volume and total protein were within reference ranges, and no viral inclusions or parasites were present on peripheral blood smears. Blood chemistry results were unremarkable except for mild hypertriglyceridemia. At necropsy, no significant findings were noted. Histopathology of the lumbar spinal cord revealed a focally infiltrative mass of mature adipocyte aggregates with invasion into the central canal with focal gray matter degeneration. To our knowledge, this is the first report of spinal infiltrative lipoma in a zebra finch. Lipomas, common in pet birds (primarily Psittaciformes), are thought to be associated with high-energy diets, obesity, and/or genetic predisposition, and are typically found in the integumentary system or intracoelomic cavity. Although uncommon, spinal infiltrative lipoma should be considered as a differential diagnosis for clinical presentations indicative of neurological and/or musculoskeletal disease in zebra finches.

### **P240 Chronic High-Dose Administration of Cyclosporine-toxicity in a Cohort of Royal College of Surgeons (RCS) Rats**

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A 7-moth-old, 170-g male RCS rat was submitted for necropsy after having developed tremors, ataxia, and hindlimb paresis. This individual was one of a 26-rat cohort that was injected subretinally with modified stem cells to prevent progressive retinal degeneration. He was receiving long-term administration of cyclosporine-impregnated water (210-mg/L) to reach a target blood concentration of 300-ng/mL, to prevent cell rejection. Gross necropsy findings included poor body condition (1.5/5), multifocal gastric ulceration, stomach dilatation, and pica of hair and bedding – no other significant lesions were identified on histopathology. Physical examination of other rats within the cohort revealed several with distended stomachs, six with malocclusion, and nearly all were thin with bedding present in the feces. One affected male was determined to have high blood concentrations of cyclosporine (2100-ng/mL), with urine dipstick samplings revealing glycosuria (500-1000-mg/dL). Consequent testing on the remaining 10 rats revealed hyperglycemia on glucometer (147-259 mg/dL) – males being more severely affected than females – and glycosuria on dipstick for several animals (100-500 mg/dL). Complete serum chemistry from one affected rat revealed hyperglycemia (203 mg/dL), elevated BUN (47 mg/dL), and hypoproteinemia (4.9-g/dL). Mild monocytosis (0.896 K/ $\mu$ l) and lymphopenia (4.096 K/ $\mu$ l) were also present. The clinical presentation of hyperglycemia, glycosuria, and poor body condition points to diabetes mellitus induced by chronic, high-dose cyclosporine exposure. Interestingly, signs of nephrotoxicity – one of the more common side effects of long-term cyclosporine toxicity administration – were not observed. Despite malocclusion and gastric ulcers not being documented as common sequelae to chronic cyclosporine toxicity administration in rats, an interaction between the strain, colony sub-strain, and the dose and duration of administration may be at play. While a lower concentration of ingested cyclosporine will be implemented for future studies, useful screening tools for rats on long-term cyclosporine administration could include point-of-care blood glucose readings, serum chemistry readings, fecal-occult-blood testing, and inspection for evidence of pica.

#### P241 Estrogen Supplementation-Associated Mortality of Mice in Estrogen-Dependent Human Tumor Xenograft Model

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Severe immunodeficient mice (NOD.Cg-Prkdc<sup>scid</sup>Il2rg<sup>tm1Wjl</sup>/YckNarl, ASID) which lack functional T cells, B cells, and natural killer (NK) cells were subjected to subcutaneous estradiol pellet implementation (D0) following human MCF-7 breast cancer orthotopic implantation (D4). Seven of thirty mice were found dead without observation of initiating clinical signs or reaching humane endpoint from D22-D35. The mortality of the experimented mice showed dose-dependent manner with low-dose (0.36 mg /90-day release pellet, 2/15, 13 %) and high-dose estradiol (0.72 mg /90-day release pellet, 5/15, 33 %). Mild to moderate cystitis and ascending pyelitis with intralésional colonies of gram-negative bacilli were found in two mice. In addition, numerous bacterial colonies in the glomerular and alveolar capillaries without apparent urinary tract lesions were noted in five mice. Two opportunistic bacteria were isolated from two moribund mice- *Escherichia coli* in the kidney and *Proteus mirabilis* in the blood. In this case report, our data show that higher dose estrogen supplementation leads to higher incidence of mortality and urinary tract problems or urosepsis in immunodeficient mice as previous researches. In previous reports, increased urethral resistance has been hypothesized as the mechanism for urine retention in mice implanted subcutaneously with sustained-release estrogen pellets. However, the previous research found that low dose of estradiol (0.5 or 0.3 mg) did not induce the death of mice and could support growth of the MCF-7 tumors in BALB/c nude or NOD SCID mice. In our case, even a low dose of 0.36 mg estradiol pellets induced 13% mortality with opportunistic bacterial infection. This is the first report to describe estrogen supplementation-associated mortality of the MCF-7

xenograft model in ASID mice, and these observations may be germane in establishing improved animal models for the evaluation of anti-estrogen-based cancer therapies.

#### P242 Struvite Urolithiasis in a Tg(KRT14-cre)1Amc/J Mouse

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An adult naïve male Tg(KRT14-cre)1Amc/J mouse presented with a rough coat and hunched posture but was alert and responsive. This mouse was used as a breeder, but never successfully contributed to the birth of any litters. The mouse was placed on diet gel and moist pellets as part of routine veterinary treatment. Three weeks later, a mass in the caudal abdomen was noted. Upon palpation, the abdominal mass was hard and granular. Differential diagnoses included neoplasia or urolithiasis. In the course of 48 hours from the time the abdominal swelling was noted, the animal became lethargic, dehydrated, and lost body condition. Humane euthanasia was subsequently elected for the mouse. Gross necropsy revealed multiple round stones within the urinary bladder. Due to the acute decline of the mouse, a urinalysis and culture were not collected, however tissues and stones were. Sample analysis of the bladder stones confirmed struvite (triple phosphate) uroliths. Histopathology was performed on the formalin-fixed tissues, which revealed the presence of bacteria within the bladder lumen, hydroureter, and prostatitis. This is the first reported case of spontaneous urolithiasis in KRT14-cre mice. Spontaneous urolithiasis is uncommonly observed in mice, but should be included as a differential for abdominal masses in rodents. The occurrence of struvite urolithiasis in this Tg(KRT14-cre)1Amc/J mouse is likely coincidental, however other transgenic and knockout mice could be utilized as animal models of urolithiasis.

#### P243 Unusual Tail Mass in a Dog

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A 7.5 year old, 20 kg spayed female, pair-housed hound mix canine (*Canis familiaris*) presented with a soft 2.5 cm by 2.0 cm mass on the proximal ventrum of the tail. No additional abnormalities were visualized in the skin and the physical examination was otherwise unremarkable. A complete blood count showed no significant findings. Dark brown serous fluid was obtained by fine needle aspirate (FNA) of the mass. Cytology of the fluid demonstrated mixed bacteria and debris. The top differential diagnosis was abscess, and other differentials included a neoplasm or cyst. The dog was treated with 200 mg cefpodoxime PO for 10 days, without observable change in the mass's appearance. A second FNA was performed that collected samples from the solid portions of the mass as well as dark brown serous fluid. The FNA of the solid material contained cellular debris, some squamous epithelial cells, and no discernable bacteria or signs of malignancy. Some bacterial rods were seen on a wet mount of the fluid. The dog was sedated and the mass was excised. The mass contained thick, brown pasty material. The dog was administered Meloxicam at 0.1 mg/kg subcutaneously for 3 days for analgesia. Gross and microscopic findings of the mass were consistent with a diagnosis of an infundibular cyst and mild mixed dermatitis with eosinophils. Infundibular cysts are benign lesions for which complete surgical excision is considered curative. Cysts are lined by normal keratinized squamous epithelium and filled with keratin and cellular debris. The mild dermatitis may be secondary to irritation caused by the cyst, however the presence of eosinophils suggests a chronic hypersensitivity reaction.

#### P244 A GnRH Antagonist as a Treatment Option for the Management of Endometriosis in Laboratory Rhesus Macaques (*Macaca mulatta*)

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Endometriosis is a chronic condition affecting females of reproductive age, characterized by the presence of endometrial tissue outside the uterus. Spontaneous cases of endometriosis also occur in macaques, exhibiting clinical abnormalities such as an abdominal mass and associated pain symptoms. While surgical removal of cysts and ovaries is often considered the most effective treatment, it may not always be feasible in cases of extensive abdominal infiltration/adhesions or when ovarian functionality needs to be preserved for long-term research objectives. As an alternative, hormone therapy aimed at blocking the effects of estradiol or its production, can be considered. Examples of such treatments include progestins, or GnRH agonists. The latter have been found to be the most effective long-term management option for endometriosis. However, their high cost poses challenges, especially in laboratory animal facilities. In this study, we explored the efficacy of Degarelix, a depot GnRH antagonist primarily used for treating prostate cancer, as a more affordable alternative to expensive GnRH agonists like Leuprolide. We tested Degarelix in two animals that were not suitable candidates for surgery and experienced recurrent endometriosis symptoms affecting their quality of life. Additionally, one female with diagnosed endometriosis and previous ovariectomy was included. All the animals received 40 mg of Degarelix every 40 days and were followed up for one year. We conducted pelvic ultrasounds and FSH, LH, estradiol, and CA-125 levels every three months. The animals treated with Degarelix exhibited a greater reduction in endometriosis-associated pain symptoms. Over time, the decreasing levels of FSH, LH, and estradiol contributed to the improvement of these symptoms. Based on the results, the depot GnRH antagonist Degarelix demonstrates promise as a treatment option for rhesus macaques experiencing endometriosis symptoms. Its affordability makes it an attractive alternative to expensive GnRH agonists, providing a potential solution for managing endometriosis in laboratory animal facilities.

#### P245 Pathologic Manifestation of *Streptococcus agalactiae* in Immunocompetent C57Bl/6 Training Mice

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Two separate groups of healthy, immunocompetent C57Bl/6 female mice, 10 weeks old and 12 weeks old were ordered from two Charles River Laboratory locations for astronaut training purposes. Each group of mice entered the empty facility, as an all-in, all-out animal turnover approach. Each cage of mice received a bottle of water, standard irradiated rodent chow and paper bedding and were housed at standard animal density. All mice were examined daily during health checks and appeared to show no apparent signs of clinical illness. As crew training started, the first group of mice was used at 16-18 weeks and the second group of mice was used at 12-14 weeks, a slightly younger age. The method of euthanasia was ketamine/xylazine overdose, followed by cervical dislocation. On dissection for training purposes, abnormalities were immediately apparent within the abdominal cavity. The spleen was rounded and enlarged 4-5x the normal size. The livers in affected animals contained several lesions that were white, multifocal, with some large, some milium. Differential diagnoses included: primary bacterial infection, primary viral infection, viral infection complicated by secondary bacterial infection, sepsis. Sterile cultures of the liver lesions were collected, urine was cultured, and splenic and hepatic

tissues were collected for pending histopathologic evaluation. Culture results revealed an overwhelming presence of *Streptococcus agalactiae* in all samples and mild presence of *Staphylococcus aureus* in liver and urine samples. Source tracing was completed on items within the primary enclosures of the mice, which were all negative for *S. agalactiae*. Group B strep has not been reported to cause pathogenic lesions in non-diabetic C57Bl/6 mice as only DBA/2 mice are specifically noted to have a unique susceptibility to Lancefield group B streptococci. These findings may have serious negative implications for others using these mice for training and/or experimentation.

#### P246 Secure Intravenous Ear Catheterization Method for Pigs

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Securing pig ear catheters often involves an extensive amount of material which adds weight to the ear while also obscuring visibility of the placement site. The goal was to develop a method that consistently and effectively secures catheters in pig ear veins, reduces the amount of material associated with traditional methods (such as wrapping the ear around a wad of gauze with tape), and increases visibility of the placement site. With the support of the vet staff, I applied methods of intravenous catheter placement learned while working as a patient care technician in a human hospital. Together, we refined and implemented a method for securing pig ear catheters that provides sufficient security and increased visualization of the catheter placement site as well as improved handler safety using a sedation protocol that often reduces the need for snaring. For our surgical procedures, sows are given 0.15 to 0.2 mg/kg acepromazine intramuscularly approximately 30-60 minutes prior to catheter placement. Topical lidocaine cream 2.5% is applied to the dorsal aspect of the ear and 0.1 to 0.25 mg/kg of morphine is administered intramuscularly approximately 15 to 20 minutes prior to catheter placement. The ear is cleaned with gauze soaked with 70% alcohol. Once dry, the SurePrep is applied liberally to the ear to allow better adhesion of the Tegaderm to the skin. The catheter is then placed intravenously and the t-port is attached to the catheter hub. Superglue is applied around the hub of the catheter where the t-port is attached to adhere the underside of the hub to the ear. The white plastic of the Tegaderm is peeled off and the Tegaderm placed over top of the entire catheter, leaving the end of the port exposed. The clear film on top of the Tegaderm is peeled off and pressed down firmly to ensure adhesion to the ear, and the t-port is flushed. This method has proven effective at securing pig ear catheters with increased visibility of the placement site. The inclusion of our sedation protocol has reduced the need to snare during catheter placement while also increasing handler safety. Furthermore, our team has maintained catheter patency for over three days using this method.

#### Husbandry and Management

##### P300 Baking Water: Watch Out Steam! Here Comes the Heat

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Water is an essential nutrient needed to sustain life. In laboratory animal research facilities, the quality of water can introduce experimental variables to research studies as well as compromise animal health. Mechanical water purification systems, such as reverse osmosis systems (RO), are most commonly used to treat animal drinking water. RO systems are capable of removing particulates, as well as biological and chemical contaminants from water sources. However, additional steps are often taken to ensure that the water is

free from microbial contamination. Chemical additives (e.g. acidification, chlorination) can be used to maintain animal drinking water that is free from microorganisms. However, the addition of chemical additives may be contraindicated for certain types of research. As an alternative, many facilities use steam sterilization as their chosen method for sterilizing water bottles for the vivarium. Technological advancements in dry heat sterilizers, ease of installation, effective heat penetration of items and non-corrosive sterilization have renewed interest in this technology. Dry heat sterilization has historically been considered an ineffective method for sterilizing water and is considered a limitation of dry heat sterilizers. Contrary to this commonly held perception, we hypothesized that dry heat would effectively sterilize water. Bottles of distilled water were inoculated with rodent feces; a control sample was removed to verify microorganism growth. The bottles containing contaminated distilled water were then "sterilized" using a bulk forced-air dry heat sterilizer. Samples were tested weekly for microorganism growth. The results of this pilot study demonstrated dry heat can be used to sterilize water indicating that this method of sterilization merits further evaluation.

### **P301 Extinction of the Conventional Mop in the Laboratory Animal Facility. Evolution of a Macro-Environmentally Friendly, One-Pieced, Streamlined Bucketless Cleaning System.**

AS Megginson\*

Animal Resources, Midwestern University, Glendale, Arizona  
Campus, Glendale, AZ

The effectiveness of the sanitation equipment in a vivarium is fundamental to the cleanliness of the facility. Supplementary considerations must include budgetary limitations as well as human and environmental impacts. These cannot divert concentration away from animal welfare and research. At Midwestern University, Glendale, Arizona Animal Resources facility, the glut of plastic-reliant and rapidly deteriorating conventional mopping system was not fulfilling any of those requirements. Since the existing system was harshly straining the budget, a less pricey heavy-duty microfiber spray mopping system was contemplated. A budgetary analysis was conducted to verify the projected savings. Upon critique, it was purchased to be implemented and tested in select animal rooms. In addition, a calculation of water and disinfectant used in conventional buckets versus the spray system bottles was also completed. A further projected benefit was a reclamation of the square footage of the space required to store the conventional mop equipment and a reduction in the amount of water, detergents, and power used to clean the equipment itself. A therapy department specialist was consulted to verify the theorized ergonomic benefit of the spray mop system. Perhaps most significantly, a quality analysis using Replicated Organism Detection and Counting (RODAC) plating was performed on the floors, which were incubated and documented by the microbiology department to verify the efficacy of both types of equipment. Technicians can now carry just one 18 oz bottle plus mop-head, truly sanitize the floor, and return the unit to the wall behind the door within the room, relieving storage. Staff currently need only empty and wipe the unit to preserve integrity. The affordable heavy-duty spray mopping system provides a hygienic floor of 38 or fewer Colony Forming Units (CFU), all while being ergonomically superior, more environmentally friendly, and all-around easier to care for. There has yet to be any need for the replacement of any mops.

### **P302 Systems and Methods for Concurrent Germfree and Gnotobiotic Mouse Infrastructure**

RW Engelman\*

Comparative Medicine, University of South Florida, Tampa, FL

Traditional infrastructure for germfree and gnotobiotic mice (i.e., isolators enclosing static cages) is space inefficient, labor intensive and involves hazardous nebulized liquid sterilants (e.g., peracetic

acid, chlorine dioxide). The infrastructure is also high risk with sterility breaks resulting in loss of the affected isolator's entire murine inventory, necessitating weeks of monitoring a replacement isolator prior to its repopulation with germfree mice, and problematic from an experimental design and implementation perspective. We combined technologies previously unaware of one another, the IsoIVC-P and QUBE, and developed highly tractable and scalable methods for long-term germfree murine colony maintenance and concurrent germfree and gnotobiotic mouse model throughput in the same vivarium room. This is a highly space-efficient practice heretofore not attempted, making microbiome studies more accessible. We took each microbially similar IsoIVC-P to the QUBE for hydrogen peroxide vapor (HPV) exterior surface sterilization prior to opening it and mouse use. After >74 weeks of experimentation and handling >1,379,693 germfree mouse-days, we determined that this novel infrastructure has a performance metric of 0.0001 sterility breaks/husbandry unit/week, comparable to the isolator "gold standard." This, while concurrently maintaining an Altered Schaedler Flora (ASF) colony and supporting multiple gnotobiotic studies involving fecal microbial transplants (FMT) to study end points without alteration in the same room, thereby improving microbiome research efficiency, eliminating hazards, and reducing risks posed by traditional infrastructure.

### **P303 New Enrichment for Squirrel Monkeys (*Saimiri sciureus*) and their Caretakers**

MD Sheets\*, G Childree, ER Barton, S Barton

LARC, university of California San Francisco, San Francisco, CA

In the wild, squirrel monkeys live in large troops with freedom to roam through wide spaces of territory to forage and carry out natural behaviors. However, in a lab setting, space to perform naturalistic behaviors can be limited and negative behaviors can become pronounced. We combat this by using different types of environmental enrichment. To prevent animals becoming uninterested or bored with the items, over time we regularly create new ones to help stimulate their behaviors. When the staff gives the squirrel monkeys fun, new enrichment, it creates a positive relationship between the animals and caretakers. The opportunity for animals to paint has proved to be one of these successful interactions. When painting, the paintbrush handles are placed through the animal caging with the tips of the brushes facing outward and attached using clips. Employees dip a small amount of non-toxic water-based paint on the tips of the brushes and hold up a canvas in front of the paint brush tips. The animals are then able to move the paintbrushes freely across the canvas. While the animals paint, it gives husbandry and veterinary staff a close view of the animals to assess them with minimal stress. With less stress, the well-being of the animals isn't compromised, both animal and caretakers are happy, and the employees get an enjoyable painting made by the animals they compassionately care for.

### **P304 Overcoming Obstacles for the Care and Use of Gambian Pouched Rats (*Cricetomys ansorgei*) in Research**

W MacCabe\*, ER Feldman

Center for Animal Resources and Education, Cornell University, Ithaca, NY

The Gambian pouched rat (*Cricetomys ansorgei*) is a large murid species native to sub-Saharan Africa. These creatures have poor eyesight but a sensational sense of smell, and for many years have been trained to sniff out land mines and detect tuberculosis in humans. Wild caught pouched rats were brought to our institution in June 2015 for reproductive and olfactory investigations due to their importance in humanitarian work. Due to a lack of literature and the uniqueness of this laboratory animal species, handling and clinical care, housing, diet, and enrichment were challenging. Though the initial wild-caught cohort required anesthesia for handling and

clinical care, implementation of a behavioral acclimation program resulted in our ability to perform many clinical and research procedures safely on awake animals, such as nail trimming, drug administration, physical examination, and phlebotomy. Modified housing was implemented to prevent escapees and reduce injuries. Nutritional changes in response to a presumptive diet induced metabolic disease resulted in resolution of clinical signs. Finally, larger caging designed with additional contents to allow environmental manipulation and species-typical behavior were developed for enrichment. This poster aims to address the challenges and our solutions associated with housing the Gambian pouched rat to create a safe environment for both the rats and human handlers.

### P305 Achieving Successful Weight Mitigation in Over-Conditioned Research Ferrets

SR Gainer, A Osborne<sup>\*</sup>

UAB, Birmingham, AL

Ferrets (*Mustela putorius furo*) are a valuable animal model for the study of human conditions and diseases because of their relatively small size and physiologic similarities to humans. A challenge in the research field is balancing species-specific behavior with research restrictions and regulations. Researchers at the University of Alabama at Birmingham utilize ferrets to study lung pathology. Standard housing is paired, same-sex ferrets in stainless steel modified rabbit caging (24x24x17) with grid flooring. Standard enrichment includes: plastic hammock, Jingle Ball or equivalent toy, paper bag with paper towel, and once weekly supervised exercise. Ferrets receive Birmingham tap water and food *ad libitum*, filled twice daily. In October 2022 two intact, male, wild-type ferrets arrived from Marshall BioResources. These ferrets were noticeably larger in stature than others in the shipment but otherwise within normal limits. From arrival to January 2023, veterinary staff noted that the ferrets were steadily increasing in weight and body condition (BCS 5/5, weights 2.4 kg and 2.21 kg). To improve the health of the ferrets, and decrease the chance of study outliers, vet staff initiated a weight mitigation plan. The program included: feed 80 g of food in the morning and only feed in the evening if the food hopper was empty, increased exercise time to 15 minutes 2-3 times weekly, minimum once weekly weigh-ins, and monthly veterinary checks. From February to April 2023, ferret ID 561 lost 530 g (22% BW) and ferret ID 560 lost 330 g (15% BW). Staff observed increased mobility, species-specific behavior, and stamina during exercise time. UAB behavioral and vet staff plan to standardize exercise efforts for ferrets on campus. This program encourages a proactive approach to optimal veterinary and enrichment needs of research ferrets, and may serve as a template for other institutions utilizing ferrets.

### P306 Maintaining a Green Anoles Colony and Creating a Green Anoles User Training Program: Challenges and Solutions

P Cheng<sup>\*</sup>, SA McErlane, C Cheng

Animal Care Services, University of British Columbia, Vancouver, , Canada

Green anoles are an excellent animal model for human eye diseases because unlike mice, they have fovea similar to that of human eyes. Reference material for the care and use of green anoles is limited. In this presentation, we describe the development of a husbandry program for the care of green anoles at our institution and the creation of a training program for users of these unique animals. We describe development of handling and restraint methods that takes into account the natural behavior of these reptiles. We describe drug therapies, quarantine and husbandry protocols for the maintenance of the health of our green anole colony. We describe an optimized care protocol for maintenance of small crickets, which are the main food source of our green anole colony. Finally, we describe the synthesis of our knowledge in the creation of a training course with online modules and practical hands-on labs for users of these

reptiles.

### P307 Chicks Dig it, Improving Chicken Housing and Enrichment in a Laboratory Setting

TL Stevens<sup>\*</sup>, A Tresler

Comparative Medicine, University Of Oklahoma Health Science Center, Oklahoma City, OK

Appropriate caging and housing systems are vital to the physical, physiological, and behavioral needs of laboratory animals. Inadequate housing that does not allow the expression of species-typical activities or provide appropriate space allotment can be detrimental to animal well-being and research outcomes. Chickens, *Gallus gallus domesticus*, are routinely housed at our institution, the University of Oklahoma Health Sciences Center (OUHSC). Historically at OUHSC white leghorn chicks are received at 1-2 days of age and housed in wire-bottom, heated brooder cages containing 20-25 chicks per brooder with limited enrichment options. We began experiencing an increase in pecking behavior with the chicks as early as 4-5 days of age, which would often continue even once the chicks were relocated into bigger brooder caging. After a chick received an injury involving open wounds and blood the other chicks would become attracted and obsessed with the injury site and would continue to peck at the affected chick, leading to removal from the flock for veterinary treatment, single housing, and at times even euthanasia. Evaluation by the OUHSC Behavioral Management and Enrichment Team (BMET) quickly elucidated that the housing arrangement was not allowing the chicks to elicit normal poultry behaviors such as scratching and foraging, as well as allowing the chicks to spread out and hide from more dominant chicks. The BMET developed a plan of floor housing for the chicks to help deter pecking incidences, as well as promote the natural behaviors of chickens. The larger floor space combined with the ability to forage, scratch, hide, and perch, as well as increased occupational enrichment and cage complexities have greatly improved our chicken's well-being and comfort. The improved floor housing better promotes the ability to express natural behaviors, leading to a significant decrease in pecking injuries and euthanasia, in addition to improved research outcomes.

### P308 An Identification Guide for Vole Species

S Bruggeman<sup>\*1</sup>, CG Mahan<sup>2</sup>, E Machtinger<sup>3</sup>

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Proper identification of wild voles trapped for research down to the species level is critical to avoid noncompliance (e.g., change in species without prior IACUC approval) and for accuracy in scientific publications. However, many vole species have a similar external appearance, overlapping territories, and are commonly captured via the same trapping techniques. Four vole species are found in Pennsylvania: the southern red-backed vole (*Clethrionomys gapperi*), the meadow vole (*Microtus pennsylvanicus*), the woodland or pine vole (*Microtus pinetorum*), and the rock vole (*Microtus chrotorrhinus*). Here, we describe their habitat distribution, physical and morphological characteristics and provide a photographic guide for species identification to ensure researchers are certain of the species being used and publications correctly identify the species of interest.

### P309 Vivarium Modernization of a Nonhuman Primate Pre-Clinical Safety Facility

P Kowal<sup>\*</sup>, J Davies, L Singer

Comparative Medicine, Pfizer, Groton, CT

The Pfizer Groton CT site recently undertook an 18 month project to convert a wing consisting of ten rooms of dog kennels to a contemporary vivarium designed to house nonhuman primates (NHPs). The renovation was a complete transformation of the existing footprint. The ten dog rooms were converted to nine large holding rooms including state-of-the-art novel caging with adjacent ante rooms, 3 procedure rooms, and a large ancillary equipment storage area. The renovation includes unique solutions for finishes, including fiberglass reinforced walls (FRP), NICO ceiling (interlocking FRP tiles with soundproof insulation incorporated). In addition, automated diamond textured doors, natural light sun tubes, stainless steel air filters, LED lighting, retractable power cords and ergonomic hoses were installed, along with hands free doors and sinks. Comparative Medicine (CM) further opened the space by incorporating smart glass. Using liquid crystal technology, this feature changes from transparent to opaque with the flip of a switch. This technology was used between the ante room and holding room allowing NHPs more visibility and provides personnel an unobtrusive means to perform clinical observations. Furthermore, another inventive design concept was developing a viewing room using smart glass that allows visitors to observe NHPs without having to satisfy medical clearances.

### **P310 Zip It! Mark Zebrafish Larvae, Juveniles and Adults with Fluorescent Elastomer.**

S Frederickson<sup>\*1,2</sup>

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There are many experiments that require individuals or small groups to be distinguishable from others. Rodent models use traditional marking methods like tissue clipping or tattoos that are ineffective in zebrafish because of their regenerative properties. Visible Implant Elastomer is a flexible plastic that cures after injection and has been used to mark wild and aquacultured fish, including zebrafish juveniles and adults. We improved on this procedure to mark larval zebrafish as young as 2 dpf. This mark remains visible in an average of 72% of larvae at one month, when they can be injected with enough material to remain visible for the remainder of the fish's lifetime. The improved procedure, Zebrafish Injectable Plastic for Identification Tagging (ZIP IT), could be a powerful tool enabling zebrafish larvae to be tracked and data to be gathered in ways previously not possible. This method could also be applied to other species of fish or even other kinds of animals, like amphibians or insects, to benefit the broader scientific community.

### **P311 Utilizing Microsoft Office Applications for Inventory Management**

AC Admire<sup>\*</sup>, T Grgurich

Comparative Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK

Inventory management helps organizations optimize stock levels, minimize stockouts, and improve efficiency of their ordering process. One way to enhance the tracking of supplies quantity and order management is by developing a novel inventory management system using Microsoft Forms, Automate, and Excel. The proposed solution aims to address the limitations of traditional inventory systems by leveraging the capabilities of these Microsoft platforms. Our inventory management system utilizes Microsoft Forms, a user-friendly survey and form creation tool, to gather and input inventory data. Users can submit information about stock levels and order requirements through customized forms. This streamlined data entry process has enhanced our accuracy and efficiency in the ordering process. Microsoft Automate, a powerful workflow automation platform, is utilized to automate various inventory management tasks. The system is configured to trigger automatic notifications and alerts based on predefined thresholds for stock levels. Microsoft Excel, a spreadsheet program, gives us the ability to sync and collect

the data from Forms and Automate to generate a report and/or analytics which provides the calculations necessary to indicate which items need to be ordered. During weekly inventory audits, when inventory falls below a specified quantity, the system will automatically indicate the amount to order from the predefined stock, ensuring timely replenishment. A good inventory system also provides reporting capabilities. From the Excel file, our facility has been able to generate reports on various inventory-related metrics such as turnover rate, stock accuracy, and usage. These insights allowed our facility to become more proactive in ordering the proper amount of goods instead of reactively ordering when there is a deficit. Our facility has been able to predict usage based on historical inventory levels and give us an instant overview of our current inventory status. The development of this inventory management system utilizing Microsoft Forms, Automate, and Excel presents a promising approach to improve the tracking of goods quantity and order management.

### **P312 Creation of a Premature Piglet PICU (Piglet Intensive Care Unit) Using Common Vivarium Supplies**

S Kozlov<sup>\*1,2</sup>, LV Kendall<sup>2</sup>

<sup>1</sup>Inotiv co, Fort Collins, CO; <sup>2</sup>Laboratory Animal Resources, CSU, Fort Collins, CO

Premature piglets are a common model for studying preterm infant development, including neuromuscular, gastrointestinal, respiratory dysfunctions, and anthropometric and physiological characteristics. Setting up a PICU can be a challenging and expensive endeavor. Key considerations include providing thermal and humidity support, oxygen therapy, and maintaining umbilical cord/orogastric access for fluid therapy, and nutritional needs. Utilizing equipment commonly found in most vivariums, we constructed individual incubators using rat cages topped with ¼ inch Plexiglas cut to fit for lids and placed absorbent pads on top of an anti-slip matting cut to fit the inside of each rat cage. Humidified oxygen support was provided individually to each incubator using the bubblers attached to a 5-outlet oxygen manifold supplied by a H-tank or central oxygen supply. Groups of five incubators were placed on top of warming devices on a counter within an insulated tray constructed from building insulation. Temperature and humidity monitors were attached to the inside of each incubator with the display facing outwards to allow for monitoring without disturbing the microenvironment. Additional humidification was accomplished with room humidifiers and by separating the PICU from the main entrance to the room using sheets of heavy-duty plastic suspended from the ceiling. Total parental nutrition, IV fluids, and medications were delivered using a variety of syringe pumps used throughout our facility. Challenges faced in the PICU included C-section and resuscitation, instrumentation of nasogastric tubes and umbilical catheters, maintaining humidity and temperature in the incubators, medical issues due to dehydration. Specific issues encountered included meconium impaction, GI perforation, specific issues related to our model, and how we responded to precocious piglets removing their catheters and GI tubes.

### **P313 Thinking Outside the Cage: Innovative Opportunities for Staffing**

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Current events such as COVID-19 fallout, The Great Resignation, and early retirement have presented staffing shortages for many institutions. Jobs that require in-person attendance and traditionally have lower wages have had a more difficult time retaining staff. Paired with all-time high compassion fatigue and burnout in the industry, the result has been a suffering laboratory animal field. To continue to develop current staff, individuals would need to be hired to help with laboratory animal technician duties such as cage wash,

cage component preparation, sanitation, and other odd jobs to help facilitate vivarium daily operations. Working with a staffing agency to hire temporary staff would mean constantly having to train someone and then always having to start over when new temporary staff are hired. The question becomes how to find someone and/or an organization to assist with vivarium functions. Here at The Hormel Institute, University of Minnesota, laboratory coats and scrubs have been laundered by Cedar Valley Services, Inc. for many years. Cedar Valley Services, Inc. assists individuals in activities of work and community living in Southern Minnesota. While speaking with the organization, it was discovered that Minnesota Department of Employment and Economic Development (MN DEEDS) Vocational Rehabilitation Services Department works with companies to set up paid internships with individuals who are interested in their specific vocation. MN DEEDS will sponsor the first 130 hours of the internship, after which the company will finance the last 130 hours of the internship. While collaborating with MN DEEDS, a Laboratory Attendant position was created to closely align with the needs of the department. A Laboratory Attendant Skills Checklist was created to allow all staff to be involved in training when available and picture tutorials were produced to help with common duties. Currently, the program has exceeded all expectations. The first intern here at The Hormel Institute has far surpassed what was expected of the internship. This success has evolved into the permanent hire of the first intern and the addition of several interns as the company continues to grow.

#### **P314 In-House Developed Digital Tools Providing Point-of-Care Reporting Applications Improve Operational and Veterinary Efficiencies in Laboratory Animal Care Program**

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Digital tools improve efficiency of communication, reporting and record keeping in laboratory animal care programs. Typical modes of communication and documentation such as phones, paper forms, and electronic files can result in delays in animal care, loss of vital information, and challenges in the collation of information for effective oversight and management of the care of the animals and facility. Commercial digital solutions are available, but usually come at a high cost, limited customization capabilities, and challenging user interfaces that slow adoption by animal technicians who are most likely to see and report concerns. Using organization-wide readily available digital tools and provisioning mobile devices, we created custom mobile applications for our animal care staff to submit cage-side reports regarding veterinary or facility related issues. The average time to submit mortality reports was reduced by 66.6% ( $p < 0.0001$ ) and health issues by 21.9% ( $p = 0.003$ ) as compared to previous methods of reporting dead or sick animals. Time savings was retained even after adding photo requirements with savings of 48.1% ( $p < 0.0001$ ) and 17.0% ( $p = 0.01$ ) respectively. Reports are instantaneously recorded to a centralized location for easy access and can be connected to visualization software or automated workflows with customized triggers to escalate issues without additional steps to be taken. The time saved by animal care staff can be allocated to other husbandry and care activities while veterinary and operational staff receive standardized reports and earlier notifications granting our program increased efficiencies and better, faster outcomes.

#### **P315 Consistency of FMT Colonization of Gnotobiotic Mice in IsoIVC-P**

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Since large experimental gnotobiotic murine cohorts become

segregated into multiple IsoIVC-P, we were interested in determining the fecal microbiota in specific pathogen free (SPF) fecal microbial transplant (FMT) gavage-administered mice for consistency of colonization. Forty-four, 22 male and 22 female, germfree 8-10-week-old BALB/c mice were assigned to one of two cohorts, with 12 mice, 6 male and 6 female, administered sterile phosphate buffered saline (PBS) by gavage, and 32 mice administered SPF FMT by gavage, each weekly. PCR for bacterial 16s rRNA and microbial cultures and shotgun sequencing of fecal pellets of each mouse was performed prior to study, and fecal microbiota shotgun sequencing of each mouse performed at weeks 1, 3, and 5. Comparisons of colonization showed that the SPF FMT colonized each individual mouse of the FMT administered cohort similarly (i.e., no individual variability), whether mice were housed in the same or in a different IsoIVC-P (i.e., no cage effect). Among SPF FMT gavage administered mice, the greatest number of OTUs that were different both within and between groups was from weeks 1 and 3 and the fewest between weeks 3 and 5, suggesting stabilization of the microbiota transfer over time. Overall, female ( $n = 44$  samples) and male ( $n = 40$  samples) mice administered SPF FMT had a differing relative abundance of three OTUs: *Kineothrix* sp000403275 (FDR  $p$ -value=0.040; 0-22.8, 0.9 and 0-16.2, 2.7 [min-max, median of female and male mice, respectively]), *Bacteroides uniformis* (FDR  $p$ -value=0.042; 0-37.5, 0 and 0-0, 0), and *Roseburia* sp. 1XD42-69 (FDR  $p$ -value=0.042; 0-3.1, 0.7 and 0.1-4.4, 1.0). Evenness and diversity did not differ between weeks 1 and 3 ( $p = 0.064$  and  $0.313$ , respectively), but did differ between weeks 1 and 5 ( $p = 0.002$  and  $0.006$ ) and weeks 3 and 5 ( $p = 0.005$  and  $0.016$ ). Richness differed between each timepoint ( $p < 0.05$ ). Large experimental gnotobiotic murine cohorts can be successfully housed in multiple IsoIVC-P.

#### **P316 Reusable Polyester Bags for Autoclaving Rodent Caging**

J Smachlo<sup>\*</sup>, G Okenka

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Autoclave bags for sterilization are commonly used in animal care facilities for both clean and soiled cages. Our institution primarily uses polypropylene bags as the secondary containment for autoclaving caging and associated material from ABSL-2 spaces. However, after multiple cycles of autoclaving in polypropylene bags, polysulfone rodent cages begin to display varying degrees of permanent cloudiness. This can impede the ability to perform daily rodent health checks while minimizing disturbance of the animals and increases the need for more frequent cage replacement. Additionally, single-use polypropylene bags generate excessive waste. The use of reusable bags for autoclaving cages also presents an opportunity to institute more environmentally friendly practices and reduce operational costs. A pilot study was conducted to assess whether the use of 100% polyester reusable bags for autoclaving polysulfone mouse cages achieved appropriate sterility and reduced or slowed the onset of cage cloudiness compared to polypropylene bags. Twelve polysulfone cages with minimal to no cloudiness were selected for use. A baseline picture was obtained of each cage before the first autoclave cycle. Six cages were autoclaved daily in a polypropylene bag and six in a 100% polyester reusable bag. Pictures of all cages were obtained after each autoclave cycle to monitor the development of cage cloudiness. Sterilization strips, biological indicators, and chemical indicators were included in each bag every cycle to verify appropriate sterilization was achieved. Over the eight days of the pilot study, all cages autoclaved within both styles of bags achieved appropriate sterilization. Cage cloudiness between the two groups was similar with an average ranking of 1.5/5 on a cloudiness scale, where 0 was baseline. Preliminary results indicate that the use of 100% reusable polyester bags did not slow the progression of cage cloudiness compared to polypropylene bags. Further data will be collected to assess the progression of cloudiness over a 30-day period. The lack of difference in cloudiness between the two bag materials presents an opportunity to further investigate the application of reusable autoclave bags to promote greener practices and reduce



daily waste.

### P317 Flooded Cage Response Cart

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Many research facilities have automated watering systems providing consistent, high-quality, variable-free drinking water to their rodent housing racks. Though automated watering systems provide mice with on-demand access to clean, fresh water, 24 hours a day, 7 days a week, there is the risk of flooding. Drinking water is released from the watering valve at a controlled rate when the mouse licks, bites, or noses the stem of the watering valve. Water flow stops when the stem is released and returns to normal position. Uncontrolled water leakage into a cage can occur due to plugging/clogging of the water valve with bedding or nesting materials, causing the stem to remain in the water-flow position. It can also be caused by a malfunction of the water valve or even the rack. Unfortunately, any of these can result in a rapidly flooding cage. Rapid response to a flooded cage is critical for improving the chances of saving mice. Preparing and gathering essential materials to remove mice from flooded cages, dry the mice, and place mice under heat, can take upwards of 8 – 10 minutes. The Flooded Cage Cart was developed as a timesaver and lifesaver by having all items needed on the cart for immediate response when a flooded cage is found, and mice can be quickly assessed. Having everything you need in one central location at all times significantly shortens response time. For mouse health and welfare, it is imperative to remove mice from a flooded cage, dry them and return body temperature to normal as quickly as possible. The Flooded Cage Response Cart is mobile and contains all necessary resources in one location to rapidly respond to a mouse cage flood and provide them with essential care before being returned to the IVC housing rack.

### P318 The Long and Short of It: Anogenital Distance in African Spiny Mice (*Acomys cahirinus*) as “Telltale” Sexual Differentiation

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Anogenital Distance (AGD) is a fundamental observation utilized in laboratory animal husbandry and medicine, providing a rapid method for sexing mice (*Mus musculus*). However, AGD has not been characterized previously in African Spiny Mice (*Acomys cahirinus*). *Acomys spp* demonstrate unique regenerative capacities and is the only known rodent to menstruate, increasing their popularity in research. Yet they are also readily prone to sloughing skin with manipulation, emphasizing the utility of non-invasive exam characteristics. The AGD of adult (> 3 months, 50 ± 4 grams) spiny mice was measured (n= 20) by the length of the perineum. Anesthetized mice were sexed based on the ability to extrude the penis and the presence of nipples, and calipers were used to measure the AGD. Additionally, photos were collected of the perineum, digitally processed, and scaled with in-frame rulers to measure AGD. AGD was found to significantly differ ( $p < 0.05$ ) between female and male spiny mice, indicating sexual dimorphism as reported in other species, and measured to be  $0.5 \pm 0.11$  and  $1.4 \pm 0.18$  [cm], respectively. A male:female AGD ratio of 2.8 was observed, similar to reported AGD ratios in *Mus musculus*. No statistically significant ( $p < 0.05$ ) differences were determined between the caliper and digital methods, reinforcing results and suggesting either use in future practice or studies. The presence of nipples has historically been suggested as the only reliable method of sexing spiny mice. However, this requires handling and can be challenging depending on mouse age, parity, viewer experience, and season. As such, the ratio of AGD represents a reliable visual method for sexing adult African Spiny Mice while avoiding unnecessary handling or anesthesia.

### P319 Evaluation and Time and Cost Savings Analysis of Extended Sanitation Interval of Caging Accessories and Automated Watering Valves

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Although the Guide suggests changing rodent cage accessories every 2 weeks, it also states that “decreased sanitation frequency may be justified if the microenvironment in the cages, under the condition of use, is not compromised.” Increased use of individually ventilated cages (IVCs) for rodents has led to investigation of extended cage sanitation intervals. The purpose of this study was to evaluate extended sanitation intervals of cage accessories (automated watering valve, wire bar lid, and filter top) of mouse IVCs at our institution. We hypothesized that there would be no significant difference in relative light units (RLUs) measured by ATP luminometry of these cage accessories at control time 14 days compared to each time interval: 28, 56, and 84 days. We extended the study for automated watering valves to 168 days and hypothesized that we would see no significant difference in RLUs. We also hypothesized that a time-and-motion study performed by moving to a sanitation interval of 84 days for all components would result in significant time and cost savings. A total of 24 cages containing 4 or 5 mice each were used for swabbing cage accessories. An ATP luminometer was used to detect organic matter by quantifying the amount of ATP present, and results were given in RLUs. There were no significant differences ( $P > 0.05$ ) between 14 days and each other time point for all cage accessories, including the automated watering valves extended study. Additionally, the time and cost savings analysis found that extending the sanitation interval of cage accessories to once every 86 days for a mouse census of ~22,000 cages would save ~7,000 technician hours annually for a total labor cost savings of ~\$240,000. Overall, extended rodent cage change of accessories is a feasible alternative that decreases workload of animal care staff without compromising sanitation.

### P320 Iodine Immersion of Pups Coupled with Cross Fostering to Eradicate MNV and *Helicobacter* from Mouse Colonies

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Eradication of diseases from mouse colonies benefits scientific research and animal welfare. Previously described techniques, such as surgical rederivation, may be costly. The purpose of this study was to assess the efficacy of iodine immersion coupled with cross fostering for eradication of Murine norovirus (MNV) and *Helicobacter spp*. This study also aimed to modify the existing iodine immersion technique described for *Helicobacter spp*. to prevent pathogen transmission and cannibalism. This study hypothesized that iodine immersion of pups would be as effective at clearing MNV as *Helicobacter* in a mouse colony. Within 48 hours of birth, 34 newborn litters of mice of various strains were immersed in warmed, diluted iodine, and transferred to a Swiss Webster foster mother negative for MNV and *Helicobacter*. The presence of MNV and *Helicobacter* in donor dams, weanlings, and adult animals were confirmed through fecal PCR. Out of the 27 litters from MNV and *Helicobacter* positive mothers, all 27 were effectively cleared of MNV and *Helicobacter* at weaning and eight weeks following weaning. Iodine immersion coupled with cross fostering is an effective and cost saving method to eradicate MNV and *Helicobacter spp*. in colonies affected by both pathogens.

### P321 Enrichment Database: A Comprehensive Resource to Unify the Use of Enrichment to Improve Animal Welfare

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The use of enrichment for animals housed in a laboratory setting is crucial in supporting the animal's physical and psychological well-being while also promoting natural behaviors and reducing stress. Since rodent strains and stocks display individualized phenotypes, their enrichment requirements will naturally follow suit. In facilities which house large numbers of different animal lines, there can be uncertainty regarding the specific required enrichment items for each line with the diversity of available options to provide to the animals. Similarly, as new hires begin the training process or as unfamiliar animal lines are introduced into facilities, staff may struggle with understanding enrichment requirements. In order to avoid confusion and to unify systems in place for enrichment, veterinary staff at a large, multi-site animal vendor developed a digital enrichment database. The enrichment database acts as an online repository designed to provide detailed descriptions of enrichment devices required for individual lines. In addition to providing descriptions, it also provides implementation guidelines for individual enrichment devices and allows staff to search and filter for individual lines. Overall, it aims to improve the quality of life for laboratory animals by promoting effective use of enrichment strategies and promotes harmonization of husbandry for individual lines which are produced at more than one location. Acting as a valuable resource for animal care technicians, researchers, and other laboratory animal professionals, the enrichment database has proven to be a user-friendly, comprehensive resource that promotes the proper use of enrichment for animals in the laboratory animal setting.

### P322 Refinement in Vampire Bat Husbandry

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As of November 2022, a new research animal was welcomed into the Large Animal Facility at The Ohio State University, *Desmodus rotundus* commonly known as Vampire Bats. Within research there was a lack of documents providing guidance for vampire bat care. The research, veterinary, and husbandry team had to work together to develop a plan. As a team, we had to devise our own guidelines considering their welfare, the team's flexibility, and previous consultations with zoo veterinarians experienced with vampire bat care. We discussed their transportation, housing, space requirements, sanitization, enrichment, and feeding methods. The husbandry team was tasked with providing food every morning, which was defrosted beef blood, and verified that the environmental conditions of the enclosure were within the established range. At the end of the day, the feeding supplies were removed from the enclosure and cleaned. Afterwards the enclosure's floor was cleaned in a manner that prevented their escape and was safe for the animals. For the first three months it was a success and the colony had adapted to our care and procedures. Shortly after, several females gave birth to new pups, and we began to find unresponsive pups and adult bats. Injuries were found on all of them once they were inspected for a postmortem. Because of this it was theorized that this may be due to resource guarding, fighting, and the birth of new pups to the colony. Our procedures were reevaluated by the research and veterinary team, as a result the feeding time was extended, and small towels were hung to provide additional hiding places and textures for them to cling to. Vampire bats have an extended feeding time and access to more hiding locations, which reduced the incidence of injuries.

### P323 Mouse Allergen Exposure on Single-Use Versus Multi-Use Disposable Gowns: A Pilot Study

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During the SARS CoV-2 pandemic, the widespread shortage of disposable gowns necessitated reuse of these gowns at some institutions. Cost savings and decreased waste associated with disposable gown reuse were incentives for continuing to reuse gowns, after the supply issues were rectified. One concern of multi-use gowns was increased personnel exposure to the rodent allergen Mus m1. We compared single-use and multi-use disposable gowns, hypothesizing that the reuse of disposable gowns during rodent clinical case management increases the exposure to Mus m1. At the barrier mouse facility, each disposable gown received 10 exposures per test day. Exposure was defined as opening a cage and examining the mice for 2 minutes. This exposure simulated the daily average number of clinical cases and mouse contact time, in this facility. For the single-use condition, 3 gowns were each used one time on 3 separate days. For the multi-use condition, gowns were each reused for 3 days within one week. From each gown, two sites were sampled: the right arm and the center of the front torso. All experimental groups included 3 samples (3 arm sites single-use, 3 torso sites single-use, 3 arm sites multi-use, 3 torso sites multi-use). Samples were processed using the Multiplex Array for Indoor Allergens (MARIA) and Charm PocketSwab ATP tests to detect the level of Mus m1 and organic matter respectively. There was no significant difference of Mus m1 levels between single-use and multi-use gowns; however, Mus m1 and organic debris are present on multi-use gowns hanging in the room. Within the arm sample location, the multi-use gowns had greater ATP levels than single-use gown samples. Thus, recommendations were made to use sanitizable Tyvek sleeves with disposable gowns to reduce the impact of allergen exposure from the multiuse gowns stored in the rooms between uses.

### P324 Utilizing Cage-Side Scales for Efficient Weight Collection in Non-Human Primates (NHPs): A Comparative Analysis

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Obtaining accurate and regular body weight measurements is essential in the management of non-human primates (NHPs) in research. Traditionally, animals are sedated or jumped and moved to a scale away from the enclosure. These processes can be time-consuming, stressful for the animals, and increase the potential for injuries to animals and staff. To refine this procedure, 17 macaques were operantly conditioned to voluntarily enter a chair and be rolled to a scale, and later trained to voluntarily shift into a scale secured to their home cage. The objective of this pilot study was to evaluate the feasibility and reliability of cage-side scale use as a non-invasive alternative for weight collection in non-human primates. This was done by comparing the number and duration of training sessions required for animals to voluntarily perform these behaviors at a mastered level, as well as the reliability of voluntary participation. It was hypothesized that the total time to condition an animal to shift into the cage-side scale for successful weight collection would be lower in duration than the chair weighing method, and that the rate of voluntary participation would be higher. Conditioning was considered complete when the animal obtained the criteria of maintaining a calm body state throughout the weight collection process. To determine participation reliability, the frequencies of successful weights and the refusal to participate in sessions were collected and compared for both chair and cage-side sessions. Subsequent chairing session data was also reviewed to determine the extent of any behavioral regression that may have occurred following weight collection. Preliminary analysis reveals a shorter conditioning period between first introduction and successful weight collection for animals using the cage-side scale when compared to chaired weighing. The animals also showed a reduced latency to participate, fewer instances of fleeing, and a lack of regression recorded in subsequent sessions during cage-side weight collection. The use of a cage-side scale proved to be a practical and reliable method for

collecting weights of non-human primates in a way that increased agency and allowed them to voluntarily participate in care to optimize wellbeing.

### **P325 Avoiding Breeding Burnout: An Enriched Life**

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Promotion of species-specific behavior requires a little understanding, creativity, and time spent to bring undeniable rewards to research animals and the humans providing their care. Not only does promoting species-specific behavior enrich animals' lives, but it also improves animal welfare and increases our understanding of the basic needs of the species; thus, we can implement strategies to meet those needs. Zebra Finches (*Taeniopygia guttata*) and Budgerigars (*Melopsittacus undulatus*) are gregarious species requiring a substantial amount of enrichment to thrive in captivity. Keeping these species in a laboratory setting provides numerous enrichment hurdles. These species are commonly used for behavioral research, and unenriched birds may exhibit undesirable behaviors and health issues. Conditions such as poor breeding performance, feather plucking, lethargy, muteness, among other problems may result in birds which are unsuitable for research. At Cornell University, we examined the impact of various enrichment on the quality and quantity of a colony of breeding Zebra Finches and their offspring produced. By providing unique opportunities for species-specific enrichment that promote species-specific behaviors in breeding, feeding, social interaction, bathing, and structural opportunities, we have been able to reduce the number of breeding pairs needed to obtain the same number of offspring. By following these strategies, we experienced a four-fold increase in production, and a dramatic increase in the quality and well-being of parents and offspring. We are excited to expand and adapt these enrichment strategies for the benefit of other bird and non-avian species throughout the diverse Animal Care and Use Program at Cornell University.

### **P326 A Mutually Enriching High School Student Internship Program**

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Boston University Animal Science Center (BUASC) was contacted by a local agricultural high school in the spring of 2022 requesting support for student externs, as a previously scheduled externship program became unexpectedly unavailable. The opportunity to provide an in-depth look at all aspects of our animal care and research program was accepted with enthusiasm as this could be mutually beneficial to both parties. Students were required to apply to and interview for acceptance to the program through their school's administration, which ensured a baseline of interest with the majority expressing plans to pursue careers in research or veterinary medicine. A pilot one-week experience was generated for twelve high school juniors and a teacher chaperone. Students were granted access to a wide variety of departments (including Occupational Health, Environmental Health & Safety, and IACUC) that interact with BUASC. Students also learned about the career paths our staff have taken and participated in hands-on labs and routine husbandry tasks in rodent barrier and aquatics facilities. Feedback was positive and our participation was again requested for 2023, for which a two-week experience was provided for ten high-school juniors. Recruitment of additional BUASC staff to participate involved mentor training and mandatory background checks since the students were all minors. Staff who volunteered were asked to oversee shadowing opportunities in their facilities and to teach students about day-to-day activities. Additional activities for the second year included research conversations with investigators and preparation of enrichment items for primates. This collaboration has only been in

place for two years, but it is hoped that it creates an additional pipeline of job applicants amidst a highly competitive local hiring market. Other institutions may consider similar high school-level experiences for both improved recruiting and job satisfaction by participating staff.

### **P327 Feed Placement Preference in Golden Syrian Hamsters**

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Research and Development, Inotiv, San Antonio, TX

Golden Syrian hamsters have undergone a resurgence in popularity as a research model following the SARS-CoV-2 pandemic and their susceptibility to the disease. Unfortunately, the husbandry literature concerning the feeding location of hamsters has not been recently re-examined, which has led to facilities housing hamsters in cages designed for other rodents. Although the literature recommends feeding hamsters on the floor, citing narrow feed hopper bars as contraindicated for a hamster's wider muzzle, facilities may feed hamsters in an elevated hopper for various reasons. Ground feeding may lead to increased food waste from it being soiled by dirty bedding as well as increased labor time for animal technicians to ensure animals have adequate amounts of edible feed or scraping dirty cages with feed waste stuck to the cage bottom. To better understand the desired method for feed placement for hamsters, an initial study was conducted to evaluate feed placement preferences. This study utilized socially housed, age-matched male and female hamsters (housed by sex) and provided water and feed *ad libitum*. One group was ground fed, the second fed in a conventional raised feed hopper, and the third, provided feed on the ground and in a hopper. Feed was weighed before and after providing to the animals, with any abnormal interaction being noted, such as grinding. Preference results were assigned based on amount of feed removed from original placement. This team proposes that hamsters are adaptable and will consume food from any location in the cage and adequate husbandry can be provided regardless of feed placement. Future research into animal preferences to update standard husbandry practices is needed to ensure animal research models can safely express species-specific behaviors. The animal manipulations described in this presentation have been approved by Inotiv's IACUC and are verified as original work of the author listed.

### **P328 Long-term Seroconversion Risk Among a Cohort of Cynomolgus Macaques**

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AAV drug development has been aided by use of preclinical animal models to assess vector safety and efficacy. However, AAV vector administration is ineffective in animals carrying neutralizing antibodies from a previous exposure. Seronegativity is determined by prescreening animals for anti-AAV antibodies using either a cell-based neutralizing antibody (NAb) assay or an ELISA-based binding antibody (BAb) assay. However, even NAb negative animals risk seroconversion while awaiting study start which can lead to reduced gene transfer. Here, we undertook a longitudinal study over 6 months to assess seroconversion risk among a cohort of cynomolgus macaques. Animals were prescreened and twelve AAV9 seronegative macaques (6/gender) were procured, shipped, and housed (2/cage) before study start. Animals were bled monthly, and serum investigated for anti-AAV9 antibodies. While animals were NAb negative before shipment, two out of the twelve animals (Female A and Male B) were AAV9 NAb seropositive before study start. Animals were positive for the study duration with no fluctuations in titer. Cage mates co-housed with the seropositive animals did not seroconvert suggesting a lack of transmission. BAb were marginally higher in the animal with higher NAb titer. The seroconversion of

two animals to AAV9 led us to investigate NAb and BAB against a panel of AAVs belonging to different clades (AAV1, 2, 3B, 5, 6, and 8). Animal Male B had cross-reactive NAb to AAV8, albeit at lower levels, while Male C had higher NAb to AAV3B, and lower cross-reactive titers against AAV1 at all time points. IgM binding antibodies were higher in one animal (Female D) against all AAVs at study start that declined below detection levels by 2 months. Surprisingly, there was no concordant increase in IgG levels against any AAV in this animal. Similarly, IgG levels peaked in two animals after 4 months without an expected prior increase in IgM levels. These findings suggest the risk of seroconversion of a naïve animal co-housed with a seropositive cage mate appeared to be low. Finally, while NAb titers were more stable, IgG and IgM binding antibodies fluctuated more during the study and were less predictive of seropositivity.

### P329 Effect of Novel High-Fat Diet Feeding Methods on Food Wastage, Hair Coat Grease Accumulation, and Weight Gain in C57BL/6nrc1 Mice

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Soft-pelleted, high-fat diets are greasy and crumble easily. Due to their poor consistency, high-fat diets often fall through the wire bar lid resulting in food waste. Mice fed high-fat diets frequently experience hair coat grease accumulation. Our study compared the performance of six different feeder designs: a commercially available wire bar lid feeder, a commercially available modular feeder, a novel wire bar lid feeder, a novel floor feeder, and two novel wire bar lid inserts. Novel feeder designs varied the spacing between wire bars. Male C57BL/6Ncr1 (n=120; 4/cage) mice, were randomly assigned to one of the six different feeding conditions and were fed a soft-pelleted, high-fat diet for 12 weeks. Mice were individually weighed and scored for hair coat grease accumulation twice weekly. Feeders and cage bottoms were weighed once weekly. Mice gained the most weight when fed using the commercially available wire bar lid feeder (23.8 ± 1.8 g) and the novel wire bar lid feeder (18.8 ± 2.3 g). Weight gain was significantly less when mice were fed using the other feeder designs (commercially available modular feeder 15.5g ± 2.1 g; novel wire bar lid inserts 15.7 ± 3.4 g, 10.5 ± 1.8 g; novel floor feeder 14.0 ± 3.9 g; *P* < 0.001). Due to diet wastage, the commercially available wire bar lid feeder required replenishing 2-3 times per week and cages necessitated more frequent cage changes. Mice housed in cages with commercially available wire bar lid feeders also had considerable hair coat grease accumulation. By comparison, cages with novel feeder types did not require diet replenishment. In conclusion, careful consideration of feeder selection is crucial in balancing weight gain and resource management when creating diet-based mouse obesity models. Optimal feeder design should minimize food waste and mouse hair coat grease accumulation while promoting weight gain.

### P330 An Attempt to Standardize the Appropriate Breeding Environment by Using Environmental Enrichment Strategies in Naturally-Aged Mice

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Our facility has kept many naturally aged mice used in gerontology and geriatric researches. Social stress (e.g., fighting, etc.) and chronic stress-related inflammation resulted in increased risk for age-related diseases. On the other hand, changes in the characteristics of the environment (e.g., enrichment, etc.) have been shown to have a large

effect on animal welfare. However, the use of fixed environmental enrichment did not offer optimal long-term results, especially in females. In this study, we evaluated the effectiveness of a monthly rotary enrichment system in mice used in gerontological research. Male and female mice (n:15) were used per enrichment group (EE), C57BL/6 (C57BL/6NcrSlc(B6N), C57BL/6J(B6J)) mice (4-weeks-old) were kept over their lifetime. Three environmental enrichment systems were studied: the control group without environmental enrichment (NEE), the fixed enrichment group (FEE), and the rotational enrichment group (REE). Four enrichment shafts were used in both enrichment groups: Nesting material (Enviro-dri), safe harbor retreat nest, swing and cardboard tunnel. Physiological (body weight and survival rates), behavioral (rotarod tests, etc.), and biochemical (urinary corticosterone (CORT)) were performed from 3 months old (MO) to 15 months old every three months. B6N FEE male body weight (Ave.45.2 g) were relatively higher than NEE (Ave.42.3 g) and REE (Ave.40.4 g) at 12 months old. Rotarod test results showed that motor coordination in FEE (176.4±44.5 sec) and REE (227.3±33.4 sec) were not lower than NEE (128.0±23.0 sec) in both strains from 12 months old. The occurrence of hair loss tended to be higher in the experimental groups in both strains, but dermatitis and other skin disorder incidences were lower than NEE. The CORT were relatively lower in the 3-6 months old B6J female REE (202.0±114.2 ng/ml) than in the NEE (429.1±278.2 ng/ml). Results showed that enrichment reduced abnormal behaviors, incidence of skin problems, and stress levels in young mice. The survival rates were higher (10-20%) in EE than NEE. These results suggest that REE was highly useful specifically in strains with high stress base levels. In the future we will continue to investigate various enrichment methods combined with different densities of box animal breeding.

### P331 Use of Running Wheel Enrichment to Decrease the Incidence of Food Grinding Behavior in CD-1 Mice (*Mus musculus*).

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Mice and rats are the most common laboratory animals utilized in research worldwide. In a laboratory setting, mice in particular have been known to grind up and deplete food resources, which has many negative consequences that include increased costs, aberrant research data, and may indicate a deficiency in core biologic or physiologic needs, such as energy expenditure. We hypothesized that the addition of a running wheel as a novel enrichment item would decrease the incidence of food grinding in laboratory mice at our institution. Food pellets were weighed daily in 13 cages of mice known to grind their food. Experimental cages were enrolled in the study for 8 weeks. At week two, a running wheel was placed in the cage and it remained for 4 weeks before removal. Other enrichment devices present for the entire duration of study were a chew block, red hut, and nesting puck. Control cages had all the same enrichment with the exception of the running wheel for the entire duration of study. Food pellets were weighed daily in 8 separate cages of mice not exhibiting grinding behavior and averaged to determine a normal value of food usage. Our findings show that mice known to engage in food grinding behavior demonstrated a decrease in grinding behavior shown as total reduction of average food usage (ingested or grinded) from 84.9 g/cage/day to 46.2 g/cage/day following introduction of novel enrichment. This resulted in an average reduction of ~46% between all experimental cages. Furthermore, our data suggests that there may be a lasting impact on food grinding behavior following removal of the running wheel, and possible implications for the necessity of increased energy expenditure in laboratory mice.

### P332 Effects of Non-Aversive Handling on Home-Cage Activity During Cage Change in Mice

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Cage changing has shown to cause alterations in mouse behavior including increased activity, changes in sleep and increased anxiety. Increases in activity have been found to spike immediately after cage change and then decline over many days. During a cage change, handling a mouse by the tail to move it to a new cage is still common practice in many animal facilities. Non-aversive handling techniques, such as tunnel handling, have been found to reduce anxiety when compared to tail handling. Our study aims to investigate if using non-aversive tunnel handling at cage change alters the in-cage activity during and after cage change, and if activity levels change over time as mice become more familiar with tunnel handling. Using digital ventilated cages, we compared the activity of two groups of mice with no previous exposure to tunnel handling. All mice were group housed and provided with corn cob bedding, a nest, and a plastic tunnel. One group was exclusively tail handled during bi-weekly cage changes and the other group was exclusively tunnel handled; no other experimental interventions were performed. Data was collected 24 hours a day for 20 weeks and animal activity levels were compared. Results from this study will provide further information on the impact of non-aversive rodent handling and determine if these benefits extend to routine husbandry practices such as cage changing.

### **P333 Relationship Between Temperament, Social Rank and Diarrhea in Group Housed NHPs**

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Diarrhea is a common health issue in captive nonhuman primates and incidence increases after relocation to a new social group. To determine if specific temperaments predict diarrhea incidence after relocation from pair to group housing of 8-9 individuals, Human Intruder Tests (HIT) were performed on juvenile (1.63-3.81 years) cynomolgus macaques ( $n = 529$ ) of both sexes while animals were in pair housing. Sixteen observed behaviors were recorded and subjected to factor analysis that identified two dimensions: boldness vs. fearfulness and friendliness vs. aggressive. An additional temperament, anxiety, was also identified. Behaviors for each temperament were summed to create five temperament variables. Once the animals were relocated to group housing, weekly visual observations of each group were made to determine social rank of each individual and its stability over weeks. Temperament and social rank variables were tested to see if they distinguish between animals that develop diarrhea versus those that do not. Among those exhibiting diarrhea, the relationship between temperament, social rank, diarrhea severity and days of diarrhea were examined. Sex was included in the analyses as there was a trend for more males to develop diarrhea than females ( $X^2(526, N = 529) = 3.1278, p = .08$ ). Males that exhibited diarrhea after relocation tended to have higher composite scores in the fearful category on the HIT than those without diarrhea (55% vs 44%), whereas females with diarrhea tended to have lower proportion of fearful scores (46% vs 58%). Among animals with diarrhea, more days of diarrhea after relocation correlate with higher boldness scores on the HIT, particularly among females ( $r = .032; p < 0.05$ ). Greater social rank stability associated with more severe diarrhea ( $r = .032; p < 0.05$ ). The data suggest that behavioral observations are beneficial in colony management to reduce diarrhea rates.

### **P334 A "Beta" Environment for Beta Fish (*Betta splendens*)**

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Environmental enrichment is essential to enhancing animals' welfare in a laboratory setting. At Columbia University, we strive to provide a variety of enrichment to all the species housed there. There has been a lot of research into the positive effects of enrichment on many different species. However, there has not been as much research on the effects of enrichment on aquatic species. At Columbia University, we house a large variety of aquatic species in many different settings. Specifically, we house over 300 betta fish. Due to their aggressive nature, Betta fish are typically housed individually with visual dividers from conspecifics, meaning they receive very little mental stimulation. We wanted to add enrichment to their tanks to enhance their environment. We added physical and sensory enrichment to each tank. We used a polypropylene bar as a floating base, drilled holes in each piece, and added artificial plants. The piece hangs upside down so the plants are floating in the water, reminiscent of floating vegetation found in the wild. We then printed paper with a natural green environment and laminated each sheet. We placed the sheets between tanks in place of the white dividers so the betta fish would have visual enrichment on each side of their tanks. Our approach to enrichment detailed in this abstract demonstrates that there are cheap and easy ways to enrich the lives of betta fish. Although we have not collected data on the behavioral effects the betta fish display in the enrichment presence, we see them interact with the enrichment.

### **P335 Characterization of Palpebral Foreign Material Accumulation in RNU Rats on Paper Square and Corn Cob Bedding**

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RNU (*Foxn1*<sup>RNU</sup>-Nude) rats are an increasingly valuable model for evaluating human cell therapies in a rodent context owing to the immunodeficiency conferred by a mutation in the *FOXN1* gene. The *FOXN1* protein plays an important role in the development of nails, hair, skin, and the immune system and its absence results in impaired fur growth and thymus development. Consequently, RNU rats lack eyelashes and have been reported to accumulate dust beneath the palpebra and develop periorbital abscesses. Beyond the initial reports regarding dust accumulation in this strain, there are no publications describing ophthalmic issues in RNU rats on different bedding products. In our facilities, RNU rats are routinely housed on a paper square product and were observed with severe blepharitis and periorbital swelling attributable to the presence of foreign material beneath the palpebra. We found 80/83 animals with material lodged beneath the palpebra ( $n = 74$  with both eyes effected,  $n = 6$  for one eye effected). Extraction of the material was performed on all animals and the samples collected ranged in appearance from white fibrous squares to linearized brown masses that were consistent in texture with porphyrin-stained bedding. We hypothesized that paper square bedding was lodging beneath eyelids due to the shape and conformation of the substrate (flat, flexible) and that bedding with a different shape and conformation (round, inflexible) would reduce the incidence of this issue. To test this hypothesis, we ran a pilot study comparing the incidence rate of palpebral foreign material accumulation on animals bedded on 1/8th inch corn cob bedding ( $n = 8$ ) and the paper square bedding product ( $n = 8$ ). All animals had foreign material extraction performed prior to placement on one of two bedding types on day 0 and incidence of palpebral foreign material accumulation was measured on days 1, 3, 5, 7, 14, and 21. We anticipate that the completion of this pilot will confirm our hypothesis and will yield valuable data for the laboratory animal community housing this increasingly relevant rat strain with special housing needs.

### **P336 Behavioral and Wounding Rate Measures as Predictors of Pairing Success in Laboratory Swine**

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Pigs are social animals that thrive in group settings. Accordingly, pair housing is one of the most valuable components of behavioral management for laboratory swine, improving animal welfare and enhancing translational research. During transport and arrival at a research facility, they may be separated from their familiar social groups and placed in proximity to unfamiliar pigs. This disruption of their social dynamics can induce stress and anxiety. When attempting to pair pigs, caged interactions can result in aggression and injury during the introduction process. There is little published, empirical data regarding the behavior of laboratory swine during social introductions and so data guidelines describing what criteria determine incompatibility are lacking. To develop metrics for use when pairing swine, we examined behavioral parameters during the pairing process and measured during the first week of a social introduction. We created a behavioral ethogram and wound scoring system, based on size and location of each wound. We recorded affiliative and aggressive behaviors cage-side during continuous one-zero observations every minute for 20 minutes, both during the pairing event and one week post pairing. Wounds were scored at baseline prior to pairing and at time points 0.33h, 4h, 24h, 48h, 72h, 168h post pairing. Wounds were scored by severity using a rating scale, including number and body part. We predicted that affiliative behaviors and low wounding rates within the first 72 hours would predict longer term (1 week) social compatibility. To date, we have collected data on 15 pairings. Preliminary data revealed that most social wounding, indicated by a wound score above 7, was inflicted by the 48-hour timepoint. Most wounding was characterized by bite attempts and lifting of the subordinate animal. All pigs, regardless of wounding, remained socially paired at the 72-hour mark. If initial wounding is found to predict pairing success, this can offer a quick assessment metric in lieu of behavioral observations. Moving forward, this study will validate observational measures (behavior and wounding rates/severity) to develop a simple and practical assessment tool for facilities to use when socializing swine or assessing swine compatibility.

### **P337 Evaluation of Improvements in an Innovative Disinfection System Using Gas Phase Nucleic Acid Digestion Technology to Support Laboratory Animal Management in NCGG**

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Sterilization of animal testing facilities is performed not only on articles (breeding equipment, instruments, and laboratory equipment), but also on breeding rooms and laboratories to prevent the development of pathogenic microorganisms. Each sterilization method has its own characteristics and there have been reports that some methods caused corrosion and the accumulation of residues on breeding equipment (rubber products, etc.) and electronic equipment. We evaluated a new system of combined gas equipment containing formaldehyde gas components with methanol as raw material, which allows decontamination with no corrosion and no residue. Equipment costs (initial costs, running costs), workability, etc., will also be introduced. The method was performed in two facilities of different sizes: the disinfection room (DR, 10 m<sup>3</sup>) and the Infectious Diseases Laboratory (IL, 50 m<sup>3</sup>). The decontamination devices used were a steriXcure (SEALive.Inc) gas generator and a gas decomposition device. To evaluate the sterilization of each room, chemical indicators (CI) (F-Sign) and biological indicators (BI) (*Bacillus atrophaeus* incubated for 7 days) were placed in four locations in the DR and eight locations in the IL. Gas decontamination was performed for 4.5 hours in the DR and 7 hours in the IL. CI results showed the effective sterilization in both rooms. In addition, BI were negative from day 1 to day 7 (spores SAL<10<sup>-6</sup>). These results confirmed the adequate decontamination of both rooms. Furthermore, no residues or corrosion of instrumentation,

electronic devices or experimental equipment were observed. The running cost of the decontamination system is as low as \$10 per operation using only methanol, which is the lowest among sterilization (decontamination) equipment. In the future, we would like to try the decontamination effect of air conditioning ducts. Gas phase nucleic acid digestion method is effective for complete DNA degradation, and therefore for disinfection of COVID-19 research laboratories and is expected to become a next generation sterilization solution. Currently, the manufacturer is developing a decontamination system using ethyl alcohol as a raw material.

### **P338 Tenrec Ecaudatus Enrichment**

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The family Terecidae consists of four sub-families and twenty-nine species. Native to Madagascar, they inhabit almost every ecological niche on the island. The tenrec in general is a small mammal that weighs between 10 g to 2 kg, most are terrestrial but some do inhabit water ways and arboreal habitats. Common tenrecs are the largest of the species and hold the record for the most animals in a litter (up to 30). They live in a variety of environments such as grasslands and urban areas. In the research setting, there is very little information on their behavior or enrichment preferences. In order to provide effective enrichment to our newly acquired group of Common tenrecs, we reviewed the literature, and observed our animals in their home cages. We noticed they were very interested in finding and sourcing food, so we focused on novel enrichment strategies to encourage foraging behavior. Simultaneously, we also investigated interest in behaviors like climbing and tunneling.

### **P339 Why Handling Laboratory Livestock Should Not be a Rodeo Event and the Journey to More Humane, User Friendly Techniques**

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One of the greatest challenges for researchers, veterinarians and animal care staff is finding alternatives to the traditional handling methods typically taught to animal science and veterinary students. Young domestic swine, minipigs, sheep and goats can be some of the more difficult animals to handle, especially for procedures like blood sampling, IV infusions, IM injections, oral dosing and hoof trimming. This poster describes the journey from rodeo wrestling methods to more humane and less stressful techniques for handling and restraining livestock in the laboratory. One example is our relocation of the typical site for intramuscular injections in swine from the highly innervated ham muscle to the lightly innervated neck area. The side of the neck has much thinner skin than the upper rear leg, allowing for use of much smaller needles. Minimal innervation, combined with smaller needles greatly reduces the pain experienced by the animal, making such injections significantly less stressful for both the pig and the veterinarian or technician. Another example is the use of more humane, minimum stress restraint to perform oral dosing in variably sized swine. This allows for the insertion of a stomach tube without the need for a "swine mouth speculum" typically recommended for such procedures. Illustrations of these and other more humane procedural methods will be included in this poster for not only swine but sheep and goats as well.

### **P340 Validation Study of Liquid Sterilants for Sterilization of Flexible Film Isolators**

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Flexible film isolators have been used as housing for germ-free (GF)/axenic, gnotobiotic, and rodents with unknown health statuses for

many years. For practical reasons, it may be feasible to re-use the flexible film bag between projects. To ensure a high level of biosecurity, it is imperative that bags are cleaned and sterilized prior to re-use. To validate the use of various liquid chemical sterilants we tested six different sterilants: peracetic acid 2%, chlorine dioxide 1:5:1, chlorine dioxide 1:3:1, hydrogen peroxide 4.25%, commercial cold sterilant (peracetic acid (0.08%), hydrogen peroxide (1%), acetic acid (<10%)), and sodium dichloroisocyanurate dihydrate (100 ppm). Isolator bags designated for recycle and less than 5 years in age were cleaned with a mild detergent and a dilute bleach solution, rinsed with water, and then dried. The isolator bag was inflated, and isolator shelving and cages assembled. Two, 22-oz spray bottles were filled with each test sterilant and both bottles were used to thoroughly sprayed all surfaces of the isolator bag, structural components, and caging inside of the bag. A fan was connected to the isolator to assist in ventilation of the bag and the bag was left to dry. The dryness of the bag was assessed every two hours until visible moisture was no longer seen. Ten flexible film isolator bags per sterilant were tested for a total of 60 bags. Sticky swabs and Amies agar gel swabs were collected across various surfaces within the isolator both before and after the sterilant was applied. A selection of prevalent rodent pathogens PCR assays and bacteriology cultures were performed to confirm sterilization prior to reuse. Results showed that chlorine dioxide 1:5:1, chlorine dioxide 1:3:1, and the commercial cold sterilant all resulted in negative PCR and culture results post-sterilization. The peracetic acid 2%, hydrogen peroxide 4.25% and sodium dichloroisocyanurate dihydrate all had variable positive results post-sterilization. Based on these results and as a performance standard fit to the needs of our facility, we concluded sterilizing isolators with chlorine dioxide 1:5:1 is a viable option for effective sterilization of isolators designated for reuse.

#### **P341 Running Wheels as a Potential Treatment for Stereotypic Behaviors in Mice**

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The provision of Environmental Enrichment to research mice has evolved greatly and become widespread in recent years. Soft bedding, nesting material, gnawing devices, and running wheels have all shown to elicit natural behaviors in captivity. Unfortunately, little evidence for reducing or eliminating stereotypies appear in the literature. To evaluate specific types of enrichment that may influence stereotypies we utilized video monitoring to quantify levels of stereotypy before and after providing multiple types of enrichment. Cages were video recorded to capture behaviors during different points within the day/light cycle and scored in binary fashion (present/absent) for circling and flipping behaviors. Multiple strains, including C57Bl/6, Balb/c, CD-1, and NOD, were assessed during this period. The types of enrichment implemented after the observation of stereotypies included running wheels, suspended lofts, and double the standard nesting material (~16g) alone or in combination. Because the analysis unit was a cage, and multiple animals within the same cage displayed multiple behaviors, the observed data were correlated within the same cage. Therefore, before analysis, behavior data needed to be aggregated into a single number for each cage. One appropriate aggregation method is the standardized area under the curve (sAUC). Accordingly, data analysis and sample size calculations are based on summary statistics obtained from sAUC. Four treatment-behavior combinations provided measurable means and standard deviations to determine sample sizes needed for statistical significance. These sample size calculations were based on assumptions of the one-sided Type I error rate at 0.025 or 0.05, between 80% - 95% power. Sample sizes needed for significance varied greatly between the different treatment-behavior combinations and statistical power. The required sample sizes for extra nest and extra nest/wheel treatment groups ranged from 53-573 while wheel/circling and wheel/flipping groups ranged from 7-41. Based on these results it appears that running wheels may have an impact on the levels of stereotypic behaviors in mice. Further

investigation into this hypothesis is ongoing.

#### **P342 Portable Emergency Euthanasia System**

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Producing and caring for laboratory rodents and rabbits is full of many challenges. Many facilities strive to find innovative, efficient solutions to everyday challenges while ensuring the welfare of animals and safety of staff. One challenge we sought to address at our facility was the ability to quickly and humanely euthanize animals that are identified with clinical conditions that warranted immediate euthanasia without disrupting the daily activities of staff. A simple, easy to use, AVMA compliant device was developed, and a small pilot study was conducted to test its reliability and efficiency. Sixty mice and rats (30 male, 30 female) varying in age from 21-180 days were exposed to CO<sub>2</sub> in groups of five. The development and implementation of this device for emergency euthanasia can allow for a more immediate response for animals in need of emergency euthanasia at the time of an observation of pain or distress without having to wait for assigned staff to respond or to transport the animal to a euthanasia room. Additionally, it can provide staff an assurance that animal welfare and humane care is paramount to our operations. The prototype device is 9.4 liters in volume and is made of durable clear food-grade plastic. It is outfitted with an 8-liter vial of CO<sub>2</sub> and a fresh air exhaust relief valve for venting. The animals are placed in the device and the lid secured, and the CO<sub>2</sub> is activated. After the first minute, saturation of 40% was reached and by minute 8, the chamber reached 70% saturation and remained at this level until opened. Animals were observed throughout the entire euthanasia process and death confirmed by observing the animals for lack of movement and cessation of breathing following a 10-minute exposure to fresh air. During CO<sub>2</sub> exposure all animals remained calm with the majority becoming recumbent within 60 seconds of introducing gas. In conclusion, following 12 minutes of exposure, we confirmed cessation of breathing in all animals indicating a reliable, effective device. In addition, no animal welfare concerns were observed, and the device meets AVMA guidelines.

#### **P343 Enzymatic Presoak on Primate Foraging Toys is More Effective When Compared to a Rinse and Sanitize Strategy**

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The provision of manipulable foraging toys is important to the Environmental Enrichment Program because they allow animals to exercise species typical extractive foraging behavior. However, foraging toys often include consumable foods to increase the animals interest that are sticky in order to bind the foods together and to the toy. The elements used as binding agents (i.e., apple sauce, yogurt, peanut butter) require the animal manipulate the toy to access and remove the food, but also create a significant challenge for cleaning these devices after each use. A common strategy to clean and disinfect toys is to first remove any leftover substance from the toys manually and then sanitize the toys at high temperature (>140 degrees) in an automatic cage washer. After a trip through the cage washer toys are swabbed and tested for fomites using ATP testing. If a passing grade is obtained, toys are returned to colony usage. Frequently, various toys remain visibly soiled after going through the cage washer. A company that specializes in sanitization processes was hired to review our sanitization procedures and to provide suggestions for areas in need of improvement. One recommendation was to have toys removed from individual cages, presoaked in an enzymatic cleaner, placed in a designated rack for smaller items prior to being run through the cage washer. To determine the effectiveness of this suggested strategy we compared our current "WNPRC Rinse and Go," cage wash procedure vs. pre-soaking items in an enzymatic

cleaner for at least 20 minutes, rinsing, then cage washing. All toys were then swabbed using surface ATP tests following removal from the cage washer. For this comparison we included both dirty cage and foraging toys to cover all ranges of toys at our facility that we use a pre-rinse cage wash sanitization method. Observation: Rinsed toys still had crud and didn't come out of the cage washer as clean as to toys that went through the soaking routine suggested by sanitation solutions. Together, these observations suggest that an enzymatic presoak is more effective when compared to a rinse and sanitize as strategy. Suggested labor benefits include saving staff time and water usage with using a rinsing and cage washing method.

#### **P344 Battle with the Bots – Optimizing Your Semi-Automatic and Automatic Fish Feeding Systems**

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Despite the widespread and growing use of zebrafish as a model in biomedical research, standardized feeding protocols have yet to be established. Instead, individual institutions typically implement feeding procedures based on available resources – typically labor and technology. To improve efficiency and standardization in zebrafish feeding, semi-automatic and automatic fish feeding systems are available. In our facility, we sought to validate the use of two types of technologies, a semi-automatic solid feed dispenser and an automatic fish feeding system, for implementation in our zebrafish feeding program. To do this, weights of feed dispensed by both systems were assessed, with manufacturer-recommended settings providing a starting point for application. Despite controlled use of the same type of technology with the same diet and associated settings, the variability in the amount of feed dispensed was notably greater than what was expected. Though these systems may improve efficiency in labor of feeding, further refinement was warranted to improve standardization; this was achieved in our facility through labor-intensive end-user adjustments exploiting the technologies' inherent capabilities and limitations. In addition to mechanical settings, we assessed several parameters that could potentially influence variation, such as the use of desiccant packs, feeding aperture size, amount of feed present in the device at the time of feeding, and the type of diet. After extensive validation and internal refinement of both systems, we reached optimization, established acceptable usage protocols, and created a quality control program to continuously monitor the performance of these systems in our zebrafish feeding program. Here, we describe the process we performed to achieve targeted feeding amounts for adult zebrafish using a semi-automatic solid feed dispenser and an automatic fish feeding system.

#### **P345 Evaluation of a Novel Electric Shock Trap for Rodent Pest Control in Animal Facilities**

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An effective rodent pest control program is an important aspect of any animal care and use program as it minimizes the risk of pathogen transmission to ensure the SPF status of rodent colonies and protect human safety, especially as to prevent zoonoses. There are many commercially available rodent trap options but four big factors to consider in choosing one include efficacy, costs, easy maintenance, and animal welfare assurance. Live capture traps require significant monitoring and could have questionable efficacy. Meanwhile, mechanical kill traps can be inhumane. According to the AVMA Guidelines, kill traps do not always render a rapid or stress-free death, and thus, use of live trap followed by euthanasia is preferred. However, the Guidelines also state that although newer technologies are improving kill trap performance in achieving loss of

consciousness quickly, individual testing is recommended to be sure the trap is working properly. Here, we evaluate an electric shock trap as an option for vermin control in animal facilities. This trap utilizes a high voltage shock train to quickly render animals unconscious and continues until respiratory and cardiac arrest. This system also has a robust set of digital integration, notification and management features. We evaluated the trap's ability to induce irreversible loss of consciousness and eventual death with minimal pain and distress. This was performed by placing a modified trap (allowing visualization of the animal's interaction within the trap) in a plexiglass open field (24"x 12"x 8") and allowing animals to freely interact with the trap. Assays were videotaped by an overhead and a side camera. We measured time to trap, time to induce loss of consciousness, and time to death using male and female mice wild-type B6 and BALB/c mice. Video recordings were reviewed by two personnel to measure time points. Our results indicated that the trap has a 100% kill rate of animals that trigger the trap. Mean time to trap was 50 seconds, mean time to unconsciousness was 14 seconds, and mean time to death was 25 seconds from trap activation. In conclusion, electric shock traps should be evaluated carefully and once considered effective and humane, could provide a viable option for vermin control in animal facilities.

#### **Laboratory Investigation**

##### **P400 A Comparison of Three Buprenorphine Formulations for Management of Acute Post-Operative Pain in Mice**

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Buprenorphine is a widely used opioid analgesic for managing post-operative pain in mice. However, there are different formulations available, and their comparative efficacy remains unclear. This study aimed to compare 3 buprenorphine formulations, buprenorphine – HCL subcutaneous (SC), buprenorphine extended-release (XR) injectable solution SC, and buprenorphine transdermal solution (TD) for the management of post-operative pain in mice. Singly housed female C57BL/6 mice (N=9) were implanted with a carotid artery blood pressure monitor with SC telemetry placement under an IACUC approved protocol, and randomly assigned to receive one of the 3 formulations (N=3 per group). Pain behavior was assessed using the Mouse Grimace Scale, nesting behaviors and body weight trends. The mice were assessed by the same individual for consistency and assigned a numerical score based on the 5 grimace scale criteria, nesting behaviors and percent body weight loss. A score of 0 represented no pain behavior observed and a score of 1 or 2 represented varying degree of pain behavior observed. The lowest score a mouse could receive was 0 and the maximum was 14. In addition to the assigned buprenorphine formulation, all mice received meloxicam 5 mg/kg SC on the day of surgery and for 3 days post-operatively for 96 hours of coverage. The results showed that all three formulations attenuated post-operative pain behaviors assessed by the 3 above methods. The TD formulation was found to be the most effective, resulting in the lowest scores and therefore provided sustained analgesic effect over 96 hours. The buprenorphine-HCL group was used as the control group to compare the two other formulations. The buprenorphine-HCL formulation required multiple doses to maintain adequate pain control, the buprenorphine XR and TD formulations provided more consistent coverage with fewer additional doses needed. In summary, all formulations of buprenorphine were effective at controlling post operative pain in mice, but the transdermal formulation provided the most effective pain control with the least amount of intervention. These results provide valuable insights for in vivo researchers and veterinarians when choosing an appropriate buprenorphine regimen for post-operative pain management in mice.

##### **P401 COVID-19 Pandemic Effects on the Activity Levels of Yucatan**



## Mini Swine

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During the COVID-19 pandemic, only essential personnel were allowed to interact with our cardiology research animals, substantially decreasing the animals' amount of socialization time. There was a noticeable change in the activity patterns of Yucatan mini swine, as recorded by activity monitors. The limited interaction and socialization resulting from pandemic restrictions led to an increase in their activity levels. This unique circumstance provided an opportunity to explore if the level of interaction affected the swine's activity levels. To investigate this hypothesis, we analyzed the data of Yucatan mini swine already enrolled in two separate cardiac model studies: chronic heart failure (CHF) (N=3) and atrial fibrillation (Afib) (N=3). Each swine was equipped with activity collars to measure their daily activity levels. Three interaction protocols were observed and compared: pre-pandemic, pandemic, and re-entry. These protocols were consistent for both the CHF and Afib models, ensuring uniformity in the assessment. The primary focus was on the swine's "passive activity" levels, measured between the time 10:00 and 14:00, representing isolated activity without staff-induced interaction. Passive activity is the most important timeframe to analyze for this study as it is a representation of the swine's activity with no interference from staff or feeding time. During staff induced activity, the swine are bathed, given treats, and play with toys and treats, leading to an increase in activity. We want to analyze the differences in the swines' isolated activity, hence we only analyze the passive activity time frame for this study. The passive activity time frame is the same for both cardiac models, and the schedule is consistent for each day of the week. For the CHF swine, the average passive activity area under the curve was significantly increased ( $p < 0.005$ ) during the pandemic ( $57.09 \pm 2.9$ ) compared to the pre-pandemic period ( $47.23 \pm 2.42$ ) and the re-entry period ( $50.44 \pm 1.61$ ). There was no significant difference between the pre-pandemic and re-entry periods ( $p < 0.5$ ). The Afib swine displayed similar trends, with higher passive activity during the pandemic compared to pre-pandemic and re-entry. The increase in activity levels during pandemic periods suggests underlying physiological and psychological changes in the swine due to reduced socialization with researchers. This study highlights the importance of enrichment and interaction with research animals, as it sheds light on the impact of humans on animals. Furthermore, it emphasizes the broader implications of the COVID-19 pandemic on research and highlights how variability may impact research study results.

### P402 Evaluating Sub-Renal Capsule Cavity Tumor Growth in Immunodeficient NSG-MHC I/II Double Knock-Out Mice

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The sub-renal capsule site promotes tumor growth by supplying abundant blood and nutrients. Our study aimed to establish a reliable mouse model using MDA-MB-231-2A-Luc-GFP (MDA-Luc) cells, mixed with Matrigel, implanted under the kidney capsule in NSG-MHC I/II double knock-out (DKO) mice with delayed graft-versus-host disease onset. For the first and second studies, we engrafted  $1 \times 10^6$  MDA-Luc cells, while the third study examined three different cell concentrations:  $5 \times 10^5$ ,  $1 \times 10^6$ , or  $2 \times 10^6$  MDA-Luc cells. Daily post-operation observations were conducted to ensure the well-being of the mice for six days following surgery. Tumor burden was assessed twice weekly using in vivo imaging system, along with monitoring body weights and clinical signs. On day 17, mice were euthanized, and kidneys were weighed. Tumor shapes and total flux

intensity (p/s) were compared among mice engrafted with  $1 \times 10^6$  cells across the three studies. In the first study, 93% of mice developed tumors growing beyond the capsule, while in the second study, the figure was 20%. All mice in the third study exhibited well-encapsulated tumors. Mice engrafted with different cell concentrations showed dose-dependent tumor growth until day 10. However, on day 14, we observed that group differences diminished, suggesting tumors reached the maximum volume allowed under the renal capsule. Lung metastatic tumors developed in 20% of mice, and 7% developed tumors on the right kidney, leading to a significant decrease in body weight. Our study demonstrated MDA-Luc tumor growth under the renal capsule and observed metastatic patterns in DKO mice. This model offers significant benefits to researchers interested in combining sub-renal capsule tumors with humanization, particularly considering the advantages of using DKO mice. Furthermore, it is crucial to utilize this model development platform specifically for renal cell carcinoma to create orthotopic models that accurately replicate tumor burden and disease progression.

### P403 Comparing Tumor Growth and Erosion Rate Following P16MC38 Subcutaneous Versus Mammary Fat Pad Implantation

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Murine tumor models are commonly used to understand the biological processes that take place during tumor growth and for the preclinical development of antitumor therapies. The rate of tumor growth, erosion, and ulceration can differ depending on the site of cell implantation and tumor cell line. Here we evaluate tumor growth and erosion rate of mouse colon carcinoma cell line P16MC38 implanted subcutaneously (SC) or in the mammary fat pad (MFP). Forty female 7-week-old C57BL/6J (stock#: 000664) were evaluated for this study. One million MC38 cells were delivered to each mouse, 20 mice were injected subcutaneously, and the other 20 mice were injected in the mammary fat pad. Bodyweights, clinical observations, and tumor caliper measurements were monitored 3 times a week after cell implantation. Mice were euthanized via CO<sub>2</sub> asphyxiation as they reached any of the following humane endpoints – tumor volume over 2000 mm<sup>3</sup>, bodyweight loss over 20%, tumor ulceration, or if they reached the predetermined study end date of 21 days post cell engraftment. Tumor growth differed significantly between the two injection sites with 90% of SC tumors ulcerated whereas only 10% of MFP tumors ulcerated. Eighty five percent of MFP tumors reached or surpassed 2000 mm<sup>3</sup> within 21 days, while only 0.5% of SC tumor reached this same endpoint volume. Our study illustrates the different growth trajectories of tumors cell lines, specifically P16MC38, may have depending on the site of implantation. Understanding the expected tumor growth curve and expected tumor erosion rates for a given tumor cell line and implantation location allows researchers to choose the best implantation site for their study design.

### P404 Effects of Exogenous Erythropoietin on Rabbit (*Oryctolagus cuniculus*) Hematological and Biochemical Parameters

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Preventing anemia in rabbits used to feed arthropod colonies and for disease models or studies is vital for preserving their health and wellbeing. Erythropoietin (EPO) is an endogenous hormone produced mainly by the kidneys in direct response to low pO<sub>2</sub> and thus indirectly to low hemoglobin levels. EPO is commercially available for clinical treatment of anemia in humans, primarily as related to chronic kidney disease or chemotherapy. However, the use of exogenous EPO to prevent anemia in rabbits has not been studied

extensively. This study measured CBC and chemistry parameters in apparently healthy New Zealand White rabbits receiving EPO (n = 16) or saline (n = 2) to test the hypothesis that all doses would increase hematocrit and hemoglobin, with greater effects at higher doses and after multiple doses. Rabbits received either a single injection or 3 consecutive injections 24 hours apart of EPO subcutaneously at 150 or 1,000 IU/kg to determine whether these dosages produce a sustained increase in hematocrit. Baseline bloodwork was drawn on Day 0, followed by initial dosing and serial sampling on days 7, 14, 21, and 28. Blood was collected from marginal ear veins. All experimental groups showed an increase in hematocrit after dosing, followed by a gradual decrease towards baseline after day 7. Analysis of CBC and chemistry parameters showed statistically significant elevation in hematocrit after administration of 1,000 IU/kg EPO for 3 consecutive days only at sampling day 7 (mean = 48.75%) compared to controls (mean = 41.75%). Histopathology assessed for presence of injection site reactions, cellular changes in hematopoietic organs, and evidence of adverse effects related to EPO dosing, such as thromboembolism. Results showed that EPO is well-tolerated by rabbits at doses between 150 and 1,000 IU/kg, as indicated by lack of histopathologic changes in tissue, including no evidence of injection site reactions. These results indicate that a subcutaneous dosage regimen of 3 consecutive injections of 1,000 IU/kg EPO can increase hematocrit in apparently healthy, nonanemic rabbits for one week.

#### P405 Comparative Mutational and Gene Expression Analysis in Canine and Human T-cell Lymphoma

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Naturally occurring cancer in companion canines recapitulates many attributes of counterpart human neoplasms. Lymphoma is one of the most common cancer types in dogs. Some breeds have a higher risk of lymphoma, and specifically, boxers have approximately a 4- to 10-fold increase in risk of developing T-cell lymphoma (TCL) compared to other breeds. Canine lymphoma shares many clinical and phenotypical similarities with human non-Hodgkin lymphoma, and recent studies have also shown molecular similarities between canine and human B-cell lymphoma. However, it is unclear if canine and human TCL share similar biology, including genetic mutations. For this, we investigated canine TCL lymph node and peripheral blood samples to evaluate common genetic mutations associated with TCL in boxers and compare them with those associated human TCL. An association analysis via logistic regression was performed, and SNPs with  $p < 0.015$  were selected. The Ingenuity Pathway Analysis (IPA) identified the hematologic malignancy as the most statistically significant ( $p = 0.00005$ ) disease category associated with high-frequency SNPs. High-frequency SNPs were present in 15 genes involved in hematologic malignancy. In addition, canine TCL was associated with high-frequency SNPs in 27 genes, including eight that are known to be involved in human TCL. One of these genes was HDAC4, which serves as a target for HDAC inhibitors used as a therapeutic in humans. We are currently in the process of conducting RNA-seq of canine TCL samples to further validate these findings. These results offer an opportunity to inform and accelerate cancer drug development for both human and veterinary patients.

#### P406 Bacterial and Infectious Agent Profiling in an Academic Colony of *Xenopus Laevis*

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To better elucidate diagnostic challenges for uncommon models, a panel of gastrointestinal, liver, spleen, kidney, and toe web skin samples were collected from adult *X. laevis*. One hundred samples (equal amounts of male and female) were assayed for the presence of *Salmonella* species, *Ranavirus*, *Pseudocapillaria xenopi*, *Batrachochytrium* (*B.*) *dendrobatidis*, *Mycobacterium* (*M.*) *chelonae*, *M. marinum*, *M. goodii*, *M. xenopi*, *M. liflandii*, *Chlamydomphila* genus, *Aeromonas* (*A.*) *hydrophila*, *A. dhakensis*, *Pseudomonas aeruginosa*, and *Cryptosporidium* species. In the male frogs, only *A. hydrophila* was detected in the gastrointestinal sample. By contrast, *A. hydrophila* was detected in the gastrointestinal fraction and *B. dendrobatidis* in the toe skin fraction of the female frog cohort. Additional environmental samples were also collected to evaluate detection in host-independent samples. In the detritus, tank bottom with algae and sump lich, *A. hydrophila* was detected. For a more comprehensive look at the commensal bacteria colonizing the gastrointestinal tract, V3-V4 16S rRNA profiling was performed. Overall, similar totals of observed taxa were seen between female and male frogs with female frogs showing more variation in the total number of observed taxa. Abundant levels of *Romboutsia sedimentorum* and *Turicibacter sanguinis* were found in all samples. *Cetobacterium somerae* was found in all frogs but varied wildly in the relative abundance. Lastly, *Clostridioides difficile* was identified in all frog samples. Supporting research model expansion requires constant evaluation and re-evaluation of diagnostic testing and commensal profiling. In this study, we demonstrated detection capability of aquatic infectious agents and insight into the commensal populations of *X. laevis*. Profiling the microbial community aids in understating of *X. laevis* health under normal conditions and works to protect husbandry workers from potential zoonotic agents.

#### P407 Washed up and Wasted: A Promising Alternative for Collection of Influenza Viral Load in the Ferret

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High morbidity and mortality associated with human influenza virus has far-reaching public health and economic impacts. Influenza research frequently requires the use of animal models, with ferrets distinguished among other small research animals by their development of physiological symptoms similar to humans. Ferret sample collection using a saline nasal wash technique provides information on viral load of the upper respiratory tract but requires anesthesia with isoflurane gas that brings behavioral agitation and excessive salivation. Because the use of anesthesia requires fasting prior to anesthetic treatment, it is possible that the required fasting may adversely influence animal weight measurements that are used to evaluate infection-induced morbidity. Considering this, alternative techniques that collect a sufficient viral load for sample testing while avoiding the need for anesthesia and fasting may alleviate isoflurane-related agitation and weight fluctuation. Studies were performed to determine if oral swabs provided a sample suitable for viral load monitoring that did not require anesthesia or fasting. Samples from 16 male ferrets were collected via oral swabs of the soft palate followed by nasal washes. Timepoints at day 2 and day 4 post-infection were tested for viral load using plaque assays. Nasal wash samples on day 2 post-infection (dpi2) returned an average 6.69E+03 Foci-forming units/milliliter (FFU/mL) among the 12 (75%) samples in which virus was detected, with no virus detected in samples gathered at dpi4. In comparison, oral swabs collected on dpi2 showed an average of 1.20E+03 FFU/mL among only 7 (43.8%) detectable samples. At dpi4, oral swabs returned an average 6.55E+02 FFU/mL from 6 (37.5%) of the animals sampled. These findings indicate a higher titer of detection among a greater percentage of animals through nasal wash at early timepoints, whereas oral swabs offer detection for a longer duration post infection. This suggests an efficacy of oral swabs for detecting viral load at timepoints dpi2 and

dpi4, making them a promising alternative collection technique that does not require anesthesia.

#### **P408 Meloxicam Attenuates Heat Hypersensitivity in Red-eared slider Turtles**

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Meloxicam is one of the most commonly used analgesics for reptiles. However, there is limited efficacy information in red-eared sliders. Here, we evaluated two dosages of meloxicam. We hypothesized whether 1 mg/kg meloxicam attenuated thermal hypersensitivity more effectively than 0.4 mg/kg meloxicam in red-eared sliders. Twenty-one red-eared sliders (female = 17, male = 4; weighing 700±75 g) were randomly assigned to 1 of 3 groups: 1) Saline; 2) 0.4 (Melox-0.4) mg/kg meloxicam; 3) 1 (Melox-1) mg/kg meloxicam. All injections were administered subcutaneously in the right forelimbs. Using the Hargreaves thermal hypersensitivity device, used commonly in rodent pain assays, radiant heat was applied to the plantar surface of both hind limbs and withdrawal response to heat (heat latency) was determined at 0.5, 2, 4, 8, and 24 h post-meloxicam injection. Compared to Saline group, 1) Melox-0.4 attenuated (increased heat latency) hypersensitivity at 0.5, 2, 4 and 8 h; 2) Melox-1 only attenuated hypersensitivity at 8 h. Melox-1 did not attenuate thermal hypersensitivity more effectively than Melox-0.4. This study indicates that 0.4 mg/kg meloxicam effectively attenuates heat hypersensitivity up to 8 h in red-eared sliders.

#### **P409 Extending MS-222 Mediated Anesthesia in Zebrafish (*Danio Rerio*)**

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The emergence of Zebrafish as a model species took advantage of the species' adult size, hardiness, time to maturity, relative ease of housing, and genetic similarities to humans to make them an ideal model across many disciplines. However, zebrafish husbandry lacks standardization in many facets and researchers are left desiring some commonplace procedures available to other model organisms. One considerable disadvantage to using aquatic species is the limitation on handling, imaging, and surgery. Historically, tricaine methanesulfonate (MS-222) has been a preferred mode of sedation and anesthesia but extended anesthesia on the surgical plane level has not been examined. To determine if extended sedation of zebrafish can be achieved while being potent enough to maintain plane III of anesthesia while out of water is possible, a trial was conducted. After an initial induction dose of 150 ppm MS-222, reduced concentrations of MS-222 (70, 50, and 30ppm) were run over the gills of the sedated fish. This was done by intubating the sedated fish with a size 22 catheter connected to an IV infusion medical pump containing the reduced dose of MS-222. Fish were checked for response to external stimuli every ten minutes with a gentle tap to the caudal peduncle using sterile forceps followed with a mist of system water. After 30 minutes of continuous MS-222 dosing, fish were moved back to system water to start recovery. Time to induce, intubate, recover, stimuli response, and operculum beats per minute were measured during the trial. Of the 30 fish this trial was ran on, 30 survived the anesthesia and resumed normal behavior. Response to external stimuli was less in the fish receiving 70 ppm maintenance doses, with 30% of individuals responding to the first external stimulation (30 ppm = 60%; 50 ppm = 30%), and no responses to the second (30 ppm = 30%; 50 ppm = 20%), and third attempt (30 ppm = 20%; 50 ppm = 10%). Additionally, it took on average 84, 140, and 266 seconds to recover from a 30, 50, and 70 ppm MS-222 maintenance dose respectively. In summary, the ability to handle zebrafish outside of water for procedures would greatly improve the usages of

zebrafish as a model organism and can be achieved for at least 30 minutes through intubation.

#### **P410 Atipamezole Results in Increased Heart Rate and Decreased Blood Pressure in Cynomolgus Macaques (*Macaca fascicularis*)**

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Atipamezole, an  $\alpha$ -2 adrenergic receptor antagonist, reverses  $\alpha$ -2 agonist anesthetic effects. There is a dearth of information on the physiological effects of these drugs after administration in cynomolgus macaques. Here, we assessed atipamezole's physiologic effects. We hypothesized subcutaneous atipamezole administration causes post-injection hypotension. Five cynomolgus macaques (3 male, 2 female) were sedated with ketamine (4 mg/kg) and dexmedetomidine (0.02 mg/kg) intramuscularly. Forty-five minutes later, atipamezole (0.5 mg/kg) was given subcutaneously. Anesthetic parameters (heart rate, blood pressure [systolic (SAP), diastolic (DAP), and mean (MAP) blood pressure], body temperature, respiratory rate, and %SpO<sub>2</sub>) were monitored prior to and every 10 min (through 60 min) post atipamezole injection. While the heart rate was significantly increased from injection at most time points; SAP, DAP and MAP were significantly decreased from injection at 10 minutes. Temperature was also significantly decreased at 10 minutes post injection. No other parameters were significantly altered from injection time. This study indicates subcutaneous atipamezole causes increased heart rate and decreased blood pressure from parameters under sedation with ketamine and dexmedetomidine. These findings are clinically important to raise anesthetist awareness to properly support and treat as needed.

#### **P411 African Grass Rats (*Arvicanthis niloticus*) as a Spontaneous Model of Type II Diabetes and Metabolic Syndrome in Humans**

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In humans, metabolic syndrome is characterized by a spectrum of metabolic and cardiovascular diseases that predispose individuals to acute cardiovascular events, type II diabetes, kidney disease and nonalcoholic steatohepatitis, among other conditions. While the incidence of metabolic syndrome varies according to age, gender, ethnicity and socioeconomic status, it currently affects about 20-25% of the human population, a figure that has been increasing. Numerous animal models are used to study metabolic syndrome, including genetically engineered, diet-induced and chemically-induced rodent models; however, these models all lack the natural progression, risk factors and full spectrum of disease recognized in humans. African grass rats are diurnal rodents that develop spontaneous disease mimicking human metabolic syndrome when raised in a laboratory setting on standard rodent chow. To validate and better characterize this model, we collected histomorphologic, clinical chemistry and biomarker data from aged (n = 12) and young (n=3) African grass rats on standard rodent chow. By as early as 2-3 months of age, grass rats develop gross and histologic lesions, clinical chemistry abnormalities and biomarker expression patterns similar to those seen in humans with this disease. Characteristic pathologic findings included atherosclerotic plaques, chronic progressive nephropathy, pancreatic remodeling and hepatic steatosis. Clinical chemistries revealed hyperglycemia with glucosuria, hypercholesterolemia, elevated triglycerides and elevated liver enzymes. Corresponding with these findings, serum biomarker analysis showed an inverted correlation between insulin and adiponectin. In addition, the animals had decreased serum cortisol and elevated serum ACTH along with adrenal lesions suggestive of

hypothalamic-pituitary-adrenal axis disruption and derangement in adrenocortical steroid biosynthesis, an important component of metabolic syndrome in humans that has not previously been reported in African grass rats. These findings demonstrate that African grass rats are a novel and relevant model to study the natural progression of metabolic syndrome in humans, and can provide insight into the pathophysiology of the disease where traditional animal models fall short.

#### P412 Anesthetic Sensitivity of Mouse C57/BL/6 Substrains to Isoflurane

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While genetically very similar, there are many physiological differences between the substrains of C57BL/6 mice having important impacts on the animals. Specifically, differences have been noted between substrains in response to some injectable anesthetic protocols. The goal of this project is to determine the effect of C57BL/6 substrain on isoflurane responsiveness. To test this, the minimum alveolar concentration (MAC) for isoflurane was determined from female and male mice of four substrains of C57BL/6 mice from four different vendors: C57BL/6NTac from Taconic (TAC: n=6), C57BL/6J mice from The Jackson Laboratory (JAX: n=6), C57BL/6NHsd mice from Envigo (n=6), and C57BL/6NCrl from Charles River Laboratories (CRL: n=6). Isoflurane concentrations from 1.7% to 2.2% were tested, using oxygen as the carrier gas. The surgical plane was defined as no motor response to a 300-gram force (Touch Test Device) delivered to the hind paw. MAC for each substrain was: C57BL/6NTac: 2.02%±0.11, C57BL/6J: 2.08%±0.09, C57BL/6NHsd: 2.00%±0.15, and C57BL/6NCrl: 2.04%±0.11. None of these differences were significantly different (p=0.282). Nor were the differences between males, 2.02% ± 0.10 and females, 2.06%±0.13 (p=0.441). There was no morbidity or mortality noted in any of the mice. These important results show that while there are differences between substrains to some injectable anesthetics, no significant differences in response to isoflurane, the preferred anesthetic for mice, exist. Future work will examine the substrains' responses to ketamine/xylazine/acepromazine, the most used injectable anesthetic protocol.

#### P413 2D-Analysis of Serum Proteins in a Mouse Model of ANCA-Associated Vasculitis (SCG/Kj Strain)

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[Objective] The SCG/Kj mouse strain has been used as a model of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (Proc Natl Acad Sci USA 90: 3413, 1993). We have searched for serum biomarkers of vasculitis by comparing SCG/Kj and normal controls (C57BL/6) and found apolipoprotein A2 (APOA2) as a candidate biomarker protein (Drug Des Devel Ther 13:555-568, 2019). In the present study, we further searched for serum biomarkers in SCG/Kj mice using two-dimensional electrophoresis (2DE) with fluorescence-labeled proteins (2D-DIGE). We tried to confirm that APOA2 is one of the biomarkers of vasculitis by two-dimensional Western blot (2D-WB). We also tried to find proteins other than APOA2 that are preferentially present in SCG/Kj. [M&M] Serum samples were collected from female mice of both strains at ~13 weeks of age. Albumins and IgGs were removed from the serum samples using Cibacron blue agarose beads and Protein G, respectively. Albumin-IgG-depleted serum proteins from SCG/Kj and C57BL/6 were labeled with orange (IC3-Osu) and red (IC5-Osu) fluorophores, respectively. Two differentially labeled proteins were combined and separated by 2DE under non-reducing conditions (first dimension: isoelectric focusing using IPG strips from pH 3 to 10; second

dimension: SDS-PAGE). Fluorescent gel images were captured using an image scanner. Differentially present proteins were searched for by examining a merged image of two pseudocolored images. In addition, spots reacting with anti-Apoa2 antibodies were searched by 2D-WB with unlabeled albumin-IgG-depleted serum proteins from SCG/Kj. [Results] Using 2D-DIGE, we found a spot preferentially present in SCG/Kj (pI: ~6.2; molecular weight: ~26kDa). We also found that anti-APOA2 antibodies bound this spot by 2D-WB. Some other spots were preferentially present in SCG/Kj adjacent to the spot bound by anti-APOA2 antibodies. [Conclusion] APOA2 was found to be a serum biomarker specific for SCG/Kj, confirming the association between APOA2 and vasculitis. We found some other candidate biomarker proteins by 2D-DIGE and will further investigate serum biomarkers for vasculitis.

#### P414 Medetomidine/Vatinoxan and Ketamine Effectively Anesthetizes Mice

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Medetomidine/vatinoxan is a novel anesthetic combination used in veterinary medicine. We evaluated the clinical safety and efficacy of three different dosage combinations of medetomidine/vatinoxan (Zenalpha®) and ketamine as compared to xylazine and ketamine for anesthesia of B6 mice. We hypothesized that Zenalpha® with ketamine would provide reliable anesthesia without adverse physiological effects or mortality more consistently than ketamine and xylazine. Ten-week-old male and female C57BL/6 mice were randomly administered 1 of 4 anesthetics subcutaneously (time 0, T0): 1) KX - ketamine (80 mg/kg) and xylazine (8 mg/kg); 2) KZ/2 - ketamine (80 mg/kg) and Zenalpha® (2.0 mg/kg); 3) KZ/2.5 - ketamine (80 mg/kg) and Zenalpha® (2.5 mg/kg); 4) KZ/3 - ketamine (50mg/kg) and Zenalpha® (3.0mg/kg). All mice received buprenorphine-ER (0.5mg/kg), 0.9% NaCl (5 ml/kg), and were maintained with 100%O<sub>2</sub> on a heating pad. Buprenorphine-ER was administered to provide balanced anesthesia and replicate the anesthetic regimen recommended for rodents. Following drug administration, we assessed induction by measuring the time to loss of righting reflex and loss of paw withdrawal reflex (PWR). Throughout anesthesia, the physiological parameters monitored included heart rate, respiratory rate, %SpO<sub>2</sub>, blood pressure, body temperature, and skin color. Atipamezole (1 mg/kg SC) was administered 30 minutes after anesthetic drug administration (T30) and recovery time was monitored through measurement of the time until return of paw withdrawal reflex, righting reflex, standing and ambulation. All mice were monitored for 3 days post anesthesia. Results were: 1) KX did not achieve the complete loss of PWR and 2) all KZ treatments had a loss of paw withdrawal reflex throughout 30 minutes. KZ anesthesia provides more consistent and stable anesthesia as compared to KX in mice. This data suggests that medetomidine/vatinoxan (2.0 and 2.5 mg/kg) and ketamine (80 mg/kg) provides reliable and safe anesthesia in mice.

#### P415 Effects of Refined Handling on Reproductive Indices of BALB/cj and CD-1 IGS Mice



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Current methods of mouse handling during cage change procedures

can cause stress, potentially compromising animal welfare. A prior study on breeding C57BL/6J mice showed modest increases in pup production and significant reduction in pre-weaning litter losses with tunnel handling compared to lifting mice by the tail with padded forceps. The current study describes results for two randomised controlled trials of handling method on the reproduction of additional mouse strains, BALB/cJ (a low-intermediate fecundity strain), and CD-1 IGS (a high fecundity stock). It was predicted that refined handling would have minimal effects on the high fecundity line because reproductive performance was already maximised, but greater effects on the low fecundity line. Handling method (tunnel versus tail-lift) was randomly allocated to monogamous breeding pairs of mice. Reproductive metrics (litter size at birth and weaning, numbers of litters, litter attrition, between-litter intervals, pup weaning weight, sex ratio) were prospectively monitored for 80 BALB/cJ and 77 CD-1 pairs allowed to breed continuously for 6 months. Neither strain demonstrated operational or statistically significant differences between handling methods for any reproduction metric. Because breeding pairs were relatively undisturbed, only handled during biweekly cage changes, it is likely that mice did not experience sufficient stress either to affect breeding performance or discriminate between handling methods. Nevertheless, previously documented welfare benefits for a more sensitive strain suggest that refined handling should be the default option to reduce background stress.

#### **P416 Progression of Spontaneous Colitis in MDR1a and IL10 Knockout Mice Housed in either Disposable Mouse or Rat Cages**

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Colitis, inflammation of the colon, is caused by a range of factors including infections, autoimmune disorders, and inflammatory bowel disease (IBD). The MDR1a and IL-10 knockout mouse strains are established animal models of colitis and IBD. Environmental factors, including caging and microbiome, can affect the development and severity of these models and cause high variability from one location to another. In this study, cage size and housing density were assessed for their impact on incidence and progression of colitis in these two mouse models of inflammatory bowel disease. Female MDR1a and IL-10 knockout mice were housed in either standard disposable Innovive Innocage® IVC mouse caging; 81 in2 floor space (n= 5 mice per cage) or standard disposable Innocage® IVC rat caging; 141 in2 floor space (n= 10 mice per cage). Enrichment and microbiome (via fecal matter transfer from donor mice caging) were kept consistent for all cages. Nestbuilding scores, weekly photos, body weights, modified Irwin test scores, daily observations and fecal scores were recorded in-life. Study endpoints included body weight, plasma, cytokines/chemokines (IFN- $\gamma$ , IL-1 $\beta$ , IL-6, KC/GRO, TNF- $\alpha$ ), fecal scores (0-3), hemocult scores (0-3), and colon analysis (weight/length ratio, cytokine/chemokine protein expression, gene expression, and histopathology). Results: There was no significant difference in colitis scores or cytokine/chemokine levels between mice housed in mouse or rat cages. Differences were observed in the histological evaluation of the colons. Mice housed in rat cages exhibited increased exploratory behavior and active engagement with their environment. Conclusion: Utilizing rat instead of mouse cages resulted in more positive social activity and a healthier environment for animals. However, both mouse and rat disposable cages resulted in similar progression of spontaneous colitis in MDR1a mice and IL10 mice.

#### **P417 Decellularized Graft for Repairing Severe Peripheral Nerve Injuries in Sheep**

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Peripheral nerve injuries resulting in a severe nerve defect require surgical repair. Despite the advances in microsurgery, regeneration of the injured peripheral nerve and the subsequent functional recovery are usually incomplete. The gold standard of repair, the autograft, has several limitations such as limited supply, loss of function of the donor nerve and morbidity in the extraction area. Therefore, new alternatives must be developed, and animal models are still necessary. In this study using the sheep as a model we have assessed nerve regeneration through a long gap nerve injury (5 cm) in the peroneal nerve repaired with a decellularized nerve allograft. A nerve gap 5 cm long was made in the peroneal nerve of ten sheep. The repair was performed by an autograft (AG) or by a decellularized nerve allograft (DCA). Functional tests were performed once a month, and electrophysiology and echography evaluations at 6.5- and 9-months post-surgery. Nerve grafts were harvested at 9 months for immunohistochemical and morphological analyses. The sheep allowed to perform long-gap nerve injuries with no relevant clinical signs. Besides, all the animals reached the end of the experiment in good conditions. The decellularization protocol eliminated the cells while preserving the extracellular matrix of the nerve. No significant differences were observed in functional tests. Reinnervation of the tibialis anterior muscle occurred in all animals, with some delay in DCA compared to AG groups. Histology showed a preserved fascicular structure in both AG and DCA, however, the number of axons distal to the nerve graft was significantly lower in the DCA compared to the AG group (\* $p < 0.05$ ). The sheep is a useful model for investigating long-gap peripheral nerve injuries and to test new therapeutic alternatives. Although, the decellularized graft assayed supported effective axonal regeneration when used to repair a 5 cm long gap in the sheep.

#### **P418 Evaluation of the Cellular and Humoral Immune Response of a Vaccine Candidate against COVID-19 with Different Alum Adjuvant Formulations**

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Vaccine development is a major contribution of immunology in the history of humanity against illness. Different platforms are currently being used for the development and delivery of vaccines such as inactivated virus, viral vector (adenovirus), recombinant protein, live-attenuated, and peptide vaccines. Recombinant proteins are the antigens of choice for generating modern vaccines, however, formulating these vaccines with adjuvants is necessary because they are poorly immunogenic. In Mexico, only alum adjuvants are approved for use in human vaccines. To evaluate the humoral and cellular immune response of a recombinant protein (20 and 60 $\mu$ g) with two alum adjuvants (Adju-Phos and Alhydrogel) and PHAD® in 8 different formulations, we inoculated C-57BL/6J female mice (N=56) subcutaneously (seven animals per experimental group). Based on the results, we added two more groups with 60 $\mu$ g of

vaccine, Adju-Phos, and without PHAD®. We used an immunization scheme of two doses (Day 0 and 21) and euthanasia performed 10 days after. Antibody titers were determined by indirect ELISA, lymphoproliferation assays, and the determination of cytokine production in serum and cells were performed by flow cytometry, and neutralizing antibodies were assessed by simulated neutralization assays. Higher titers of IgG, IgG2 $\alpha$  and IgG1 antibodies were observed in the formulation with 60 $\mu$ g protein and Adju-Phos with PHAD®, as well as specific CD4+ and CD8+ T cells. We also observed the highest levels of TNF- $\alpha$  and IFN- $\gamma$  with this formulation, in addition to the presence of neutralizing antibodies against the Delta and Omicron variants of SARS-CoV-2. The formulation with 60 $\mu$ g protein and Adju-Phos plus PHAD® was shown to be antigenic and immunogenic in mice vaccinated with a two-dose schedule and had a Th1 rather than a Th2 cell response. These results demonstrate novel findings because we have created this chimeric protein where we included 10 peptides that are present in the RBD of the most predominant COVID variants thus far. A receptor-binding domain (RBD) is a short immunogenic fragment from a virus that docks to a specific receptor to enter cells and thus lead to infection. This offers another platform for vaccine development based on recombinant vaccines with this class of adjuvants.

#### **P419 Subcutaneous Injection of Alfaxalone-Xylazine-Buprenorphine in Mice to Induce a Surgical Plane of Anesthesia and Echocardiographic Evaluation**

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Alfaxalone has gained increasing popularity as an injectable anesthetic for use in dogs and cats due to its minimal cardiovascular side effects. Data for mice are limited and demonstrate strain- and sex-associated differences in dose response. In a dose-comparison study of alfaxalone-xylazine-buprenorphine (AXB) in CrI:CFW(SW) mice, subcutaneous injection of 50mg/kg alfaxalone-10mg/kg xylazine-0.1mg/kg buprenorphine HCl consistently achieved a surgical plane of anesthesia (loss of toe pinch) for 48.6 $\pm$ 4.7 and 60.8 $\pm$ 9.6 minutes in females and males, respectively. The same dose and route of AXB induced surgical plane of anesthesia in C57Bl/6NCrI (females: 43.4 $\pm$ 14.1 min, males: 51.1 $\pm$ 10.7 min), NCr-Foxn1<sup>nu</sup> (females: 75.8 $\pm$ 34 min, males: 80.3 $\pm$ 40.4 min) and NOD.Cg-Prkdc<sup>SCID</sup>Il2rg<sup>tm1Wjl</sup>/SzJCr (females: 68.3 $\pm$ 27.9 min, males: 65.7 $\pm$ 12.1 min) mice. No significant difference in duration of surgical plane of anesthesia was seen between male and female CrI:CFW(SW), C57Bl/6NCrI, NCr-Foxn1<sup>nu</sup>, and NOD.Cg-Prkdc<sup>SCID</sup>Il2rg<sup>tm1Wjl</sup>/SzJCr mice (two-sided unpaired Wilcoxon rank test,  $p > 0.05$ ). In an echocardiography study ( $n=5$  per group) of CrI:CFW(SW) mice under AXB anesthesia compared to 100 mg/kg ketamine-10mg/kg xylazine (KX), AXB induced less bradycardia (295.4 $\pm$ 29 bpm) compared to KX (185.8 $\pm$ 38.9 bpm) group ( $p=0.0012$ ). There was no significant difference ( $p > 0.05$ ) in cardiac output (CO), ejection fraction (EF), end-diastolic volume (V;d), end-systolic volume (V;s), or fractional shortening (FS) between the two anesthetic groups. These results suggest that subcutaneous administration of 50mg/kg alfaxalone-10 mg/kg xylazine- 0.1mg/kg buprenorphine HCl is a viable anesthetic option to induce a surgical plane of anesthesia in CrI:CFW(SW), C57Bl/6NCrI, NCr-Foxn1<sup>nu</sup>, and NOD.Cg-Prkdc<sup>SCID</sup>Il2rg<sup>tm1Wjl</sup>/SzJCr mice, regardless of sex. Additionally, AXB may be a better injectable anesthetic option compared to KX when there is concern for adverse cardiac effects in mice, however more studies are needed to evaluate cardiac function.

#### **P420 Volume-Associated Clinical and Histopathological Effects of Intranasal Instillation in Syrian Hamsters: Considerations for Infection and Therapeutic Studies**

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Syrian hamsters are a key animal model of SARS-CoV-2 and other respiratory viruses and are useful for the evaluation of associated medical countermeasures. Delivery of an infectious agent or intervention to the respiratory tract mirrors natural routes of exposure and allows for the evaluation of clinically relevant therapeutic administration. The data to support instillation or inoculation volumes are important both for optimal experimental design and to minimize or avoid effects of diluent alone, which may compromise both data interpretation and animal welfare. The purpose of this study was to investigate four intranasal (IN) instillation volumes in hamsters (50, 100, 200, or 400  $\mu$ L). Groups of sixteen 5- to 6-week old Syrian hamsters (8 male, 8 female) were instilled intranasally (IN) under isoflurane anesthesia, with 50, 100, 200 or 400  $\mu$ L of DMEM, divided bilaterally between the nares. The animals were monitored daily, and a subset were serially euthanized at one of four pre-determined time-points (1, 3, 7, and 14 days post-instillation). Weight, temperature, oxygen saturation, CBC, radiographs, and respiratory tissue histopathology were assessed to determine changes associated with instillation volume alone. With all the delivery volumes, we found no notable differences between instilled and non-instilled controls in all of the parameters assessed, except for histopathology. In the animals instilled with 200 or 400  $\mu$ L, inflammation associated with foreign material was detected in the lower respiratory tract indicating that higher volumes may result in aspiration of nasal and/or oropharyngeal material in a subset of animals, resulting in IN instillation-associated histopathology. Overall, we have shown that studies in Syrian hamsters should limit instillation volumes to 100 $\mu$ L or less to mitigate the volume-associated histological changes.

#### **P421 Development of a Delayed-Type Hypersensitivity Model for Assessing Immunomodulatory Effects in Rhesus Macaques**

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Delayed-type hypersensitivity (DTH) is a T cell-mediated response that can be used to assess the immunomodulatory effects of drugs in nonclinical species. Historically, DTH modeling has commonly been conducted in rodents and has shown sensitivity to various immunosuppressive agents. With more biologics being pursued as therapeutic candidates, nonhuman primates (NHPs) are often the only relevant species. In the present study, we established an NHP-based intradermal (ID) challenge DTH model. Effects of two antigens, tetanus toxoid (TT) or keyhole limpet hemocyanin (KLH) were evaluated. Our results showed that although a robust anti-TT antibody response was detected in serum, ID challenge of an available TT vaccine with adjuvant resulted in acute inflammation characterized by local neutrophil infiltration. In contrast, KLH provided opportunities to optimize the antigen and adjuvant concentrations. All 10 animals demonstrated a robust anti-KLH serum antibody response. Histopathological analysis of ID challenge sites revealed signatures of a DTH reaction most prominently demonstrated by an infiltration of CD3+ and CD68+ cells. An anti-inflammatory agent, dexamethasone (1mg/kg), was able to reduce the DTH response, indicated by decreased number of CD3+ and CD68+ cells. Additionally, the use of laser speckle contrast imaging (LSCI) was developed to assess dermal blood flow as a method to quantify DTH responses. Preliminary results from LSCI evaluation detected a significant increase in dermal blood flow at ID challenge sites, which was attenuated with dexamethasone pretreatment. A correlation between dermal blood flow and histologic findings

indicates that LSCI could be utilized as a non-invasion method for assessing DTH responses in the presence of immunomodulatory molecules. Gene expression profiling of skin biopsies to determine DTH-induced transcription was also evaluated as a novel assessment tool. Our results show a KLH-based DTH model in NHPs can readily detect drug induced immunosuppressive effects. Novel technologies including LSCI, and gene transcription provided a quantitative assessment of DTH responses which can increase the sensitivity and specificity of the model for evaluating immunomodulatory effects of biologics during nonclinical development.

#### **P422 Non-invasive In Vivo Imaging of Reactive Oxygen Species (ROS) Activation in Neuroinflammation and Pain**

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Reactive oxygen species (ROS), such as superoxide, are a class of highly reactive oxygen-containing molecules that play an important role under normal physiological conditions. However, the overproduction of ROS leads to oxidative stress and neuronal injury, hallmarks of several neurodegenerative diseases including but not limited to Alzheimer's disease (AD), Parkinson's disease (PD), and Amyotrophic Lateral Sclerosis (ALS). In addition, the overproduction of ROS is thought to contribute to peripheral nerve injury-associated neuropathic pain. Therefore, non-invasive ROS imaging techniques would allow us to evaluate potential therapeutic candidates targeting the overproduction of ROS. For this study, dihydroethidium (DHE), a blood-brain-barrier (BBB) penetrant fluorescent probe used to measure superoxide levels, was utilized for imaging. To image brain ROS activity in C57BL/6 mice (female, 5 mice per group) following a lipopolysaccharide (LPS, 5 mg/kg, intraperitoneal [IP]) challenge, DHE (20 mg/kg, IP) was injected as an imaging substrate (excitation 500-530 nm/emission 590-620 nm). DHE fluorescence signal from the brain at 4hr post-LPS injection was significantly higher in the LPS group than in the saline-treated group. We then examined Luminol (a non-BBB penetrant probe) to estimate the peripheral ROS activity in C57BL/6 (male, 10 mice per group) hind paw following saline-mineral oil (control) or Complete Freund's Adjuvant (CFA, 0.5 mg/ml, 25 µl in the right hind paw), a model of neuropathic pain. For this study, Luminol (200 mg/kg, subcutaneous)-based bioluminescence imaging (BLI) was performed following CFA administration. Luminol-BLI provided a non-invasive, specific, and highly sensitive optical readout of phagocyte-mediated myeloperoxidase (MPO) activity in vivo. CFA administration in the hind paw resulted in a significantly higher average Luminol signal at the injection site than in the control group. In summary, DHE- and Luminol-based longitudinal non-invasive in vivo imaging studies can be used to evaluate the efficacy of compounds targeting oxidative stress without the need of euthanizing animals at multiple time points.

#### **P423 Comparison of Novel and Traditional Bleeding Techniques in Neonatal and Juvenile Mice**

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Terminal blood collection is common in toxicology, developmental, and immunology studies involving neonatal and juvenile mice. Implementation of non-terminal blood collection techniques, such as the submental bleed technique that has been recently described for adult mice, offer opportunities to reduce animal numbers and refine current methods. To the authors' knowledge, the submental bleed technique has not been described in neonatal or juvenile mice. This

study compared submental bleeding techniques with another commonly used method, submandibular venipuncture, in neonatal and juvenile mice. CD1 mice, ages 7, 14, 21, and 28 days, were randomized into submental (N=16), submandibular (N=16), or control (N=8) groups based on sex, with equal numbers of each sex represented. Each mouse was weighed, restrained via manual scruffing, bled per their group (or only restrained in the case of control animals) and then decapitated for terminal blood collection. A subset of mice (N=8) with two representatives from each age group were bled with one of the techniques, and tissue was collected after 48 hours for representative histopathology samples to determine gross or histopathologic evidence of trauma. Serum corticosterone and blood collection volume were measured. Preliminary results show a significant difference in the volume of blood collected on days 14 and 28, with the submandibular technique yielding a significantly higher blood volume collected (approximately 18uL more on days 14 and 17uL more on days 28). No significant differences were seen in corticosterone levels between the two bleeding techniques across all ages and sexes. Trauma was seen histologically for all samples except day 21 submandibular bleed. We conclude that the submental bleed technique can be a viable non-terminal blood collection method in neonatal and juvenile mice, especially when smaller amounts of blood are needed. Future studies may further evaluate parameters pertaining to animal welfare and blood sample quality.

#### **P424 Novel Bedding: Does Hemp Material Have the Physical and Chemical Properties Appropriate for Housing Laboratory Animals?**

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There are various types of rodent bedding materials used to house laboratory animals. Types of bedding material will affect the animal's microenvironment and potentially alter physiological responses. Rodent bedding should supply a comfortable environment for animals and personnel working animal rooms. Hemp, Cannabis botanical, is a rapid growing plant whose fiber, hurds, seeds, flowers and leaves have been used for multiple purposes for years. Hemp hurds are ligneous woody tissues which are used for animal bedding and construction. Hemp bedding has been used for agriculture species for years primarily due to its high absorptive capacity. The objective of this study is to evaluate properties of hemp bedding products from the USA, Netherlands and France for microbial, pesticide residues, heavy metals, aflatoxin, endotoxin, cannabinoid, dust content and moisture to see if it is a suitable bedding option in the laboratory animal industry. Assays were conducted using mass spectrometry for cannabinoids, aflatoxins, pesticides, and heavy metals; kinetic method for endotoxin; Mettler Toledo analyzer for moisture; ROTAP shaker for dust content; and conventional microbiology testing for microbes. All samples were negative or at acceptable levels for cannabinoids, aflatoxin, pesticides and heavy metals. The hemp bedding samples met contract lab specifications for moisture, dust content and pathogen microbial screen and were comparable to our hardwood animal bedding levels. Total aerobic bacteria ( $\geq 1.3 \times 10^6$  cfu/g), coliform (1,000 – 6,700 cfu/g) and endotoxin levels (8,496 – 336,976 EU/g) yielded high readings. Appropriate sterilization of hemp bedding via steam autoclave or irradiation will eliminate live microbial content; however, endotoxin levels are resistant to sterilization processes. Hemp bedding's high levels of endotoxin makes it less than an optimal rodent bedding for conducting respiratory and immunological studies. Absorptive capabilities and dust content of hemp bedding make it a good candidate for use with rodents; however, additional testing is needed.

#### **P425 Interaction of Juvenile Stocking Density and Larval Diet on Adult Size and Sex Differentiation in the Laboratory Zebrafish (*Danio rerio*).**

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Zebrafish are an increasingly utilized research model. Despite evidence suggesting environment is a significant driver of behavioral and morphologic phenotype, consistent husbandry standards have yet to be established. For example, tank stocking density impacts growth, reproductive efficiency, and larval survival. Yet there is considerable variation between institutional standards on age-specific stocking density. Such standards have been difficult to establish in part due to interactions with other variables such as diet. In practice, larval and juvenile zebrafish are often held at higher average stocking densities (10-40 fish/L) than adult fish (5-10 fish/L), with reduction in stocking occurring at various time points between 15 and 90 days post-fertilization (dpf). To better characterize the impact of juvenile stocking density on growth potential, we assessed the interaction between age, diet, and 30 dpf stocking density on adult standard length, weight, sex, and survival. Wildtype AB zebrafish were housed and fed in one of three larval care paradigms: rotifers without flow, rotifers with flow, or manufactured diet. Tanks were stocked at 10 fish/L until 30 dpf at which time a subset of each larval care group was transitioned to 5 fish/L or maintained at 10 fish/L. For all larval care paradigms, fish housed at 5 fish/L were significantly larger (mass  $p < 0.0001$ ; length  $p < 0.0001$ ) at 90 dpf than those housed at 10 fish/L. No significant difference in 30-90 dpf survival or sex differentiation was observed. Collectively, these data suggest reduction in housing density to 5 fish/L at 30 dpf is correlated with increased size of adult fish.

#### P426 Effectiveness of Three Injectable Anesthetics in Sedating Pacific Bluefin Tuna

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The sole FDA approved anesthetic agent for tuna is MS-222, a compound added to water requiring full immersion. Immersive anesthetics, however, are not a viable option for larger tuna due to the volume of water required. Here, we trialed the use of alternative injectable anesthetics to sedate three 50-kg Pacific bluefin tuna. Three injectable anesthetics included tiletamine/zolazepam (T), tiletamine/zolazepam/ketamine/xylazine (TKX), and dexmedetomidine/ketamine/xylazine (DKX). Each fish was individually caught in a sling within their home tank and injected with one of the three anesthetics intramuscularly. The first fish was sedated with T (23.6 mg/kg). The second fish was sedated with TKX [T (4.4 mg/kg), K (17.6 mg/kg), X (6.2 mg/kg)]. The third fish was sedated with DKX [D (0.07 mg/kg), K (37.8 mg/kg), X (11.1 mg/kg)]. Sedation criteria were documented as time to: disorientation (swimming in the opposite direction of the school and slower swimming pattern), loss of righting reflex (inability to orienting in an upright position), and immobility (incapable of moving). Other clinical behavioral observation (hyperexcitation) was also monitored. The T and DKX fish had the shortest time to disorientation (2 min and 1 min, respectively) and the TKX had the longest time to disorientation (9 min). All 3 tuna showed similar time to loss of righting reflex ( $31.7 \pm 3.3$  min). The TKX fish had the shortest time to immobility (36 min), followed by the DKX fish (47 min) and the T fish (114 min) had the longest time to immobility. The TKX, but not T and DKX, tuna showed significant hyperexcitation. There were no other abnormal clinical behaviors observed. These data suggest that: 1) T, TKX, or DKX effectively sedates Pacific bluefin tuna; 2) DKX and T cause the least hyperexcitation.

#### P427 Development of an Acute *K. pneumoniae* Lung Infection Survival Animal Model for Evaluating Therapeutics in Immunocompetent Mice.

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The cyclophosphamide (CP)-treated acute *Klebsiella pneumoniae* (Kp) lung infection mouse survival model is commonly used to evaluate efficacy of antibiotics and biological agents in drug development. However, because CP can cause neutropenia and suppress the immune system, CP-treated mouse survival models are not suitable for evaluating certain biological agents such as monoclonal antibodies and bacteriophages that need neutrophil cells and a healthy immune system to facilitate clearance of bacteria. For validation, a Kp lung infection Balb/c 6 weeks age mouse survival model without CP was developed. First, four doses of a hypervirulent Kp43816 strain,  $1.0 \times 10^3$ ,  $1.0 \times 10^4$ ,  $1.0 \times 10^5$  and  $1.0 \times 10^6$  CFU, were inoculated into the mouse's lung through nose nostril, causing 100% death (humanely euthanized according to clinical scores) on post-infected day 3, 4, 3 and 2, respectively. This proves that acute infection is possible without the use of CP. Second, to prove the model can be used to test therapeutics, a positive control experiment was conducted using polymyxin B. Twenty mice were then randomly divided into four groups and infected with Kp43816. Group A was inoculated with  $1.0 \times 10^3$  CFU and Group B with  $1.0 \times 10^4$  CFU both groups were treated with saline. Group C was inoculated with  $1.0 \times 10^3$  CFU and Group D with  $1.0 \times 10^4$  CFU. Groups C and D were treated with polymyxin B (5.0 mg/kg) BID I.P. (twice a day) for three days. The survival rates for Groups A and B were 0%, while Group C was 80%, and group D was 60%, demonstrating that polymyxin B significantly increased mice survival rates ( $p < 0.05$ ). In conclusion, we successfully developed an acute *K. pneumoniae* lung infection mice survival model for evaluating therapeutics without the use of CP.

#### P428 Examining Inflammatory Signaling in Astrocytes as a Key Modulator of the Neurovirulence of Western Equine Encephalitis Virus in a Novel Mouse Model of Parkinson's Disease

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Parkinson's disease (PD) is the second-most common neurodegenerative disease after Alzheimer's disease, constituting over 10 million ongoing cases in humans worldwide. Neuroinvasive infections with mosquito-borne alphaviruses such as Western equine encephalitis virus (WEEV) induce a post-encephalitic parkinsonism with neurologic sequelae that closely resemble PD due to persistent neuroinflammation. Although the standard for these studies has been intranasal inoculation with WEEV, footpad injection more closely resembles natural mosquito infection and activates the same brain regions as PD by entering the CNS through circumventricular organs (CVOs) where the blood-brain barrier is naturally absent. In addition, transgenic mice lacking NFkB inflammatory signaling in astrocytes were shown to have neuronal loss and reduced gliosis, suggesting that astrocytes play a critical role in initiating PD-like pathology following WEEV infection. In this pilot study, we used a Cre-LoxP system of gene deletion to generate an astrocyte-specific IKK2 knockout model using tamoxifen-inducible Cre ERT2-recombinase mice. Three male and three female *Aldh1l1-Cre/ERT2* BAC transgenic mice were administered 75mg/kg tamoxifen intraperitoneal every 24 hours for 5 consecutive days to induce this genetic knockout and immunohistochemistry was performed on whole brain samples. Astrocyte-specific deletion of IKK2 *in vivo* was demonstrated by co-immunofluorescence labeling of paraffin-embedded brain sections for GFAP<sup>+</sup>/IKK2<sup>+</sup> astrocytes, and for IBA1<sup>+</sup>/IKK2<sup>+</sup> microglia. Future studies will utilize this mouse model to interrogate the neuroinflammatory and neurodegenerative consequences of systemic inoculation with WEEV in astrocyte-specific conditional IKK2 knockout mice.

#### P429 Fecal Screening for SARS-CoV-2 from Urban White-Tailed Deer (*Odocoileus virginianus*) on the NIH and NIEHS Campuses



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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has been the largest worldwide pandemic in over 100 years infecting more than 700 million people. As with most emerging infectious diseases, SARS-CoV-2 is believed to have originated in animals and spread to humans. During the course of this pandemic, multiple domestic, wild and captive animal species were found to be infected with SARS-CoV-2 and likely the result of “reverse zoonosis” passing from humans to animals. Many health officials were concerned that these susceptible animal species could act as a viral reservoir for continued human infections. Beginning in 2021, reports emerged suggesting a high percentage SARS-CoV-2 infections in white-tailed deer (*Odocoileus virginianus*) across the U.S.A. White-tailed deer are ubiquitous throughout the US and often live in close proximity to urban populations. Both the National Institutes of Health (NIH) in Bethesda, MD and the National Institute of Environmental Health Sciences, in Research Triangle Park, NC, reside within large urban populations and also have a significant wild, white-tailed deer population on their campuses. We collected 50, separate fecal droppings from white-tailed deer located on both campuses and tested them via PCR for the presence of SARS-CoV-2. We were unable to detect SARS-CoV-2 in any of the samples collected for testing. Based on our results, the populations of white-tailed deer on either NIH campus were not infected with SARS-CoV-2 and do not pose a risk to the human population.

#### **P430 Carprofen Attenuates Postoperative Hypersensitivity after Plantar Incision in Immunodeficient NSG Mice**

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The immunodeficient NSG mouse was recently reported to have continued postoperative hypersensitivity after buprenorphine administration. Here, we investigated the efficacy of Carprofen, a nonsteroidal anti-inflammatory agent, for attenuating postoperative hypersensitivity in NSG mice. The aim of this study was to assess whether a high dose of carprofen (25 and 50 mg/kg) would attenuate postoperative hypersensitivity more effectively than the commonly recommended dose (5 mg/kg) for an incisional pain model in NSG mice. Male and female NSG mice ( $n = 40$ ) were randomly assigned to 1 of 4 groups and received subcutaneous injections once a day for 2 days: Saline (1 ml/kg); 5 mg/kg carprofen (Car5); 25 mg/kg carprofen (Car25); and 50 mg/kg carprofen (Car50). Mechanical and thermal hypersensitivity assessments were performed 24 h prior to surgery and at 4, 24, and 48 h afterward. Plasma carprofen concentration ( $n = 56$ ) was measured on D0 (at 2, 4, 12, 23 h), D1, and D2 after the first, second, and third doses, respectively. Fecal occult blood tests ( $n=15$ ) were performed daily and following euthanasia, tissue was collected for histopathological examination. The Saline group exhibited both mechanical and thermal hypersensitivity throughout the study. Car5 did not attenuate mechanical or thermal hypersensitivity at any time point. Car50 attenuated thermal hypersensitivity only on D1. Car25 effectively attenuated mechanical and thermal (except at 4 h) hypersensitivity. Positive fecal occult blood was detected in Car25 (25%) at 48 and 72 h, no other samples tested positive. Histopathological abnormalities were observed only in Car50 mice [gastric ulceration (50%), ulcerative enteritis with villus blunting and fusion (25%) and renal lesions (75%)]. Plasma carprofen concentration consistently maintained in all groups for 12 h. Our results indicate that Car25 attenuated postoperative mechanical and thermal hypersensitivity more effectively than Car5 or Car50 for an incisional pain model in NSG

mice.

#### **P431 High-dose Carprofen as a Postoperative Analgesic Therapy in Female CD1 Mice is Inadequate**

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The minimization of pain in laboratory mice is a scientific and ethical necessity since pain can influence experimental results. Carprofen is a common NSAID used for postoperative pain management; however, the standard dosage of 5 mg/kg may not provide sufficient analgesia. We hypothesized that a higher dose of carprofen will decrease pain-associated behaviors. Female CD1 Mice received an ovariectomy or isoflurane anesthesia-only sham surgery, then were given high- (10 mg/kg), low-dose (5 mg/kg), or saline subcutaneously every 12 hours ( $n= 10$  per treatment) starting right before surgery. The behaviors indicative of pain: orbital tightening, arched posture, rearing, and grooming were assessed by the same two trained observers cage-side prior to surgery, with the addition of wound licking and at 4, 8, 12, 24, and 48 hours postoperatively. All surgery groups had significantly increased scores in orbital tightening, arched posture, and wound licking compared to the anesthesia-only groups at 4, 8, 12, and 24 hours postoperatively. The surgery groups treated with carprofen had significantly lowered arched posture scores than the surgery group treated with saline only at the 8 hours postoperative timepoint. No significant differences were found between treatment groups for rearing and grooming behaviors at any timepoints. These results indicate that 5 and 10 mg/kg subcutaneous carprofen doses every 12 hours do not provide sufficient analgesia to mitigate postoperative pain in female CD1 mice.

#### **P432 Two Sentinel-Free Soiled Bedding Matrix Exposure Methods Outperform Soiled Bedding Sentinels for Mouse Colony Health Monitoring**

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Soiled bedding sentinels (SBS) for rodent colony health monitoring have limitations including inconsistent disease detection, use of animals and are costly and labor intensive. Sentinel-Free Soiled Bedding (SFSB) health monitoring is an alternative that overcomes the limitations of SBS by using PCR testing of sample collection matrices exposed to soiled bedding. The purpose of this study was to compare two SFSB in-cage matrix exposure methods to soiled bedding sentinels to detect pathogens in a colony of mice housed in Animal Care Systems Optimouse cages over a 12-week monitoring period. One matrix was shaken in pooled dirty bedding, while the second matrix type was exposed to all soiled cages on the rack using a scraping method around the perimeter and into the center of the cage. This strategy was used to evaluate SFSB performance in four racks located in three different rooms. We hypothesized that matrix agitated in an SFSB cage and matrix exposed to all soiled cages would detect *Rodentibacter spp.*, *Helicobacter spp.*, and murine norovirus (MNV) as well or better than SBS. Prior to study initiation, colony mice were confirmed positive by PCR for all three pathogens at varying prevalence rates. Realtime PCR testing on technical triplicates of the two matrix exposure methods were compared to SBS in the four different colonies. All SBS failed to detect *Rodentibacter* and *Helicobacter*, and 25% failed to detect MNV by PCR and serology. Alternatively, agitated and all-cage-exposed matrices detected MNV, *Rodentibacter spp.*, and *Helicobacter spp.*

with few exceptions when monitoring low prevalence colonies. Exposing the matrix to all cages had negative ergonomic, workflow, and labor challenges compared to the agitated matrix SFSB method. Thus, agitating the matrix in composite soiled bedding at two-week intervals provided the most efficient and user-friendly method while providing superior pathogen detection compared to SBS.

#### **P433 Different Effect of Exercise on Metabolic Profile in SPF and GF Mice**

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Microbiota has been known to have a significant impact on metabolic turnover in humans and animal models. Fecal microbiota transplantation has been shown to transmit exercise effects. However, the full extent of exercise effects under germ-free (GF) conditions has not been fully elucidated. Metabolic profiles were determined through a forced treadmill test after 4 weeks of wheel running training in 8-week-old male SPF (Specific Pathogen-Free) and GF C57BL/6N mice. To assess improvements in exercise performance, respiratory capacity was measured using an exhaustion test in a chamber equipped with a treadmill. Measurements such as oxygen consumption, carbon dioxide consumption, energy expenditure (EE), oxygen/carbon expenditure ratio (RER), heat production, and activity were recorded. During the test, the VO<sub>2</sub> (Oxygen uptake) peak of trained SPF mice was significantly higher compared to untrained SPF mice and both untrained and trained GF mice. Additionally, the VO<sub>2</sub> peak of GF mice did not improve after training. Furthermore, throughout the treadmill test and recovery period, trained SPF mice exhibited a significant increase in VO<sub>2</sub> levels, whereas GF mice consistently showed low VO<sub>2</sub> levels during running (21min) and recovery (20min). The higher levels of blood lactate in GF mice compared to SPF mice during training, along with the elevated VO<sub>2</sub> levels in trained SPF mice compared to GF mice, suggest a fundamental difference in glucose metabolism between the two groups. Additionally, in an additional co-breeding experiment, it was observed that the lower oxygen uptake in GF mice recovered to the same level as SPF mice during the fatigue test. These findings indicate that microbiota plays an essential role in VO<sub>2</sub> capacity. In conclusion, SPF and GF mice demonstrate divergent gains in aerobic capacity following exercise training, which can be quantified by real-time measurement of respiratory rate using a forced treadmill test.

#### **P434 Optimizing the Glass Bead Sterilization Protocol Focusing on Removal of Organic and Bacterial Intraoperative Contamination**

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No standard glass bead sterilization (GBS) protocol exists to effectively sterilize rodent surgical tools after heavy bacterial exposure, (e.g., cecal contamination). Various detergents are used to remove gross debris from surgical tools. We hypothesized that adding a detergent to GBS protocols will enhance removal of gross debris and potential bacterial nidus for intraoperative patient contamination. This hypothesis was tested by adding either a multi-enzyme detergent, neutral pH detergent, or chlorhexidine scrub step before GBS of forceps or needle drivers exposed to cecal contents. We modeled intraoperative contamination by humanely euthanizing mice (mixed sex, predominantly C57/BL6) via CO<sub>2</sub> inhalation and exposing sets of forceps and needle drivers to the cecal contents for 3 minutes. Immediately after, tools were wiped several times with a paper towel. The contaminated tips were soaked in either a multi-enzyme or neutral pH detergent (T = 5min), chlorhexidine scrub (T = 2 min), or no pretreatment solution (control). Tools (all treatment groups) were then scrubbed using a clean brush. The control tools were briefly soaked in saline before scrubbing. After rinsing with sterile water, all tools were exposed to GBS for 60 seconds at 500 °F. Tool sets were then swabbed for

bacterial culturing. Swabs were plated onto either sheep blood agar (n=23) or chocolate agar (n=20) for aerobic culturing or *Brucella* agar (n=20) for anaerobic culturing. A subset of tools was sampled to determine if organic material was present after treatment using an ATP luminometer (n=21). To date, no growth has been found on any sheep blood or *Brucella* agar plates. Two out of twenty forceps in the control group yielded growth on chocolate agar, but all other tools from all groups did not. The neutral pH detergent group yielded no test-positive tools via luminometer, while the multienzyme detergent group yielded a single test-positive tool. The number of tools testing ATP-positive in the chlorhexidine and control groups were significantly higher (n = 4 for each). Though each group represents a significant improvement of GBS protocols, both detergent groups were superior for their lack of positive bacterial culturing and limited ATP-swabbing positive results when compared to GBS alone.

#### **P435 Assessing Chronic Stress in Cats: Analytic Validation of an Assay to Measure Hair Cortisol**

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Evaluating stress in shelter and institutionally owned cats is important to help guide improvements in their welfare and environment. Measuring cortisol levels is the gold standard for assessing stress in most mammals. Acute stress is typically assessed by measuring cortisol in feces or urine, while hair cortisol concentrations are used to evaluate chronic stress. Although there are many available methods for measuring cortisol, few are validated for use in cats. We evaluated the utility of a commercially available enzyme-linked immunosorbent assay (ELISA) kit designed for human saliva for measuring hair cortisol concentrations in cats. Hair samples were collected from 29 cats representing two populations (a local shelter and institutionally owned) and cortisol was extracted. Analytic validation of the ELISA was assessed via linearity through serial dilution of samples and the calculation of the observed to expected ratio (O/E). Accuracy was demonstrated via spiked recovery and the associated O/E. Target acceptance criteria for the O/E were 80-120%. Intra- and inter-assay precision were determined through calculation of the coefficient of variation (%CV; target <10%). O/E for linearity of the ELISA was 81.5-119.0%. O/E for spiked recovery was 80.3-98.4%. The %CVs for intra- and inter-assay variability ranged from 6.4% to 9.5% and 5.4% to 14.6%, respectively. We found no significant difference in hair cortisol concentrations with the ELISA between the two populations. We demonstrated that this ELISA is an analytically valid method for measuring hair cortisol concentrations in cats. Measurement of cortisol in hair samples could provide a non-invasive method to monitor chronic stress and acclimation in cats that live in confinement for prolonged periods. Further studies are needed to clinically validate this biomarker.

#### **P436 Grinding Much? Does Grinding Rodent Feed Pellets Improve the Sensitivity of Microbial Testing?**

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The US Food and Drug Administration (FDA) and the Department of Agriculture (USDA) both recommend the grinding or stomaching of food for human consumption prior to testing for *Salmonella* isolation. The Quality Assurance Laboratory (QAL) routinely tests all rodent feed for *Salmonella*, and for total microbial load prior to use. The QAL also tests sterilized, pelleted rodent feed to assure proper autoclave function. Currently, the QAL cultures pellets in the thioglycolate broth that sits stationary in the incubator for seven days. Under these conditions, a natural ingredient pelleted feed does not break down thoroughly thus possibly preventing the detection of

viable microbes at the center of the pellet. The purpose of this study was to determine if grinding pelleted rodent feed improves the sensitivity of microbial detection. Our study compared bacterial growth from an autoclaved, natural ingredient, rodent feed (NIH-31) processed as intact pellets or ground. We used two different procedures to evaluate potential bacterial presence. The first was the total bacterial plate count via the pour plate method. This method allows for a direct numerical comparison of bacterial present between the two feed matrices (ground vs pelleted). The second procedure was the detection of culturable bacteria through thioglycolate enrichment broth for seven days at 37°C followed by subculturing onto blood agar plates aerobically and anaerobically. This allows for heat damaged bacteria that survived the autoclaving process an opportunity to repair and restart the active growth. The total aerobic bacterial count was below the maximum concentration level of 200,000 CFU/g for the unautoclaved and 0 CFU/g for the autoclaved pelleted and ground NIH-31 diet. After culturing the pelleted and the ground diet in thioglycolate broth, there was no difference in the microbiological counts. All samples were negative for microbial growth. These results indicate that grinding the autoclaved rodent feed prior to microbial testing does not improve the testing sensitivity, thus reducing the labor, time and handling contamination risk when testing for sterility.

#### **P437 Assessing Loss of Righting Reflex Induced by Exposure to Carbon Dioxide Gas in Three Mouse Strains**

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The use of CO<sub>2</sub> gas to euthanize laboratory rodents is a common technique, though there are concerns that nociception (at a CO<sub>2</sub> concentration of ≥ 37% in rats) and pain (≥ 32.5% CO<sub>2</sub> in humans) may occur. Whether rodents are conscious at CO<sub>2</sub> concentrations associated with nociception and pain is unknown. The loss of righting reflex (LORR) test was used as a proxy to identify loss of consciousness in 3 mouse strains exposed to CO<sub>2</sub> (C57Bl/6; 31.8 ± 6.9g, CD1; 31.5 ± 4.2g and 129P3J; 20.8 ± 3.5g, 16 females and 16 males per strain). A custom built, motor-driven, rotating cylinder was used to assess LORR at increasing concentrations of CO<sub>2</sub>. Following rotation into dorsal recumbency, two different LORR testing methods were assessed: 1-Paw assessment (righting reflex present if animal contacted the cylinder with 1 paw) and 4-Paw assessment (righting reflex present if animal contacted the cylinder with 4 paws). LORR data were analyzed with Probit regression and strain dose response curves compared using 1-way ANOVA with a Tukey correction. EC<sub>95</sub> values (CO<sub>2</sub> concentration where 95% of the population could be expected to achieve LORR) for 1-Paw assessment were: C57Bl/6; 30.7% (28.3-34.9) (median, 95% confidence interval), CD1; 26.2% (25.4-27.3), 129P3J; 20.1% (19.8-20.7). The EC<sub>95</sub> for C57Bl/6 mice was greater than for 129P3J mice ( $P = 0.0007$ ). 4-Paw EC<sub>95</sub> values were: C57Bl/6; 22.8% (21.9-24.1), CD1; 25.3% (24.6-26.2), 129P3J; 20.1% (19.7-20.7). 129P3J mice had lower EC<sub>95</sub> values than CD1 mice ( $P < 0.0001$ ). No significant difference was found between other strains ( $P > 0.05$ ) in 1-Paw or 4-Paw assessment. Only C57Bl/6 mice showed a difference in 1-Paw vs 4-Paw methods ( $P = 0.024$ ). These results indicate a greater potential for nociception and pain before unconsciousness is induced during CO<sub>2</sub> euthanasia in C57Bl/6 animals.

#### **P438 Standardized Loss of Righting Reflex Method to Identify Carbon Dioxide Gas Concentrations Inducing Unconsciousness in Sprague-Dawley and Long-Evans Rats**

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Euthanasia of laboratory rats is commonly performed using an overdose of CO<sub>2</sub> gas. Exposure to CO<sub>2</sub> gas is associated with aversion in rats, with the potential for nociception. It is unclear if unconsciousness as a result of CO<sub>2</sub> exposure occurs at concentrations below those associated with nociception (approximately 37%). A standardized loss of righting reflex (LORR) method, using a rotating, motorized cylinder, was applied to identify CO<sub>2</sub> concentrations associated with unconsciousness in two rat strains (Sprague-Dawley, 316.5 ± 73.6g [18 females, 10 males]; Long-Evans, 249.7 ± 48.3g [14 females, 14 males]). Animals were paired or group housed under controlled conditions with experiments performed during the lights on period. The order of animal use was randomized, and experimenters were unaware of animal identification. At the end of the experiment, CO<sub>2</sub> concentration was increased while animals remained unconscious, until death occurred. LORR was defined as no paws in contact with the cylinder for 15 seconds after rotation into dorsal recumbency. Data were analyzed with Probit regression and dose response curves plotted. Curves were compared with a two-tailed t test and  $p < 0.05$  considered significant. Values of EC<sub>95</sub> for LORR were 35.0% (34.0 – 36.5%, median, 95% confidence interval) for Sprague-Dawley, and 29.2% (20.0 – 31.5%) for Long-Evans, with a significant difference between strains ( $p < 0.05$ ), but no difference between sexes ( $p > 0.05$ ). These results suggest a potential for unconsciousness to occur before nociception during CO<sub>2</sub> gas exposure.

#### **P439 Etomidate Effectively Euthanizes Zebrafish (*Danio rerio*)**

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Zebrafish (*Danio rerio*) represent one of the world's most commonly used laboratory species. The leading methods of euthanizing zebrafish (MS-222 and rapid cooling) have many drawbacks including human safety, difficulty in preparation, and questionable pain/distress they cause the fish. Here we assessed the efficacy of an injectable anesthetic, etomidate, in euthanizing zebrafish. The aims were to investigate effectiveness of 2 different etomidate concentrations in euthanizing zebrafish and to see effects of 2 different immersion densities. Aim 1 - Fish (n=10 fish/group) were randomly assigned to 30-min immersion in 1 of 2 treatment groups: 6 (Eto-6) or 10 (Eto-10) mg/L etomidate. All fish were video recorded throughout the procedure and scored by 3 blinded observers experienced with zebrafish euthanasia. Parameters monitored included: 1) time to onset of anesthesia (loss of righting reflex, LORR); 2) respiratory arrest (cessation of operculum, COO); 3) aversive behaviors (erratic movement, hyperlocomotion, piping at the surface, twitching). Each fish was immersed in its assigned preparation for 30 min and then removed by net and transferred to a recovery tank. In recovery tanks, fish were monitored for signs of recovery (righting, operculum, body movement, and response to tap) once every 5 min for 60 min. Successful euthanasia was defined as absent signs of recovery for at least 60 min in recovery tanks. Aim 2 – 5 or 10 fish were immersed at similar etomidate concentrations. Results – Aim 1, fish in both groups showed: 1) similar time to LORR (1±0.5 min); 2) similar time to COO (2±0.6 min); 3) no aversive behaviors were noted. In recovery tanks, there were no signs of recovery noted in all fish. Fish in both groups were effectively euthanized. Aim 2, There were no differences in immersion density of fish. This study indicates that 6 or 10 mg/L etomidate effectively euthanizes zebrafish.

#### **P440 Validation of a Fecal Corticosterone Metabolite Immunoassay to Assess Changes in Basal Stress in the Laboratory Zebra Finch (*Taeniopygia guttata*)**



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Corticosterone, the primary glucocorticoid in birds, is released in response to stress, and its measurement can provide insight into animal physiology and welfare. Direct corticosterone measurements require blood samples; however, basal levels are difficult to determine accurately due to the stress associated with blood collection. Corticosterone metabolites are excreted in the feces, making fecal measurements a non-invasive refinement that may more accurately assess basal corticosterone, as restraint is avoided. Fecal corticosterone metabolites have been measured in many vertebrates, but antibody cross-reactivity with these metabolites must be validated for each species. Such validation has not been performed in zebra finches (*Taeniopygia guttata*), an important laboratory animal model. To validate a commercially available corticosterone ELISA kit in healthy, adult male zebra finches (4 cages, n = 11–14 birds/cage), we used two variations of a “capture/restraint” stress method, either via an established protocol with an opaque cotton bag or a modified protocol with an extended hand-capture and new home cage. Fresh basal fecal samples were collected, then each bird was hand-caught, examined, and restrained. Pooled post-restraint samples were collected at 1, 2, 3, and 5.5 hours. Approximately 30 droppings/cage were collected and pooled at each timepoint. There was no significant difference in the fecal corticosterone metabolite elevation between the two stress methods (two-way ANOVA, time\*method, p = 0.61). Results were combined and compared to unstressed, time-matched controls. There was a significant increase from baseline at 2 hours, and a return to baseline by 3 hours (two-way ANOVA, Tukey HSD, p < 0.001), demonstrating that the ELISA can detect increased fecal corticosterone metabolites resulting from physiological stress in male laboratory zebra finches. We also assessed regression curves of a dilution series, which demonstrated parallelism with the kit-provided standard curve (ANOVA, p = 0.064), thus showing robust metabolite detection and quantification within the log-logit linear range. These results demonstrate that fecal corticosterone metabolite ELISA can be used as a reliable, non-invasive test to monitor stress in adult male zebra finches.

#### P441 LINE Transposition into Murine Thyroglobulin Results in Congenital Thyroid Dysplasia

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C57BL/6 is one of the most used mouse strains in biomedical research. C57BL/6 substrains maintained at different facilities acquire spontaneous mutations, which could have phenotypic consequences. In this study we used a variety of genomic technologies to test the hypothesis that an unexpected histological finding of the thyroid gland in our wildtype C57BL/6NTac strain is caused by a new spontaneous mutation. Whole genome sequencing analysis identified a spontaneous mutation caused by transposition of a L1 long interspersed nuclear element (LINE), which inserted into an intron of the *Thyroglobulin* (*Tg*) gene. RNAseq analysis of the thyroid transcriptome of homozygotes suggested that the presence of the LINE interferes with splicing, resulting in exon 26 being excluded from most *Tg* transcripts, which causes omission of 61 amino acids from the affected protein product. This allele has been designated *Tg<sup>Δdys-Tac</sup>*. The phenotype is inherited in an autosomal dominant manner with affected mice exhibiting thyroid follicular cell dysplasia that progressed to thyroid adenoma by 12 months of age with incomplete penetrance. Consistent with previous descriptions of thyroid dysplasia, the cytoplasm of thyroid follicular cells stained positively by immunohistochemistry with an anti-Tg antibody and

the endoplasmic reticulum was dilated on ultrastructural examination. Body size and temperature were not altered, and serum thyroid hormone measurements revealed that the mutation does not affect serum T3 levels, but has a small effect on serum T4 levels at 10-16 weeks and 12 months of age. The results of this study identify the arm domain of Tg as important for its proper cellular distribution. The mouse genome contains a significant amount of retrotransposon DNA. When a LINE changes locations through retrotransposition in a germ cell, this introduces a new germ line spontaneous mutation, which can propagate in a breeding colony when going unnoticed. This study represents a rare example of an unexpected histological observation in an inbred mouse strain resulting in the characterization of a spontaneous LINE insertional event that was causatively linked to dysregulated physiological phenotypes. The LINE has been removed from our C57BL/6NTac commercial colonies.

#### P442 Xenograft Tumor Growth Variability in Nude Mice Infected with *Corynebacterium bovis* as a Single Pathogenic Agent

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*Corynebacterium bovis* is a highly contagious bacterium that causes sporadic clinical disease and chronic shedding in immunocompromised mice. Natural infection has been associated with reduced xenograft success and growth parameters, with complete engraftment failure in some cases. Despite this, 74% of surveyed NCI-designated Comprehensive Cancer Centers did not test for or attempt to exclude *C. bovis*. To protect the reproducibility of ongoing cancer studies, we aimed to study the effect of *C. bovis* on xenograft success in specific pathogen free mice. Subcutaneous tumor growth was monitored in 8-week-old male and female nude (NU/NU) mice topically infected with *C. bovis* either 2–3 weeks prior to engraftment (chronically infected group) or at the time of engraftment (acutely infected group), as compared to uninfected controls (N = 5 mice per group). *C. bovis* status was verified with fecal qPCR and aerobic skin cultures. Three established human cancer cell lines were used: SJSA-1, an osteosarcoma; HT-29, a colorectal adenocarcinoma; and A549, a lung carcinoma. Mouse weight and tumor volume were monitored, and tumors were collected and weighed once the largest tumors for each cell line approached 15 mm in length. Comparisons were made between infection groups for each sex and cell line. There was no significant difference in the average tumor weights or final volumes for any of the cell lines between infection groups, however, these values trended smaller in females than males. One significant difference was seen in growth rates: HT-29-engrafted tumors grew more slowly in acutely infected males than in the chronically infected or uninfected males, though no difference was present between the HT-29-engrafted female groups. Additionally, for both males and females, the HT-29-engrafted acutely infected groups had higher variability in volume and weight than the chronically infected and uninfected groups. Our results suggest that *C. bovis* as a single pathogenic agent may have limited effect on xenograft growth. However, mouse sex and tumor phenotype are important factors, and acutely infected individuals may have more variable tumor growth. Thus, studies at risk of acute *C. bovis* infection may need larger group sizes to avoid negative impacts on study reproducibility.

#### P443 Longitudinal Behavioral Characterization of 5xFAD (C57BL/6) Mice Confirms Sexual Dimorphism and Finds Novel Differences in Motivational/apathy Behaviors

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The 5xFAD model is a commonly used transgenic mouse line co-expressing five familial Alzheimer's disease (5xFAD) mutations that are inherited together and lead to accelerated amyloid plaque formation. Many publications report behavioral studies of 5xFAD mice; however, most of these studies test only one sex or do cross-sectional testing at a specific age. Discerning the temporal progression of AD-like learning and memory deficits in both sexes of 5xFAD mice and identifying behavioral assays that reveal subtle impairments would be useful as a guide to select appropriate ages and behavioral tests for specific research questions. Some longitudinal batteries have been reported, but they have not included frequently used water mazes. Here we describe a longitudinal battery of behavioral tests developed to chart the normal aging impairment expected in both male and female 5xFAD (C57BL/6J background) mice as compared to wildtype (WT) littermates. At approximately 9 months of age, 32 mice (evenly balanced for sex and genotype), began the first of three cycles of a 3-week battery, each cycle separated by 3 months. The battery consisted of four consecutive days of dry maze testing in week 1 (Open Field, Spontaneous Alternation, Novel Spatial Recognition, Rotarod). The following week, a 2-day 8-arm Radial Arm Water Maze (RAWM) test was done, followed by one day of Open Pool testing. The next day, mice were assessed using the Frailty Index, then individually housed with free access to running wheels for 7 days before being returned to normal housing. The resulting data provide a temporal progression of behavioral deficits in 5xFAD males and females and reveal a sexual dimorphism in behavior consistent with that seen in AD-like brain pathology, wherein females show more impairment than males at similar ages. This sexual dimorphism suggests different tests may be necessary at different ages for males and females, providing important information to investigators working with this model. This work also provides the novel incorporation of a water maze in a longitudinal study of the 5xFAD model. Interestingly, the RAWM data indicate females may express motivational/apathy behaviors sooner than in males (as measured by errors of omission).

#### **P444 Can Long-term Isoflavones Intake Alter Steroid Adrenal Secretion?**

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The hypothalamo-pituitary-adrenal (HPA) axis represents an important neuroendocrine system that regulates response to stress and several metabolic processes. The adrenal cortex plays an important role in these processes being stimulated by neuroendocrine signals and secreting different steroid hormones (glucocorticoids, mineralocorticoids, and androgens) which regulates essential functions of the organism. Isoflavones are estrogenic-like compounds that alter reproductive function in males decreasing circulating and testicular androgen levels. These anti-androgen effects of isoflavones can also affect the regulation of other endocrine organs. Therefore, the aim of this study is to evaluate the effects of a long-term administration of isoflavones on the adrenal cortex hormonal production and secretion in male Wistar rats administered with different doses of isoflavones. Seventy-five 60-day old adult male Wistar rats were administered orally using a buttoned cannula with saline solution (control group), low mixture of isoflavones (17 mg kg<sup>-1</sup> day<sup>-1</sup> genistein + 12 mg kg<sup>-1</sup> day<sup>-1</sup> daidzein) (low doses group), and high mixture of isoflavones (170 mg kg<sup>-1</sup> day<sup>-1</sup> genistein + 120 mg kg<sup>-1</sup> day<sup>-1</sup> daidzein) (high doses group), every day over a period of 5 months (20 weeks). Serum and adrenal levels of androstenedione (A4), dehydroepiandrosterone (DHEA), corticosterone (CT), and aldosterone (ALD) were determined by enzyme immunoassay. The results revealed that serum and adrenal CT and ALD levels significantly decreased in low and high doses groups with respect to control, denoting an alteration of steroid adrenal secretion. Besides, serum and adrenal androgen levels were significantly increased in

experimental groups with respect to control. These results suggest that the anti-androgenic effect of isoflavones promotes the adrenal androgen secretion causing the production of CT and ALD to be impaired. In conclusion, long-term exposure to isoflavones alter steroid adrenal production that may cause alterations in certain metabolic and endocrine-related processes.

#### **P445 Determination of Postmortem Interval in the Laboratory Mouse Under Various Environmental Conditions**

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Despite the vast use of mice in biomedical research, there is a lack of information on postmortem changes and determination of post-mortem interval (PMI) in this species. The quality of tissue collected for diagnostic purposes varies by environmental temperature and the length of PMI. As post-mortem changes such as autolysis may preclude lesion interpretation or detection of disease, it is important to have an accurate scale to determine viability of the carcass for gross or histologic evaluation. The goal of this study was to create an improved model for PMI determination by assessing autolysis rates and decomposition changes in mice under typical environmental conditions. 108 experimentally naïve C57BL/6 mice were humanely euthanized by carbon dioxide inhalation followed by cervical dislocation and divided into two experimental groups: room temperature (20-26°C) or refrigeration (4°C). PMI assessments including gross changes and histologic scoring were performed at hours 0,4,8,12, and days 2-14. Factors such as body temperature and weight change were also documented. Our data indicates room temperature carcasses had a significantly faster onset of autolysis compared to refrigerated carcasses. For most organs, autolysis rates at room temperature became apparent at two days versus five days in refrigeration. Gross changes in room temperature developed at day three versus at day six in refrigeration. Weight changes over time were significantly greater for room temperature carcasses compared to refrigerated carcasses. This study supports the clinical relevance of determining PMI and individual organ autolysis rates at various time intervals at different temperatures due to significant changes in carcasses dependent on the environment they are found in.

#### **P446 Regenerative Engineering of Complex Extremity Trauma**

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Lower extremity trauma characterized by an open bone fracture and soft tissue damage are 4-5 times more likely to result in delayed or failed bone union. There is an unmet clinical need for a regenerative approach that can facilitate both structural and functional healing of the damaged tissue. To evaluate the therapeutic efficacy of an engineered muscle treatment in facilitating functional recovery, we developed a novel mouse model of complex bone-muscle injury which consists of a 2 mm segmental bone defect of the mid-diaphysis of the tibial shaft with a dental drill, and a 20% surgically excised muscle ablation of the tibialis anterior (TA) muscle of C57BL/6J male mice (n = 39). The injured tibias were left untreated other than being stabilized with a custom 3D printed biocompatible curved resin plate. The muscle defects received an engineered muscle construct composed of differentiated primary mouse muscle cells or were left untreated. Bone healing and muscle functional recovery were evaluated by longitudinal microCT imaging and terminal *in situ* muscle physiology, respectively. On day 56, tibias and TA muscles were harvested for histological analysis. The TA muscles that

received engineered muscle scaffolds demonstrated an increase in TA muscle cross-sectional area ( $p < 0.01$ ) and maximum contractile force ( $p < 0.05$ ) compared to muscles that were left untreated, suggestive of functional myogenesis. However, decreased bone volume ( $p < 0.05$ ) and mineral density ( $p < 0.05$ ) of the bone defect was observed, indicating a possible compensatory mechanism between muscle-bone recovery. Interestingly, there was an increase in survival rate (improvement from 68% to 83%) and reduced limb morbidity (tissue necrosis, bone re-breakage, scabbing, reopening of wounds, etc.) of animals who received muscle treatments, which would merit removal from the study. Taken together, our findings demonstrate a regenerative relationship between muscle healing with functional limb recovery and survival, and further highlight the restorative potential of our engineered muscle approach for the treatment of complex lower extremity injuries.

#### **P447 Examining the Impact of Laboratory Housing Temperature and $\beta$ -adrenergic Signaling on Murine CD8 T Cell CD28 Co-stimulation**

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Per the Guide for the Care and Use of Laboratory Animals, research institutions have standardized guidelines for the housing, husbandry, and overall care for laboratory animals that they must adhere to, including ambient temperature. For rodents, standard laboratory housing temperatures fall below their thermoneutral zones, inducing non-shivering thermogenesis. This physiological response is driven by the sympathetic nervous system and increased, systemic  $\beta$ -adrenergic receptor ( $\beta$ -AR) signaling. Our lab has previously established that housing mice at a standard temperature (ST,  $\sim 22^\circ\text{C}$ ) results in significant impairment to murine CD8 T cell-dependent anti-tumor immunity, compared to mice housed at a thermoneutral temperature (TT,  $\sim 30^\circ\text{C}$ ). While a gap exists in our understanding of the mechanisms underlying the reduced CD8 T cell anti-tumor function in ST-housed mice, published and preliminary data indicate a role for the T cell co-receptor, CD28, which provides essential signals for T cell activation. To test the hypothesis that  $\beta$ -AR signaling impairs CD8 T cell anti-tumor immunity via impaired CD28 co-stimulation, C57BL/6J mice were either housed at TT for at least three weeks to reduce  $\beta$ -AR signaling or the  $\beta$ -AR agonist, isoproterenol (10 $\mu\text{M}$ ), was added to cell culture media at the time of in vitro activation of T cells (and for the duration of the experiment) to induce  $\beta$ -AR signaling. CD8 T cells were isolated from the spleens of mice ( $n=2-4$ ) housed at either ST or TT and activated in vitro with  $\alpha\text{CD3}/\alpha\text{CD28}$  stimulating antibodies for up to 7 days, then analyzed via flow cytometry, RT-qPCR, western blot, or Seahorse XF. We found that increased  $\beta$ -AR signaling did not elicit statistically significant differences in CD28 mRNA or surface expression but did lead to increased inhibitory phosphorylation of CD28 downstream signaling targets, including Lck. Interestingly, we also show that mitochondrial respiration, known to be positively regulated by CD28 co-stimulation, is significantly increased at 48 hours post-activation in T cells isolated from TT-housed mice, compared to ST-housed mice. Our findings suggest that CD28 co-stimulation is negatively regulated by  $\beta$ -AR signaling, likely contributing to the impaired CD8 T cell anti-tumor immune response.

#### **P448 Eliminating *Helicobacter* spp. from Syrian Hamster Surrogate Breeders by Using Antibiotic Cocktail**

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Syrian hamsters are important laboratory animals for antibody production, metabolic, cardiovascular and infectious disease studies. Since the pandemic outbreak of COVID-19, the Syrian hamster has been used as a good non-transgenic animal model for SARS-CoV2 infection research. Although *Helicobacter* infection rarely causes significant clinical signs in Syrian hamsters, much research has shown that some *Helicobacter* infections could influence the immune responses in Syrian hamsters. Therefore, it is essential to eradicate *Helicobacter* spp. from the breeding colony of Syrian hamsters. While, *Helicobacter*-free Syrian hamsters are not always commercially available, a medicated diet (Rat *Helicobacter* MD's™ 4 Drug Combo, BioServ) treatment has been proven to successfully eliminate *Helicobacter* spp. from mice and rats. Here, we modified the dosing strategy and used similar prescription to eliminate *Helicobacter* spp. in Syrian hamsters. In this study, four breeding pairs of 3- to 5-week-old Syrian hamsters, which were naturally infected with *Helicobacter* spp., were orally administered with clarithromycin (30 mg/kg), metronidazole (20 mg/kg), and omeprazole (0.7 mg/kg) every day for 6 weeks. An additional treatment with amoxicillin (1 mg/mL) and enrofloxacin (0.1 mg/mL) in the drinking water was simultaneously conducted. The infection status of each hamster was monitored by weekly fecal sample PCR during and for 6 more weeks after the treatment. All the PCR test results were negative for *Helicobacter* spp. through the course of antibiotic therapy. Seven of the eight subjects remained negative at experimental endpoint. Only one male hamster was detected positive in the 12<sup>th</sup> week of experiment. *Helicobacter* spp. was not detected in all offspring of treated hamsters during the course of experiment. This report demonstrates that a novel antibiotic cocktail treatment could be effective to eliminate *Helicobacter* spp. in Syrian hamsters, and the treatment could be sufficient to establish *Helicobacter* spp.-free surrogate breeder colony.

#### **P449 Effect of Granulocyte-macrophage Colony-stimulating Factor on SARS-CoV-2 Infected hACE2 Mice**

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Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine that stimulates the production of granulocytes and macrophages. While this cytokine has been described to be upregulated in response to SARS-CoV-2 as a component of the complex cytokine response, previous studies in humans and mice infected with West Nile Virus had their clinical signs alleviated following treatment with GM-CSF. We hypothesized that GM-CSF therapy may similarly alleviate clinical signs in the human ACE2 transgenic mice inoculated with SARS-CoV-2. To this end, 30 male and female hACE2 transgenic mice were intranasally inoculated with 10<sup>4</sup> plaque forming units. The day after infection, mice were treated daily with either 200  $\mu\text{l}$  saline ( $n=14$ ) or 200 mg recombinant mouse GM-CSF ( $n=16$ ) intraperitoneally. Mice were weighed daily, and clinical signs evaluated on a scale of 1 (none) to 4 (severe). Mice were euthanized if the clinical score was 4, or until 14 days post-infection. At the time of euthanasia, lung samples were collected for viral titers and histology. The plaque forming units of saline treated mice were  $1.6 \times 10^5$  compared to  $8.1 \times 10^4$  in GM-CSF treated mice. The histologic lung lesions were scored on a scale of 1 (mild interstitial pneumonia) to 3 (severe interstitial pneumonia). Lung lesion scores were 0.67 in saline treated mice, compared to 1.2 in GM-CSF treated mice. This is likely due to the increased cellular infiltration from the GM-CSF treatment. The mean survival of saline treated mice was 5.6 days in saline treated mice, and 7.4 days in GM-CSF mice. GM-CSF treatment increases the odds of long-term survival compared to saline by 21.2% and decreases the onset of clinical signs by 69.4%. These results demonstrate that GM-CSF treatment of hACE2 mice following SARS-CoV-2 infection results in a reduced viral titer, reduced clinical signs, and improved survival compared to saline

treated mice.

#### **P450 Early life dosing with Cholera Toxin B inhibits age-associated obesity in mice**

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The global rise in obesity has become a concerning public health issue, associated with various inflammatory diseases including cancer and cardiovascular conditions. This study built upon the 'Hygiene Hypothesis' concept investigating the potential of microbial products such as cholera toxin B (CTB) as an early-life vaccination strategy to prevent age-associated obesity by counteracting chronic inflammation. In order to test the hypothesis that the immune effects of CTB inhibit age-associated obesity in mouse models we used 8 males and 8 females of two different genetic backgrounds of mice, CD1 outbred stock and C57BL6 inbred mice, which were susceptible to age-related weight gain. 10 µg of CTB in 0.2 mL of Phosphate Buffered Saline (PBS) or PBS sham was administered via gastric gavage 3 times every 10 days starting at 3 weeks of age until 7 weeks of age. Mice had terminal tissue collections at 6 months. In both CD1 and C57BL6 mice, early life administration of CTB resulted in a significant inhibition of age-associated obesity. Compared to control mice, CTB-treated mice displayed reduced body weight (43g to 35g in males and 36g to 30g in females,  $p < 0.001$ ) and abdominal fat accumulation (4.94g to 1.18g in males,  $p < 0.001$  and 18.6g to 10.27g in females,  $p < 0.01$ ). Histological characterization of the adipose tissue found decreased inflammatory cell accumulations, known as Crown-Like-Structures (CLS) in CTB-treated mice when compared to the control mice (18 to 1 CLS in males,  $P < 0.0001$  and 8 to 6 CLS in females,  $P < 0.02$ ). These results were repeated by performing adoptive cell transfer experiments in C57BL6 mice. A single cell suspension of  $5 \times 10^5$  immune cells, isolated from the mesenteric lymph node of CTB-treated or control mice were injected intraperitoneally showing that the transplantation of purified immune cells were sufficient to inhibit age-associated obesity, highlighting the role of host immunity in mediating CTB's effects. Our findings demonstrate that CTB when administered in early life, inhibits age-associated obesity in mice through immunomodulatory mechanisms. This supports the hypothesis that early-life vaccination with CTB protects against developing obesity in adulthood.

#### **P451 Hypothermia Reduces Cardiac Function in Isoflurane Anesthetized Mice**

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Hypothermia is one of the most common isoflurane anesthesia complications observed in mice. While hypothermia negatively impacts the cardiovascular system, the severity of hypothermia's effect is unclear. We investigated the impact of severe hypothermia (91 °F) and moderate hypothermia (95 °F) on cardiac function in isoflurane-anesthetized mice. Using adult C57BL/6 mice ( $n = 6$ ), mice were induced with isoflurane and then placed on a water-recirculating, temperature-controlled MRI tray. The cardiac MRI scan on each animal started at 98 °F (normal, baseline), then progressed to 95 °F (moderate hypothermia), and finally, 91 °F (severe hypothermia). Cardiac functions (ejection fraction,

cardiac index, cardiac output, and stroke volume) were calculated. Physiological parameters (heart rate, respiratory rate, %SpO<sub>2</sub>, and body temperature) were monitored throughout. The baseline ejection fraction was  $70.9 \pm 2.1\%$ . Ejection fraction in the severely hypothermic group (91 °F,  $53.4 \pm 2.9\%$ ) were significantly lower than the moderately hypothermic mice (95 °F,  $60.8 \pm 3.7\%$ ). The heart and respiratory rates in severely hypothermic mice were significantly lower than those of moderately hypothermic mice. Other cardiac or physiological parameters did not differ. These data suggest severe hypothermia compromises cardiac function to a greater degree than moderate hypothermia in isoflurane-anesthetized mice.

## **Platform Sessions**

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

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

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

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Routine handling and manipulation of laboratory mice are necessary components of many research studies, but if performed incorrectly, have the potential to cause stress and physical harm to the mice. In addition to being a welfare issue, this can also lead to unintended consequences in terms of experimental outcomes. The pathological effects of these interventions are poorly documented and have been previously assumed to have a negligible effect on experimental

variables. In that context, we provide a comprehensive post-mortem overview of the main pathological changes associated with routine interventions (i.e., restraint, blood drawing, and intraperitoneal injections) of laboratory mice, emphasizing presumed traumatic osteoarticular lesions. A total of 1000 mice from various studies were included, with 864 animals being heavily manipulated and 136 being handled for routine husbandry procedures only. The most common lesions observed were associated with blood collection or intraperitoneal injections and a series of traumatic osteoarticular lesions likely resulting from restraint. Osteoarticular lesions were found in 62 animals (61 heavily manipulated; 1 unmanipulated) with rib fractures and avulsion of the dens of the axis being over-represented. Histopathology and micro-CT confirmed the traumatic nature of the rib fractures. To reduce and ideally prevent these lesions from occurring, enhanced training of research personnel on gentle mouse handling, restraint, and phlebotomy techniques could help reduce the impact on animal well-being and enhance study reproducibility.

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

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

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

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Frequent handling and manipulation of pregnant mice and their cage can lead to stress and reduced breeding performance. Nests, which can be assessed cage-side without manipulating the cage or dam, are an excellent indicator of pregnancy status and are often linked to maternal care of pups and positive breeding outcomes. Using a novel nest scoring system based on integration of nesting material, we were able to accurately assess pregnancy status for 28 matings (16 first- or second-time dams), without cage manipulation, as early as E6. Mating trios were housed together for 4 days, after which period female mice were housed singly in a clean cage and provided a pouch of nesting material (crinkled brown paper strips) and a shelter (a hut or tunnel). Nests were scored daily using a numerical scoring system from 1-4, based on nesting material manipulation and incorporation of enrichment, and nest scores were used as a basis for predicting pregnancy. Generally, pregnant females created high-

scoring (3 or higher) nests earlier in the pregnancy than non-pregnant females, who often failed to make complex nests. One of the key indicators of pregnancy was the instinctual shredding of the shelter enrichment and thorough incorporation with other nesting materials. This behavior was more pronounced in cages provided with tunnels than in cages provided with huts, corresponding to a higher predictability at earlier timepoints. Using cage-side nest scoring, pregnancy was determined with 89% accuracy at E9 or earlier. This assessment reduced the need to manipulate the cage and handle the dam during gestation, potentially alleviated handling-associated stress on animals, and was an effective method for early pregnancy prediction.

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

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

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

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In the United States, raised slats or tenderfoot flooring are the most prevalent housing modalities used in swine agriculture and research. These traditional housing methods may limit the expression of species-typical behaviors and reduce access to agency, choices the animal can make within their environment. Wild counterparts, *Sus scrofa*, naturally engage with their environment through foraging behaviors such as rooting and grazing for more than 50% of their awake day, behaviors traditional housing setups do not easily



accommodate. In a preference test evaluating straw versus no straw, research swine consistently chose the straw bedding condition. Therefore, straw bedding on a solid floor was chosen as the standard housing condition for swine at our facility. It was further hypothesized that allowing the animals to choose various types of foraging material throughout the day could increase the time spent engaging in natural behaviors such as foraging activities. To further evaluate this, five juvenile swine were taught to associate words with various foraging substrates (straw, shredded paper, beet pulp, and mushroom compost) utilizing an alternative communication button device. Videos were then collected and scanned in four different settings to measure frequencies and total durations of foraging behaviors (foraging, tail wagging, frenetic activity periods, and flehmen responses). Additionally, behaviors known to be incompatible with efficient and safe workflow by husbandry staff members were collected (pacing, biting, jumping, and freezing). When communication buttons were utilized, opportunities for the juvenile swine to engage with preferred materials increased, resulting in increased cumulative durations and frequencies of foraging behaviors. Furthermore, the frequency and duration of pacing and freezing were lower in button sessions than in other cognitive stimulating events and baseline behavior. Frequency of behaviors incompatible with efficient and safe workflow by husbandry staff members were also reduced in communication button sessions. Further research should be conducted to confirm the use of communication buttons as an enrichment device for swine and to validate the development of word associations with the button.

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

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

#### **PS8 *Chlamydia muridarum* Associated Pulmonary and Urogenital Disease and Pathology in a Colony of Enzootically-Infected**

#### **IL12rb2 deficient and *Stat1* knockout Mice**

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*Chlamydia muridarum* (Cm) has recently been reported to be unknowingly prevalent in laboratory mouse colonies, but clinical disease has only been associated with experimental infection of mice used to investigate human Chlamydial infections or following cohousing of NSG mice with Cm-shedding mice. Clinical disease and pathology were observed in 2 genetically engineered mouse (GEM) strains with impaired interferon- $\gamma$  signaling and Th1 CD4+T cell responses in a colony of various GEM strains known to be colonized and shedding Cm. Experimentally naïve, underconditioned and hunched, B6;129S1-*Il12rb2*<sup>tm1jm</sup>/J mice (n=3) were submitted for necropsy. Mild-to-moderate multifocal peribronchiolar lymphoplasmacytic and histiocytic bronchopneumonia with bronchiolar epithelial cell degeneration and intralesional Cm inclusions were observed in 2 mice; the remaining mouse had focal peribronchiolar lymphocytic and histiocytic infiltrates. Immunohistochemistry (IHC) for Cm MOMP-1 antigen demonstrated positive staining in bronchiolar epithelial cells correlating with inflammation as well as in small and large intestinal surface epithelial cells in all 3 mice. The other affected strain was a breeding pair of B6.129S(Cg)-*Stat1*<sup>tm1Dlv</sup>/J mice with poor fecundity. At necropsy, the male had severe unilateral hydronephrosis, moderate rhinitis/tracheitis/pneumonia, and minimal-to-mild gastroenteritis and typhlocolitis. The dam had severe unilateral metritis/salpingitis/periovarian steatitis, mild-to-severe bilateral hydronephrosis, severe cystitis, moderate-to-severe arteritis, mild-to-moderate rhinitis/tracheitis/pneumonia, minimal-to-mild gastroenteritis and typhlocolitis, and a renal urothelial papilloma. IHC showed positive staining in epithelial cells at the gastric limiting ridge, small and large intestines, as well as in the urinary bladder, ureter, and papilloma of the dam. Given that Chlamydia is a known cause of infertility in humans, breeding issues were likely attributable to Cm. The robust inflammation associated with Cm inclusions in the papilloma indicates the possibility that Cm contributed to the lesion. Given we previously observed significant morbidity and mortality in Cm infected NSG mice, these cases further highlight the importance of excluding Cm from laboratory mice.

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

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*Chlamydia muridarum* (Cm), a reemergent, moderately prevalent (academic colonies), Gram-negative, intracellular bacterium, that causes subclinical to severe clinical disease in laboratory mice, dependent on the host's immune status. Subclinical infection, which is persistent, modulates both innate and adaptive immune responses, potentially impacting the animal's research use. Eradication of Cm can be challenging as aberrant bodies can form in response to antibiotic therapy, resulting in treatment failure. A study was conducted to evaluate various antibiotics regimes with the aim of eradicating Cm from both immunodeficient and immunocompetent

laboratory mice. NSG mice were cohoused with experimentally infected, Cm shedding BALB/c mice for 14 days modeling a natural route of exposure. Four groups of 8 infected NSG mice were treated for 7 days with either 0.08% sulfamethoxazole & 0.016% trimethoprim (TMS) in water, or feed impregnated with 0.0625% doxycycline, 0.124/0.025% TMS, or 0.12% amoxicillin. The estimated ingested dose for a 23.5 g mouse was approximately 160/32 mg/kg, 119 mg/kg, 236/48 mg/kg, and 228 mg/kg, respectively. A control group was provided standard water and feed. All antibiotic treated NSG mice were euthanized with clinical disease consisting of dehydration, hunched posture, >20% weight loss, and dyspnea 21-40 days ( $32.6 \pm 4.2$  [mean  $\pm$  SD]) post treatment (DPTx) as compared to 14-33 days ( $23.75 \pm 5.9$ ) for the controls. At necropsy, all mice had multifocal histiocytic and neutrophilic bronchointerstitial pneumonia and/or bronchiolitis with bronchiolar and alveolar epithelial cell degeneration with prominent intralobular chlamydial inclusion bodies. Subsequently, groups of 8 C57BL/6J, BALB/c and NSG mice infected with Cm, as described, were treated with 0.124/0.025% TMS feed for 7 (BALB/c and B6) or 21 days (NSG). Immunocompetent mice were negative, whereas all NSG mice were Cm positive by PCR 14 DPTx and euthanized. These results provide insight into the difficulties of eradicating Cm from immunodeficient mice, providing additional evidence that Cm should be an excluded agent.

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

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

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

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

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

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

### PS13 Type 2 Diabetes Mellitus in *Tupaia belangeri* (Northern Tree Shrew)

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

#### PS14 Animal Research: A Risky Business?

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

#### PS15 Photobiomodulation Therapy Implementation in an Outdoor Nonhuman Primate Breeding Colony

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Photobiomodulation therapy, otherwise known as PBM therapy or laser therapy, has been shown in many species to be beneficial for adjunctive management of inflammatory conditions including acute and chronic pain. This adjunctive therapy can decrease time for wound healing and may reduce the need for additional therapeutics.

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

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

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

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

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Medical Campus, Denver, CO; <sup>4</sup>Division of Animal Resources, University of Illinois at Champaign-Urbana, Urbana, IL; <sup>5</sup>Animal Resources, University of Nevada, Reno, Reno, NV; <sup>6</sup>Office of Laboratory Animal Resources, California Institute of Technology, Pasadena, CA; <sup>7</sup>EMD Serono, Billerica, MA; <sup>8</sup>Takeda, Cambridge, MA; <sup>9</sup>Medical University of South Carolina, Charleston, SC

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

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

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

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

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Developing a diverse and inclusive laboratory animal science community can help with ongoing staffing and support concerns by ensuring a wide potential pool of potential individuals who would be interested in pursuing careers in laboratory animal science.

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

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

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

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

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Given their anatomical and physiological similarities to humans, swine are the most widely used large animal translational model in biomedical research. Despite the use of swine as a surgical model, precise dosing regimens for commonly used analgesics such as

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

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

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

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

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Isoflurane is commonly used for anesthetic maintenance in rabbits, but inhalant anesthetics can cause dose-dependent cardiovascular depression and significant hypotension, decreasing its anesthetic

quality. Another alternative, alfaxalone, has been studied for induction in rabbits with rapid onset and a short duration of action but has not been evaluated as an option to replace isoflurane for anesthetic maintenance. This study compared Total Intravenous Anesthetic Maintenance protocols (TIVA) using alfaxalone against isoflurane. Twenty-four male New Zealand White rabbits were assigned to one of three treatment groups: isoflurane alone, alfaxalone with buprenorphine, or alfaxalone with midazolam. All rabbits were premedicated with buprenorphine SQ (0.02mg/kg) and induced with alfaxalone IM (6mg/kg). Following intubation, rabbits were maintained for 1 hour on either isoflurane (2.5%) or alfaxalone Constant Rate Infusion (CRI) (0.2mg/kg/min). For rabbits on the alfaxalone CRI, boluses of buprenorphine (0.01mg/kg IV or SQ) or midazolam (0.1-3mg/kg SQ) were given upon induction or adjunctively as needed dependent on positive tail-pinch responses that were conducted at t0, 15, 30, and 45. Heart rate, non-invasive and invasive blood pressure, respiration, end-tidal carbon dioxide (ETCO<sub>2</sub>), percent oxygen saturation (spO<sub>2</sub>), and temperature were recorded every 5 minutes. Blood gas was analyzed at t0, t30, and t60. Surgical plane or anesthesia was characterized by lack of positive response to a tail-pinch and was reached in all anesthetic groups. Results showed significant reduction in mean heart rate of the alfaxalone groups while mean invasive blood pressure was increased compared to the isoflurane groups. However, respiration in the alfaxalone groups was decreased with associated increases in ETCO<sub>2</sub> levels. There were no significant differences noted between alfaxalone treatment groups. Our preliminary findings indicate alfaxalone given as a TIVA maintenance protocol could be considered as an anesthetic alternative to isoflurane with improved cardiovascular stability though respiratory monitoring or management would be warranted.

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

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

significantly longer median duration of anesthesia using either of the alfaxalone formulations. Overall, AM40 offers a more humane anesthetic than AM10 for plethysmography in cynomolgus macaques.

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

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

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

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

up to 24 hours. No skin reactions were seen in association with injection of either drug. In summary, this data supports the dosing of buprenorphine (0.1 mg/kg SC) and meloxicam (2mg/kg SC) in the NMR at a minimum frequency of once every 8 and 24 hours respectively. Further studies should be performed to evaluate the clinical efficacy of these drugs by correlating plasma concentrations with post-operative pain assessments.

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



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

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

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Angioedema, a sudden swelling often caused by allergic reactions, commonly affects the face, particularly the lips and tongue. Swelling in this area may be life threatening, especially if breathing is obstructed. In addition, angioedema in the oral cavity may affect absorption of locally administered medication. Though swelling has been induced in the skin of miniature swine (MS) using histamine injections, there is no large animal model of angioedema in this target area (i.e., oral cavity). Thus, an animal model is needed to further

investigate facial angioedema pathophysiology and treatments. It was hypothesized that histamine would induce swelling and erythema in the lips and tongue, similar to what is observed in skin irritation models. To test this hypothesis, 6 male Yucatan MS were injected in the mucosa of the inner lip (left and right) and into the tongue with 20  $\mu$ l of histamine dihydrochloride at each site and at varying concentrations. The following concentrations were used (one animal dosed at each): 25, 125, 500, 1000, 5000, and 10,000  $\mu$ g/ml. Animals were anesthetized with isoflurane to ensure accuracy of dose administration and measurements. Calipers were used to measure tissue swelling, and Draize scores were performed to monitor erythema and edema around each injection site. The animal dosed at 10,000  $\mu$ g/ml displayed the desired reaction, and an additional 4 animals were dosed at this concentration to ensure reproducibility of results. For the total of 5 animals dosed at 10,000  $\mu$ g/ml, the majority of swelling in both the lips and tongue occurred within the first 30 minutes (average change from baseline = 3.82 mm for the lips; 3.32 mm for the tongue). Lip thickness continued to mildly increase over 90 minutes, whereas swelling in the tongue decreased in 90 minutes, though not back to baseline. Draize scores showed increased erythema and edema starting at 10 minutes. Animals received diphenhydramine during anesthetic recovery to reduce any remaining swelling. All animals recovered uneventfully. Thus, histamine dihydrochloride serves as a suitable agent for inducing angioedema in the tongue and lips of MS and can serve as a valuable large animal model for assessing treatments of angioedema and investigating the impact of angioedema on drug absorption.

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

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

#### **PS30 Impact of Aspen versus Corn Cob Bedding on Reproductive**

#### **Measures in a Transgenic Mouse Model of Cystic Fibrosis**

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

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

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Simian immunodeficiency virus (SIV) infection in pigtail macaques (*Macaca nemestrina*) is a valuable model for AIDS pathogenesis, especially in the central nervous system (CNS). This model closely resembles HIV infection in humans, exhibiting diverse immune responses and disease progression between individuals. We hypothesized that differences in the adaptive immune response to SIV drive inappropriate innate activation within the CNS, leading to poor clinical outcomes such as encephalitis. SIV-specific antibody titers and cytokine production in longitudinal plasma samples and cerebrospinal fluid (CSF) were measured in juvenile males ( $n=16$ ) from baseline through 84 days post-infection using ELISA and an MSD U-PLEX assay, respectively. CNS involvement was evaluated through combined immunohistochemistry (IHC)/ *in situ* hybridization (ISH) staining of basal ganglia for apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and SIV RNA. Viral load in basal ganglia and CSF was measured using qPCR. The functionality of plasma antibodies was examined using *in vitro* neutralization. Macaques that developed encephalitis showed pronounced CNS inflammatory pathology, including elevated CSF cytokines, increased ASC expression, and higher brain viral loads. Plasma antibody response to SIV was predictive of CNS outcome as early as 21 days post-infection, with differences between outcome groups becoming more apparent over time. Plasma immunoglobulin from macaques with encephalitis displayed limited neutralization capacity compared to those without encephalitis.

These findings underscore the critical role of the early adaptive response in determining long-term clinical outcomes of SIV, particularly in relation to CNS involvement. Robust early antibody responses appear to protect the CNS from viral infiltration and nonspecific inflammatory activation. Understanding early B and T cell responses during acute SIV infection can provide insights into mitigating nonspecific inflammation and addressing chronic inflammatory changes observed in individuals with HIV. These findings contribute to our understanding of HIV CNS pathology and highlight potential areas for further research.

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

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

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

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African Clawed Frog (*Xenopus laevis*) oocytes are used in biomedical research to study cellular biology, toxicology, and embryology. Repeated survival surgical oocyte collection is commonly performed to access high quality, viable oocytes for research procedures and to reduce the number of animals used in research. Despite this common practice, there is no evidence-based limit for the total number of survival celiotomies that can be performed. To provide an improved reference a retired colony of frogs ( $n = 33$ ), previously used for surgical oocyte collection, was euthanized for gross and histopathologic evaluation. Animals received anywhere from 4–11

celiotomies (average  $6.3 \pm 2.3$ ) and were between 69–1646 (average  $427 \pm 251$ ) days from their last surgery. Body weight, residual external skin sutures, and abdominal wall sutures were counted, and all skin and abdominal wall musculature that had been operated on was collected for histopathology. Tissues submitted were examined for evidence of abnormal anatomy or changes indicative of painful processes (ex: granulation tissue, histiocytic infiltrate, fibrosis). After a standardized scoring system was developed, histopathology lesions were scored according to severity and then compared to experimentally naïve, age-matched controls ( $n = 4$ ). Results indicate that skin pathology score was significantly predicted by number of surgeries ( $P = 0.003$ ), however it was not predicted by the number of skin sutures placed ( $P = 0.14$ ). Abdominal pathology score was significantly predicted by number of abdominal sutures placed ( $P < 0.001$ ) and by the number of surgeries ( $P < 0.001$ ). Neither abdominal nor skin pathology was predicted by the number of days since the last surgery ( $P > 0.2$ ). As expected, the results indicate that as the number of surgeries and abdominal sutures placed increases, there is a greater likelihood of developing histopathologic changes that correspond to painful processes in mammalian species. This study provides an improved reference for the development of institutional guidelines for *Xenopus* survival surgical oocyte collection.

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

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

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



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

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

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Even though there have been many improvements in developing cancer therapeutics, only a small number make it through FDA approval. To combat the need for better preclinical models, we have developed PDX models of human non-small cell lung cancer (NSCLC) and canine osteosarcoma (OSA) by surgically implanting resected tumors subcutaneously in NOD scid gamma (NSG) mice (KN Suvilesh et al., Molecular Cancer (BMC), March 2022). To date, 26 NSCLC and 2 canine OSA tumors have been implanted. By using immunohistochemistry and quantifying mRNA levels, NSCLC PDXs retained expression of primary tumor-matched diagnostic

pathological markers such as PD-L1, p40, TTF, and HLADR over 10 generations. NSCLC PDX tumors and spleens in the NSG mice have also retained passaged tumor associated CD3+, CD4+, and CD8+ human T cells over several generations without causing graft versus host disease. We then used both metastatic and non-metastatic NSCLC PDX tumors to test the potential therapeutic efficacy of a novel T cell targeting molecule, anti-human CD3e monovalent Fab fragment (Mono-OKT3-Fab) and compared tumor growth results with those of established anti-human PD-L1 mAb therapy. In our non-metastatic model, 10 mice received IgG-control and 10 received the mono-fab treatment. We observed that PDX-NSG mice treated with mono-fab had a significantly reduced tumor burden ( $p < 0.01$ ) and increased survival over control-IgG-treated mice ( $p < 0.05$ ). This experiment was repeated with a metastatic NSCLC model and our mono-fab was combined with anti-human PD-L1 for comparison. We observed that 10 PDX-NSG mice treated with the combination treatment showed significantly reduced tumor burden ( $p < 0.005$ ) and increased survival over 10 control-IgG-treated mice ( $p < 0.005$ ). For our dog osteosarcoma PDX model, the passaged tumors share the same histologic phenotype as the parental tumor over three generations so far. We have also generated growth curves using caliper measurements and produced gross images demonstrating growth of canine bone tumor in NSG mice after subcutaneous implantation. In summary, our PDX models are effective at representing parental tumor characteristics and serve as an in vivo model for studying new cancer treatments.

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

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

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

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

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

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

considered and ruled out. There are many possible treatments for infection, however prolonged salt baths or other treatments require considerable planning and coordination with animal caretakers.

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

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

#### PS41 Bilateral Hind Limb Paresis in an Outbred Swiss Sentinel Mouse

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A 10-month-old female Tac:SW mouse presented with a 2-week history of ruffled fur coat and a 1-day history of unilateral periocular swelling. The next day, the mouse presented with acute bilateral hindlimb paresis and possible ataxia. This animal was housed with a conspecific in a cage receiving soiled bedding from 40 other cages weekly as part of a sentinel program. The cage mate was unaffected. Differential diagnoses for bilateral hind limb paresis and ataxia included degenerative arthritis or disc disease, autoimmune arthritis, traumatic injury, compressive nervous system neoplasia, infectious viral encephalomyelopathies, spontaneous brain infarction, and metabolic lysosomal storage disease. The mouse was euthanized and submitted for necropsy. It had a mildly ruffled fur coat and appropriate body condition (3/5). Gross findings were nonspecific and included mild thickening of the lower eyelid of the right eye with focal redness around the medial canthus and minimal clear ocular discharge, congested uterine serosal surface, and cystic

enlargement of the left ovary with clear fluid. No other gross abnormalities were identified. Histologically, there was diffuse accumulation of foamy cells (within neurons in the central and peripheral nervous system, and macrophages in several other tissues). Central nervous system lesions consistent with neuroaxonal dystrophy, such as hyaline or granular axonal spheroids, neuronal chromatolysis, and gliosis, predominantly affecting the gracile, cuneate, and caudal part of the spinal trigeminal nuclei, as well as Purkinje cell loss within the cerebellum were also observed. The widespread accumulation of foamy cells is consistent with a lysosomal storage disease. This disease encompasses an array of metabolic disorders of lysosomal dysfunction involving a steady buildup of substrates inside of the lysosomes, ultimately causing cell malfunction and death. These disorders are not widely reported in mice, making this a rare cause of neurological disease in mice. In this presentation, we will discuss the pathogenesis of several forms of spontaneous and induced lysosomal storage diseases in mice.

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

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

#### PS43 Menometrorrhagia in a Cynomolgus Macaque (*Macaca fascicularis*)

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

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

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Giant pouched rats, native to sub-Saharan Africa, are a muroid species with an exceptionally well-developed olfactory system. This feature, in addition to their small size and trainability, makes them a valuable species for applications in humanitarian work such as for odor detection of land mines, buried survivors of natural catastrophes, and diseases. The lack of literature on basic biology demands research on this valuable species to improve husbandry and clinical care. At our institution, a breeding colony of giant pouched rats is maintained to study their physiology and utility as scent-detectors. This report describes an aged (approximately 7.5-year-old) wild-caught female southern giant pouched rat (*Cricetomys ansorgei*) that presented acutely with vestibular deficits, including left-sided head tilt, ataxia, disorientation, and circling. Additional signs included poor body condition, dehydration, ocular discharge, and scaly dermatitis over the dorsum. Blood count, chemistry, thyroid panel, and urine analysis revealed elevated AST, cholesterol, phosphate, and T3. Prophylactic enrofloxacin was administered to address a potential bacterial otitis as the cause of vestibular disease. Initial treatment resolved the ocular discharge, and the animal stabilized in current condition. Despite treatment and supportive therapy, vestibular signs persisted. An intracranial lesion was suspected, and an anesthetized MRI revealed a large, focal

heterogenous mass arising from the pituitary fossa and extending into the suprasellar space causing foramen herniation, ventriculomegaly, and severe dorsal displacement and compression of the adjacent neuroparenchyma. Due to poor prognosis, humane euthanasia was elected. Necropsy revealed a pituitary prolactin expressing tumor, whose invasive behavior within the basisphenoid bone prompted a diagnosis of pituitary carcinoma. Pituitary tumors have previously been reported in various laboratory rats. Unlike reports of pituitary neoplasms in *R.norvegicus*, the pituitary mass in this pouched rat presented with uncommon malignancy. This case provides information important to our expanding clinical knowledge of a unique rodent species.

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

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

#### PS46 Creating a GLP Framework in an Academic Research Setting

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Biomedical researchers wishing to translate basic science results to human clinical trials have a need to conduct pre-clinical studies under federal regulation CFR Title 21 Part 58 Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies. These studies present many cultural and procedural challenges to an academic research laboratory and thus are often conducted at a commercial facility set up specifically for GLP work. However, an institutional animal research facility can leverage existing personnel expertise and

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

#### PS47 Ballin' On a Budget: From Stockroom to Vivarium

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

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

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

#### PS49 Cooperative Care Techniques for Large Swine in a GLP Laboratory Setting

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

effective tool for implementing cooperative care for large swine. With planning, communication, commitment of resources, and coordination, PRT can be accomplished in a GLP environment, facilitating safe and accurate data collection. Inclusion of the study and husbandry team in training activities creates a low stress environment for both technicians and animals, allowing for successful data collection with a challenging species and significantly improved animal and technician welfare.

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



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Light is an extrinsic environmental factor that profoundly influences circadian, neuroendocrine, and neurobehavioral regulation in laboratory animals. Responses to light are mediated via the retinal photoreceptors, including the classical rods and cones involved in vision, as well as the recently discovered melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) of the non-visual system. Previous studies from our laboratory demonstrated that exposure of pigmented and non-pigmented rats to blue-enriched (465-485 nm) light-emitting diode (LED) light at daytime (bLAD), compared to broad-spectrum (300-700 nm) cool white fluorescent (CWF) light, amplifies nighttime circadian pineal melatonin production and positively influences metabolism and physiology. Here, in our GLAS-supported investigation, we tested the hypothesis in mice that exposure to bLAD, compared with CWF, positively enhances integrated visual and non-visual system photic responses, shown to promote the circadian regulation of neuroendocrine and neurobehavioral parameters that are associated with optimizing animal health and wellbeing. Three mouse strains commonly used in biomedical research (C3H, C57BL/6, and BALB/c; n = 120/group; male and female) were maintained under an IACUC-approved protocol in an AAALAC-accredited facility for 12 weeks on a common lighting regimen 12L ( $68.8 \pm 5.2$  lux, within cage); lights on 0600 h):12D (0 lux) on either CWF (control) or bLAD (experimental) lighting, and were assessed for retinal photon flux ( $\text{cm}^2/\text{s}$ ), radiometric ( $\mu\text{W}/\text{cm}^2$ ), photometric (lux), and photopigment illuminances ( $\alpha$ -optic lux). Results (mean  $\pm 1$  SD) revealed in the 3 strains of mice that, although photon flux was similar between bLAD and CWF light exposure, stimulation of the non-visual melanopsin-containing ipRGCs and the visual S cones, rods, and M cones was  $43.4 \pm 0.8\%$ ,  $45 \pm 1.2\%$ ,  $28 \pm 0.7\%$ , and  $21 \pm 0.1\%$  higher ( $P < 0.001$ ), respectively, in mice maintained under bLAD. These data show that daytime exposure of mice to bLAD, compared to CWF light, has a marked positive effect on mouse retinal photic responses regulating the circadian, neuroendocrine, and neurobehavioral parameters associated with the promotion of animal health and wellbeing.

#### PS51 Current Prevalence of Nonhuman Primate Pathogens

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Nonhuman primates (NHPs) remain a critical research model for clinical trials on new therapeutics. Understanding the prevalence of pathogens in NHP colonies is essential to ensuring reliable and reproducible research results. To this end, we have summarized the results generated by our commercial laboratory since 2006 for the most monitored pathogens from over 1.2 million NHP samples across numerous colonies around the world. Efficient analysis of this large dataset was made possible by employing Excel Power Pivot.

The most recent pathogen prevalence levels comprise results from 2020 through 2022. During this period, the viruses most often monitored by serology were *Herpesvirus simiae* (B virus), simian T-cell leukemia virus (STLV), simian retrovirus (SRV), simian immunodeficiency virus (SIV), filovirus (FV), and measles virus (MV). Excluding MV, B virus was the most prevalent with just over 1% of samples testing seropositive, followed by STLV and SRV at 0.27% and 0.15%, respectively. It is noteworthy that since 2006, the seroprevalence of these three agents has trended downward. SIV and FV remain among the most tested agents but are not detected at any meaningful level. MV hovers between 50% and 70% of samples testing positive year over year, which is consistent with the push for vaccination of colonies to prevent infection. Of the bacteria for which NHPs have been screened most often since 2020, *Campylobacter* spp. was the most prevalent followed by *Shigella* spp., *Salmonella* spp., and *Yersinia pseudotuberculosis*, with prevalence levels by cultural isolation of 11.93%, 2.05%, 1.46%, and 0.98%, respectively. Prevalence for these agents by PCR was also summarized. Based on serosurveillance, the prevalence of *Mycobacterium tuberculosis* has experienced an uptick from just 0.12% in 2019 to 0.45% in 2022. Finally, recent parasite prevalence for both *Trypanosoma cruzi* and *Plasmodium* spp. was just over 5%. Prevalent agents should be included in surveillance of imported animals in quarantine and in routine monitoring of breeding and research colonies to avoid the confounding effects of adventitious infections and loss of valuable research resources.

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

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

#### PS53 Sensitivity of Polymerase Chain Reaction on In-cage Filter Paper for *Helicobacter* Species Exposure at Different Time Points

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

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

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Urban rodent populations and associated zoonotic diseases increased during the height of the pandemic. The soles of staff shoes accessing vivaria may become contaminated while traversing urban streets and serve as a source of fomite-mediated transmission of adventitious agents to laboratory rodents. While shoe covers may be employed to reduce this risk, their donning may lead to hand contamination. Shoe cleaners, i.e., motor-driven brushes that remove and collect particulates via vacuum from the top, sole, and sides of shoes, are utilized in our vivaria to reduce the potential of introducing excluded agents. Shoe cleaner debris and contact media (CM) mixed with the debris from shoe cleaners serving 5 distinct vivaria (A-E) utilized hundreds of times daily were analyzed by PCR for the presence of nucleic acid from 85 adventitious agents. To determine if the shoe debris was infectious, 3 NSG and 2 Swiss outbred female mice were exposed oronasally to a liquid suspension of debris and cohoused on

debris-contaminated bedding for 7 days from each of the 5 vivaria. A control group was similarly exposed to autoclaved debris (A-E pooled). Shoe debris and contaminated CM from each vivaria were positive for up to 51 agents. Of those agents, 47% were zoonotic and 25.5% were frequently monitored for in rodent colonies. Noteworthy agents included: Orthopoxvirus (8%), *Rodentibacter heyltii* (8%), Mouse Hepatitis virus (8%), *Sarbecovirus* (33%), *Ornithonyssus bacoti* (33%), *Spironucleus muris* (50%), Mouse Norovirus (58%), *Entamoeba* (58%), Mouse Parvovirus (67%), *Chlamydia muridarum* (100%), *Helicobacter spp* (100%), and *Tritrichomonas spp* (100%). There was a statistical difference in the odds of direct debris examination detecting more pathogens than CM in the control and 1 vivarium group, whereas there was no difference in all others. All NSG and Swiss mice remained clinically healthy, and PCR (fecal, buccal & skin samples) and sera were negative (SW) for all agents in the test panels. Preliminary histological evaluation did not reveal pathologic abnormalities. These results provide insight into the adventitious pathogens present on shoes in NYC and suggest that the debris is not infectious to laboratory mice.

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

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

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

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

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

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Soiled bedding sentinels (SBS) have historically been relied on to screen rodents in quarantine for excluded infectious agents prior to their release into a research vivarium. However, due to growing reports that many infectious agents are not effectively detected by SBS, direct animal sampling (DAS) for PCR-based quarantine has become a common alternative. Sentinel-free soiled bedding (SFSB) PCR sampling, which relies on dust collected from pooled soiled bedding, has been reported as a successful method for routine screening of research colonies. As an alternative to DAS, we investigated the efficacy of SFSB as a quarantine method. Pet shop quality mice supplied soiled bedding: one cage of (n=5) >6-month-old, one cage of (n=5) 6-10 week-old, and two cages each housing a dam and litter. DAS were collected from mice on arrival (D<sub>0</sub>); fecal pellets were collected from the dams and pups, while feces, body swabs, and oral swabs were collected from all older mice and pooled by cage. Soiled bedding from the shipping crate for each age group was divided into three equal quantities and used to expose contact media via manual agitation (D<sub>0</sub>). Shipping crate bedding was then placed with the mice of the appropriate age group in cages in a cube isolator for one week. At D<sub>7</sub>, DAS for PCR and traditional diagnostic methods were used to screen mice for infectious agents. As described for D<sub>0</sub>, soiled bedding for each age group at the end of study (D<sub>7</sub>) was divided into three replicates to expose new contact media via manual agitation. Both SFSB and DAS had more positive replicates at D<sub>7</sub> vs. D<sub>0</sub> for bacteria, parasites, and protozoa. Positive replicates for some viruses diminished by D<sub>7</sub>, likely due to normal clearance by the immune system. Overall, 12 different bacteria, 10 parasite/protozoa, and 17 viruses were detected in the mice by DAS. SFSB detected all infectious agents present in the survival samples. In general, estimated PCR copy numbers were equivalent or higher for all samples and agents at the D<sub>7</sub> timepoint. This data supports that SFSB has equivalent sensitivity to direct animal sampling and may serve as a viable option for a sentinel-free, PCR-based quarantine program.

#### PS58 Mouse Coronavirus MHV-1 Disease in A/J and NOD Mice is

### Accompanied by a Hyperinflammatory State and Fewer Tissue Repair Macrophages.

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Mouse hepatitis virus (MHV) is a betacoronavirus of mice. To understand the similarities of MHV disease with COVID-19, the immune response caused by MHV-1 was characterized in four mouse strains. NZO/HiLtJ, NOD/ShiLtJ, C57BL/6J, A/J male and female mice (18 per strain) were infected with  $1.5 \times 10^5$  pfu of MHV-1 (or media) and euthanized at 3- and 8-days post infection (DPI). Blood (plasma) was collected for cytokine analysis or flow cytometry of peripheral blood mononuclear cells (PBMC). The cytokine results for MHV-1 at 8 DPI showed no significant differences in PBMCs, recognizing the severe clinical phenotype in A/J mice precluded assessments beyond 3 DPI. Significant differences ( $p < 0.05$ ) were detected at 3 DPI in PBMCs including: 1) B6 mice had significant increases in interferon gamma and tumor necrosis factor alpha (IFN $\gamma$  and TNF- $\alpha$ ); 2) A/J mice had significant increases in IFN- $\alpha$  IFN- $\gamma$  and TNF- $\alpha$ ; 3) NOD mice had significant increases in IL-4, IFN- $\gamma$  and TNF- $\alpha$ ; and 4) NZO had significant increases in IL-6, IL-18, IFN- $\gamma$ , and TNF- $\alpha$ . The most striking feature of the cytokine data was the "cytokine storm" that occurred in A/J mice post infection. Flow cytometry results indicate neutrophils, inflammatory monocytes (Ly6C+/CX3CR1 hi) and patrolling monocytes increased after infection, except in NZO mice, which have almost no patrolling monocytes but the transition to tissue repair macrophages [CD124+(IL4Ra)] of low inflammatory status [CD119-(IFNGR1)] appears rapid in NZO mice. A/J mice produced more CX3CR1 inflammatory macrophages and fewer tissue repair macrophages. Both B6 and NZO mice had mild clinical disease and had higher levels of tissue repair (CD124+) macrophages compared to the more clinically affected A/J and NOD mice. The cytokine and flow cytometry results show A/J mice have a pre-existing inflammatory state and develop hyperinflammatory after MHV infection. NOD mice also have a pre-existing inflammatory state exacerbated by MHV infection. Both NOD and A/J have pathologic and clinical disease (A/J more severe), defects in their innate immune systems, and a C5 deficiency. The combination of a pre-existing inflammatory state and defects in innate and adaptive immunity set the stage for more severe MHV disease.

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

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Recently, we described the moderate prevalence of *Chlamydia muridarum* (Cm) infection in global academic mouse colonies. To better understand the natural biology and significance of Cm infection in commonly used inbred strains and an outbred stock, 2.7 x 10<sup>3</sup> IFU of a Cm field isolate was administered to a cohort (n=27) of 8-week-old female BALB/cJ (C) mice via orogastric gavage. After confirmation of Cm shedding via fecal PCR through 95 days post-inoculation (DPI), these mice were utilized to investigate the transmissibility, shedding kinetics, and tissue tropism of Cm following cohousing with C57BL/6J (B6) and C mice (Th1 and Th2 skewed, respectively), and Swiss (J:ARC[S]) mice (n=30 each strain). A Cm infected C mouse was cohoused with 4 naïve mice of each strain for 30 days and exposed naïve mice were followed for 6 months. Exposed (n=3/strain) and control (n=1/strain) mice were evaluated 7, 14, 21, 60, 120, and 180 DPI for CBC/chemistry, fecal

qPCR, and pathology with Cm-immunohistochemistry performed on select tissues. All B6 mice were fecal qPCR positive by 3 through 180 DPI. While only 1 of 3 C mice was positive at 3 DPI, all mice were positive by 7 through 180 DPI. 5 mice remained fecal qPCR negative at 3 and 7 DPI with only 1 of 6 mice positive at 14 DPI. All remaining 5 mice were positive by 14 through 180 DPI. No significant histologic lesions were appreciated in the lung, gastrointestinal tract, or reproductive tracts in any strain. Cm antigen was only detected in surface intestinal epithelial cells, primarily in the cecum and colon, often associated with gastrointestinal associated lymphoid tissue (GALT). Importantly, these findings indicate that 3 commonly utilized strains of laboratory mice are susceptible to chronic enteric infections with Cm, despite differing genetic background and immune response profiles. Cm's colonization of mucosal epithelial cells in the large intestine suggests that Cm may induce at least a local immune response, warranting further investigation.

### PS60 *Chlamydia muridarum* Modulates Splenic Monocyte and T-cell Response and Induces Sustained Intestinal T-cell and ILC3 Responses in Inbred and Outbred Mice

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

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



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

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

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

Items such as dig pans that elicit natural behaviors should be strongly considered as part of a behavioral management plan for tree shrews.

#### PS63 Marseille Declaration: Together We Prioritize Animal Welfare

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

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

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Zebrafish are a widely used animal model, yet there is limited understanding of their welfare needs. Despite an increasing number of studies on zebrafish enrichment, it remains unpopular among researchers to use in-tank environmental enrichment. Although supportive evidence is sparse, there is hesitancy to include in-tank enrichment due to perceived concerns of health/hygiene of the fish. To accommodate these concerns, we tested the potential benefits of enrichments presented outside the tank on 40 adult mixed-sex zebrafish. We hypothesized that zebrafish would show a preference for enriched environments and have lower cortisol levels than

zebrafish housed in barren environments. We used two experiments to test our hypothesis. In our first experiment, we either group housed (8 tanks of 4 fish) or singly housed (8 tanks of 1 fish) the fish. Over 2 weeks, using a repeated-measures factorial crossover design, we quantified if their preference for a pebble picture located under half of their tank was as strong as zebrafish's well-established preference for social contact. We used two positive controls: group housing, and visual access to conspecifics. Singly housed zebrafish displayed a significant preference for the enriched half of the tank (quantified as spending <50% of their time over the pebble picture); this preference was equivalent to the positive control of visual access to conspecifics. In our second experiment, using the same cohort of 40 zebrafish, alternating tanks received pebble enrichment or standard housing (barren) underneath the entirety of the tank floor, equally distributed between singly and group housed tanks. After one week, to quantify stress levels, we collected tank water to measure cortisol levels. Overall, being group housed decreased tank water cortisol levels by 25% and being enriched decreased tank water cortisol levels by 22%. These effects were independent and additive such that singly housed enriched fish did not differ in cortisol levels from group housed barren fish.

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

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

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

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Analgesia is one way to embrace the 3RS when working with

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

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

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

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

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Crested anole (*Anolis cristatellus*) breeding colony was established

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

#### PS69 Intermittent Lethargy and Epistaxis in a Rhesus Macaque

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

differential diagnosis for cardiac disease in adult rhesus macaques.

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

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

#### PS71 Subcutaneous Swelling in a Laboratory Ferret

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A 10 month old, 1.28kg, group-housed, experimentally naïve, neutered, male ferret was examined for a non-painful right axillary subcutaneous mass approximately 2.0x1.2x1.0cm in diameter. As the ferret was afebrile and displayed no regional lymphadenomegaly, he was placed on monitoring for suspected local inflammation secondary to rough housing. On reassessment 7 days later, the mass had grown and become lobulated, now measuring 4.1x1.6x1.3cm in diameter. An FNA of the mass found mild to moderate mixed inflammation. The ferret's clinical signs had also progressed; he was quiet, dehydrated, and febrile. Support in the form of fluids, critical care, meloxicam, and amoxi-clav was initiated. Bloodwork revealed mild neutrophilia (9.828-K/uL), monocytosis (0.546-K/uL), and basophilia (0.273-K/uL), and marked eosinophilia (13.65-K/uL). Differential diagnoses included an allergic reaction, parasitism, hypereosinophilic syndrome, eosinophilic gastroenteritis, autoimmune disease, or neoplasia. During this time, the right prescapular lymph node slightly enlarged, the right axillary mass decreased in size, additional masses were identified caudal to the left

ear and at the right proximal caudal thigh, and abdominal palpation revealed splenomegaly and enlarged mesenteric lymph nodes. Despite aggressive supportive care, the ferret became progressively lethargic and dehydrated, although no additional clinical signs developed. A diagnostic necropsy was performed. Repeat blood work revealed marked leukocytosis (47.8-K/uL), with more extreme neutrophilia (17.208-K/uL), lymphocytosis (13.384-K/uL), monocytosis (1.434-K/uL), basophilia (0.956-K/uL), and eosinophilia (14.818-K/uL) than before, while gross examination showed severe splenomegaly (35.26g, 13x3.5x1.5cm), prominent lymph nodes, and multiple firm, subcutaneous and intramuscular and subcutaneous masses. Histopathology revealed lymphoid hyperplasia and severe splenic extramedullary hematopoiesis, severe pyogranulomatous myofasciitis, and eosinophilia with severe eosinophilic gastroenteritis. This ferret's presentation of severe idiopathic pyogranulomatous myofasciitis associated with the masses and other tissues and concurrent eosinophilic gastroenteritis – two rare conditions – represents an unusual clinical case scenario.

#### PS72 High Mortality in Juvenile Rainbow Trout

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

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

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A one-year-old intact male Sprague-Dawley rat was reported to have acute facial swelling on the right cheek and blood in the ear canal. Initial differentials included: trauma, neoplasia or abscessation of the Zymbal's gland, parotid salivary gland, or extraorbital lacrimal gland, or a deep inner ear infection. The mass was 2.5x1.5x1.5cm (about 0.59 in), firm but slightly fluctuant, and warm to the touch. Under general anesthesia (2% isoflurane), the mass was lanced using a 20g needle. Approximately 1 ml (about 0.03 oz) of purulent exudate was drained from the mass. The abscess was not flushed with iodine for fear of spreading infectious material to deeper tissues or the ear canal. The rat was given a single dose of enrofloxacin and meloxicam and the husbandry staff was instructed to monitor daily. Six days later the abscess recurred, and the rat was euthanized. Necropsy revealed a solid mass immediately caudoventral to the ventral ear

canal, with purulent material filling the interior of the mass. On histopathology, the mass was identified as a Zymbal's gland adenocarcinoma, likely leading to a secondary abscess formation. Aerobic culture revealed a mixed population of bacteria, including *Proteus mirabilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus merionis*. Zymbal's gland pathology is common in rats used in research. Though abscessation was the most prominent feature in the initial presentation, it is often secondary to another underlying pathology, in this case neoplasia. The bacteria cultured in this case are common opportunistic infections. Interestingly, *Streptococcus merionis* is most often associated with gerbils and other jirds; it has previously been cultured from the oropharyngeal region of gerbils and other small burrowing rodents. Though thought to be a commensal bacterium, not much is known about its pathogenic potential. With respect to the present case, there have been no gerbils ever housed in this building. Therefore, the origin of the *S. merionis* could not be determined.

#### PS74 Mysterious Mass in a Mangabey

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

#### PS75 Subtle, Unilateral Hindlimb Lameness in a Lesser Egyptian Jerboa

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A 2-year-old female lesser Egyptian jerboa (*Jaculus jaculus*) presented with a discrete swelling on the right hindlimb. There was no known history of trauma and the animal had been singly housed since its arrival at our institution approximately 1 month earlier. The physical exam was normal except for a 2mm fluctuant swelling on the medial aspect of the right tarsus. When standing at rest, a subtle favoring of the right hindlimb was observed; however, lameness was difficult to

appreciate during normal ambulation in the cage and behavior was otherwise normal. Fine needle aspiration of the tarsal mass produced a small amount of clear, viscous, acellular material consistent with synovial fluid. Differential diagnoses included acute soft tissue injury, a synovial cyst, and chronic degenerative joint disease. Conservative therapy was initiated with 0.5 mg/kg meloxicam administered subcutaneously once daily. Despite treatment, the lameness worsened, and the jerboa was sedated for imaging. Radiographs revealed soft tissue swelling around the right hock and a small, radio dense lesion associated with the tarsal joint space. Humane euthanasia was elected due to the poor prognosis for improvement. On necropsy, there were several gross changes of the right tarsal joint, including generalized soft tissue swelling and a small, spherical outpouching from the joint space. Histologic findings included a chronic, non-healing fracture of one of the tarsal bones with exuberant cartilage formation. While this case illustrates that fractures must be considered as a differential in instances of joint swelling and mild gait abnormality in this species, the fracture of single tarsal bones is relatively rare in humans and domestic animal species. It is unclear what the inciting cause was in this animal, though this species' unique hindlimb biomechanics must be considered, as jerboas are desert-adapted rodents that demonstrate bipedal locomotion and explosive hopping as a mechanism for predator avoidance.

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

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

#### **PS77 Armenian Hamsters (*Cricetulus migratorius*): A New Host for *Corynebacterium bovis* Infection**

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

#### **PS78 Novel Demonstration of *Corynebacterium bovis*-associated Lesions and Interface Dermatitis in NIH-Foxn1<sup>tmu</sup> Rats (*Rattus norvegicus*)**

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*Corynebacterium bovis* (Cb), a short, pleomorphic, gram-positive rod, is well recognized to cause clinical disease and histopathologic lesions in immunocompromised mice and has been isolated from Sprague-Dawley and athymic nude rats. While rats can be colonized with Cb, there is no peer-reviewed literature describing clinical disease or histopathologic lesions resulting from Cb infection in this species. Clinically observable dermatitis developed in 2 naïve NIH-Foxn1<sup>tmu</sup> (nude) rats from which Cb was isolated by aerobic bacterial culture and PCR. Shared use of behavioral equipment by Cb-infected immunocompromised mice is the suspected origin of the bacterium in the colony. Affected rats presented with a pruritic dermatitis characterized by urticaria, macules, and pinpoint abrasions on the dorsal thoracoscaphular and ventral abdominal regions, as well as bilaterally thickened and flaky pinnae. Rats failed to respond to a series of treatments, including the provision of amoxicillin-impregnated feed (0.12%), application of topical silver sulfadiazine ointment, or gentle cleansing of the affected areas with chlorhexidine (4%) solution along with weekly nail trims. Histopathology showed typical Cb-associated lesions, such as acanthosis and orthokeratotic

hyperkeratosis with intracorneal and intrafollicular colonies of short rods compatible with Cb. Additionally, a distinct pattern of interface dermatitis was observed with and without an association with bacteria, characterized by a lymphocytic mural folliculitis and epidermitis, with lymphoplasmacytic and histiocytic peri-adnexal to interstitial dermatitis, and occasional neutrophilic luminal folliculitis, superficial neutrophilic pustules, and dermal fibrosis. Based on the isolation of Cb and select histologic features, which resembles Cb infection of nude and highly immunocompromised NSG mice, Cb likely contributed to the clinical disease observed in these cases. While it is possible that Cb and/or other components of the skin microbiome may have contributed to the development of the interface dermatitis, it is not possible to determine which, if any, of the clinical features are solely attributable to Cb, as interface dermatitis has also been observed in both nude mice and rats devoid of typical Cb lesions.

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

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

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

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

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



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

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

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

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

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Relative humidity (RH) is commonly measured in mouse housing rooms. It is typically assumed that measurements in the room reflect what the mice experience in the cage. However, there is limited data comparing RH measurements at the room level and inside of ventilated cages. The purpose of this study was to compare how RH levels taken at the room level compare to those inside of ventilated cages containing breeding trios and same sex groups of laboratory mice. We also assessed how RH is impacted by the age of a single mouse litter, the time since cage change, and the group size of same sex cages. RH was measured using a temperature/humidity sensor attached to a solid top caging lid. The lid was rotated across N=24 breeding trio cages, equally divided across litter ages (no litter, PD1, PD10, PD18; n=6). The lid was also rotated across N=15 same sex holding cages, containing either 1, 3, or 5 mice. Measurements in holding cages were repeated 3x per cage in relation to time since cage change (1 day, 1 week, 2 weeks). All measurements lasted for five minutes and were done following a two minute acclimation period in each location. Data were analyzed with general linear models and blocked by cageID where appropriate. In breeder cages, RH was impacted by litter presence and age ( $F_{4,20} = 29.74$ ;  $P < 0.001$ ): it was higher in all mouse cages than the room and it was higher in cages with weanlings than cages with no pups. In holding cages, RH was higher in mouse cages than the room ( $F_{2,32} = 10.36$ ;  $P < 0.001$ ). It was also impacted by time since cage change ( $F_{2,28} = 6.14$ ;  $P = 0.006$ ) and an interaction between sex and group size ( $F_{2,28} = 5.29$ ;  $P = 0.011$ ). RH was

highest in cages holding five males and lowest in cages holding a single mouse of either sex. This data shows that RH is not constant between mouse cages in a single room, nor within a single cage over time. Further, measuring RH at the room level may not be a valid assessment of what the mice experience in ventilated cages.

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

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

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

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The chronic form of COVID-19, Post-Acute Sequelae of COVID-19 (PASC), remains a significant public health concern. The B6.Cg-Tg(K18-ACE2)2PrImn/J (hACE2) mouse has been widely used to study acute COVID-19 studies, but its suitability as a model for PASC remains uncertain. The long-term goal of this research is to establish whether the hACE2 mouse is an effective model for PASC research. Previous work by our lab has shown experimental inoculation of the hACE2 mouse with SARS-CoV-2 results in persistent infection up to 16 weeks, but their infective potential was unclear. The primary objective of this study was to assess viral infectivity in persistently infected mice with the aim of transitioning them from animal

biosafety level 3 (ABSL-3) containment to ABSL-2. To this end, a sub-genomic E RNA (sgE-RNA) RT-PCR assay, an assay that detects replicating virus, and sentinel mice were employed to determine SARS-CoV-2 viral infectivity. It was hypothesized that infected mice would be free of replicating virus by 16 weeks post-infection (WPI) and that sentinel mice would only become infected when exposed to recently inoculated mice. Six- to 18-week-old, hACE2 mice (N= 78 females + 75 males) were intranasally inoculated with the USA-WA1/2020 strain of SARS-CoV-2 in ABSL-3 containment. Acutely ill mice were euthanized, and their tissues collected, while cohorts of surviving mice were necropsied at weekly intervals up to 16 WPI. Naïve, female, homozygous hACE2 sentinel mice (N =5) were exposed to previously inoculated mice at 0 DPI (N=3) and 8 WPI (N=2), and euthanized 2 weeks post-exposure, when lungs were harvested. Viral RNA and sgE-RNA were present in lungs up to 16 WPI and viral RNA was also present in the lungs of all sentinel mice. Together, these results suggest that intranasal inoculation of hACE2 mice results in persistent infection with replicating virus present up to 16 WPI, hindering removal from ABSL-3. However, the presence of viral RNA throughout the study suggests that this experimental model may be valuable for assessment of PASC pathology associated with viral persistence.

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

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

#### PS87 Group 2 Innate Lymphoid Cells Are Required for Protective

#### Immunity in Helminth Infected Mice

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

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

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The laboratory rat is currently the most used experimental model in hepatic surgical resection studies to investigate liver regeneration, chronic liver disease, and hepatic cancer. Our previous studies showed that the dietary consumption of linoleic acid (LA), an omega-6 fatty acid, stimulates the growth of rodent and human liver tumors *in vivo*. We tested the hypothesis that increased LA intake in animals fed a 5% corn oil semipurified diet (control Diet I) or an essential fatty acid deficient (EFAD; deplete in LA) diet but supplemented with an equal amount of LA as in Diet I (experimental Diet II), compared to EFAD diet alone (Diet III), would elevate plasma levels of LA and stimulate regeneration of 70% partial hepatectomized (HPX) rat livers that show metabolic similarities to hepatomas. Three groups of randomized male (N=60/group) and female (N=60/group) Buffalo rats (BUFF/CrCrI) were fed either diet I, II, or III and water *ad libitum* under an IACUC-approved protocol. After 8 weeks on the respective diets, rats were subjected to HXP. At Day 4 post-HPX (maximum regenerative period) arteriovenous (A[carotid]-V[inferior vena cava]) samples (0.2ml) were collected across regenerated livers (Diet III HPX livers did not regenerate) and measured for LA-, glucose-, O<sub>2</sub>-uptake, and lactate- and CO<sub>2</sub>-output; harvested remnant livers were measured for liver LA, total protein, cAMP, DNA content, and [<sup>3</sup>H]thymidine incorporation into liver DNA (TI-DNA). Regenerated liver A-V consumption of LA, glucose and O<sub>2</sub>, and lactate and CO<sub>2</sub> output, were significantly elevated by



over 200% in dietary groups I and II, compared to III; and, LA-, protein-, cAMP-, DNA- and TI-DNA-content were significantly elevated ( $P < 0.0001$ ) in I and II (male > female) by over 6000%, 400%, 250%, 800%, and 60% ( $I = II$ ). HPX livers regenerated to 60% original size in groups I and II, but not in III. Understanding the mechanism of LA-dependent liver regeneration in the laboratory rat will strengthen our current efforts to enhance successful surgical resection therapies in humans.

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

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

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

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Employee time is a highly valued resource, but direct training of staff either individually or in small groups exhausts the availability of both the trainers and trainees, who could otherwise be dedicated to animal care. When the veterinary group realized there was a gap due to the insufficient cadence of annual training, we developed a new method to disseminate information efficiently and rapidly in a way that was easy for staff to grasp the presented material quickly and asynchronously across multiple production sites of a large animal vendor. Welfare Wednesday was created to review the top clinical observations reported to the veterinary team through a visual aid, using one slide to outline the critical topic points. This slide is displayed on monitors throughout the vivariums and employee break rooms in conjunction with an email that includes a copy of that slide along with more detailed information. A new topic is covered weekly. The learning platform utilized to communicate with animal caretakers about these common clinical observations quickly evolved

into a way to educate the entire company on issues noted with our models and created discussion between different teams at all levels. A virtual assessment performed companywide determined that Welfare Wednesday was a successful training mechanism, with many positive responses received. This tool might be useful at institutions with large staffs and/or multiple geographically separated vivariums.

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

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

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

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

successfully maintain animals throughout their disease progression and complete numerous studies, providing valuable knowledge about human MS pathology.

**PS93 The “Aunting” System: Improving Survival in Immunocompromised Mouse Strains Post-Weaning**

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Immunocompromised mice can be challenging to breed even when housed using specific pathogen free (SPF) conditions. Breeders cannibalize pups, pups fail to thrive including post-weaning, and litter sizes are generally small for NSG and NOD.SCID strains. Important factors such as increased enrichment, temperature, and socialization can be utilized to increase production and optimize colony health. The “Aunting” system in post-weaning period provides warmth and socialization to runt pups that are often cannibalized by parents or fail to thrive when utilizing traditional delayed weaning. In addition, post-weaning auting improves weaning health while allowing breeders to continue production without having to nurse multiple litters, increasing breeding production overall. Mice are housed on ventilated racks with room temperature of 74 degrees and provided acidified water and breeder chow. At seven weeks of age, one male and two female mice are housed in breeding cages which includes bedding, nesting material and igloo enrichment items. If the first litter is unsuccessful, animals receive additional enrichment rotated to maintain novelty. On 10-14 days, pups are provided with chow on floor and a dietary gel supplement. Pups are weaned at ~20 days depending on health and appearance followed by housing with designated aunts. Enrichment items and dietary gel supplementation are maintained. Aunts, CBI7SCID females, 8 weeks of age and older, are housed four mice per cage. Depending on how many mice are being weaned, anywhere from one to four aunts are used for the auting period in single or multiple cages, without exceeding five mice per cage total. After pups are healthy enough to be removed from their aunts, the aunts are recombined into their original cage. The “Aunting” system is also effective when weaning singly housed mice and helping them adjust. After three to five days post-weaning auting, pups have gained weight with improvements in health, potentially demonstrating the importance of additional warmth and socialization versus primary nursing in a delayed wean scenario. In conclusion, weaning survival improves when post-weaning care is provided by an “Aunt” between 18-21 days of age.

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

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Researchers studying the sociality of rodents have long been confronted with the challenges of translating a rich array of natural behaviors into an ethologically relevant context that can be manipulated in a laboratory setting. Confining animals to standard cages and conducting artificial social experiments has often been the approach. To provide animals with a semi-naturalistic environment and researchers with an alternative model for sociality studies, a visible burrow system was constructed, featuring interconnected tunnels, home cages, and an open arena for rats. However, many challenges and creative solutions were needed for husbandry and health monitoring. For example, pathogen testing was conducted on two dirt-like substrates to confirm their safety for use. Extensive environmental parameters measuring temperature, humidity, ammonia, and adenosine triphosphate levels were recorded at regular intervals to gauge the effectiveness of cleaning methods, as well as to determine a threshold for cleaning that would minimally disturb the animals’ environment without posing health concerns.

An iterative process was utilized to upgrade the system from a hand-wash-only design to a rack-washable and autoclavable system. Researchers, animal care staff, and the attending veterinarian collaborated to produce new guidelines for the acclimation of animals to the system, criteria for removal, and standards for health checks. By analyzing environmental parameter data and maintaining open lines of communication among all stakeholders, the system has housed different cohorts of animals with varying experimental needs. Cages and tunnels were changed on a tri-weekly interval, and valuable behavioral and husbandry knowledge was gained from the arena and its burrows. By providing the animals a naturalistic environment that promotes social interaction, researchers can study behaviors and the neural mechanisms underlying them from a new perspective, while the vivarium continues to gain knowledge that can be applied to the construction of other novel housing paradigms in the future.

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

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

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

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Nuances of the gut microbiome have led to investigations of environmental parameters and their influences. To date, the water delivery method largely is underreported in animal research and is hypothesized to have an impact on microbiome. Water is typically provided to laboratory mice by reusable autoclaved bottle (RAB), by autowater mechanism (AW) from the housing rack, or by use of a single-use disposable plastic pouch (DPP). This study hypothesized that controlling water delivery source would positively stabilize gut microbiomes of mice following arrival from approved vendor to our facilities, within either immunocompetent (n=36 B6; 18M:18F) or immunocompromised (n=36 NOG; 18M:18F) genetic backgrounds over an 8-week study period. Mice were housed on a single IVC rack in sex-specific groups and provided with autoclaved caging/bedding and irradiated feed, while receiving one of three routes of reverse-osmosis, chlorinated water (8 cages per water source). Fecal pellets (n=2) were collected from each animal biweekly and water samples

were collected from each cage weekly or from the AW rack for analysis of potential bacterial load. Animal care was provided by dedicated staff (n=3) that performed daily checks and changed cages biweekly. The results indicated that over the course of the study, water from ~11% RAB cages (7 of 63 samples) had bacterial detection and ~4% of water samples from AW cages (1 of 25 samples) had bacterial detection. No DPP water samples had detectable bacteria during the study. Shotgun metagenomics highlighted obvious shifts in gut microbiome in all groups over the course of the study, regardless of water delivery source, except for NOGs on RAB. No clinical concerns were reported, except one cage of fighting male mice; histologic examinations of gastrointestinal tracts and organs from representative mice (n=12) were unremarkable. While microbiome shifts ultimately stabilized within cage cohorts by 8 weeks, the changes in gut composition over time, despite strict control of husbandry elements, indicated that nuances in water delivery methods still result in microbial variability and should be documented in animal studies involving microbiome assessments.

### PS97 Neuroblastoma Cell Line Engraftment in 48 Hours Post-Fertilization PF Zebrafish Larvae

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

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

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Adeno-associated virus (AAV) capsids are used for central nervous system (CNS) gene therapy. Direct administration of AAV into the brain is invasive and often associated with local transduction. However, some conditions require widespread diffusion throughout the CNS; intravenous (IV) administration of AAV represents an attractive alternative. Newborn and juvenile animals are believed to have more permeable blood-brain barriers (BBBs), and age-dependent variation in neuronal transduction has been described with AAV9, the neurotropic gold standard, and an engineered variant

AAV.CAP-Mac. We aimed to better characterize this phenomenon following systemic dosing in rodents. We dosed C57BL/6J mice (n=106) with AAV9 or AAV.CAP-Mac (5E13 genome copies/kg) IV. Animals were dosed once at P1, P9, P16, or P25 and sacrificed after 14 days. We hypothesized that the amount of vector and efficiency of transduction in the CNS would be highest in animals dosed at P1 and would decrease as age-at-dosing increased. Immunohistochemistry showed the greatest transduction of neurons and astrocytes in the brain of mice dosed at P9, supported by vector genome quantification, with both capsids. Following this peak, transduction of the brain decreased with increasing age of administration. Conversely, transduction of the liver (a potential site of AAV toxicity) by both capsids was lowest when dosed at P9. Superior BBB-crossing properties for AAV.CAP-Mac compared to AAV9 were not observed at any age. AAV.CAP-Mac demonstrated less neuronal and more endothelial transduction compared to AAV9, particularly at P16 and P25 dosing. Transduction of thoracic dorsal root ganglia was also lower by AAV.CAP-Mac compared to AAV9. Overall, our results demonstrate that CNS transduction via IV dosing of AAV9 and AAV.CAP-Mac is highest in young B6 mice when performed at P9. Because P9 in mice better correlates to the brain development of a newborn human than does P1, groups studying neonatal gene therapy with AAV9 via the IV route in mice to target the CNS should consider dosing at P9 rather than P0-1. This would also allow for baseline assays and genotyping to be performed prior to dosing, more efficient injection technique and anesthesia, and less disruption of neonatal mice immediately following birth.

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

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

Y-chromosome encoded antigens, analogous to the H-Y antigens, defined previously in mice and humans. This finding demonstrates the importance of including both sexes in animal research.

### PS100 Comparing Different Strategies to Reduce Hepatocellular Damage in Obese Common Marmosets

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

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



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Light emitting diode (LED) lighting is a new energy-efficient technology that is quickly replacing standard fluorescent lighting in animal research facilities as vivaria upgrade or are newly constructed. The impact of LED lighting on the health and welfare of research animals has not been thoroughly investigated. The goal of this study was to compare the effects of LED and fluorescent lighting on fecundity of C57BL/6 mice, paired at 6-8 weeks old, over a six-month period under four conditions: standard fluorescent (10 pairs), low intensity (~half normal intensity) LED with instantaneous light-dark phase transitions (8 pairs), normal intensity LED with instantaneous light-dark phase transitions (9 pairs), and LED with gradual light-dark phase transitions (9 pairs). We hypothesized that there would be no difference in fecundity between these lighting conditions. All breeding pairs under all lighting conditions successfully mated and became pregnant during the study period. The production of the breeding pairs was not significantly affected by type of lighting or intensity as there were no significant differences

between treatments in litter numbers (average  $4.6 \pm 1.4$  litters per dam), or litter size at birth or weaning (average  $7.3 \pm 1.7$  pups per litter). While pup weights at parturition were nearly identical across treatments averaging  $1.4 \pm 0.04$  g/pup, there was a significant effect seen with lighting treatment on weanling weight with the fluorescent control group having the smallest weanlings averaging  $9.3 \pm 0.6$  g. A higher than anticipated frequency of dystocia occurred during the study with significantly greater frequency in the normal intensity instantaneous and gradual phase transition LED treatments. The increased frequency of dystocia correlated with an overall significantly decreased survival of pups to weaning in the gradual phase transition LED treatment. For all treatments, only 6% of dystocias occurred in primiparous females, while 85% of dystocias occurred in multiparous females where the interval between litters was less than or equal to 25 days. The significant differences in weanling weight, dystocias and pup survivability between lighting conditions warrant further investigation as vivaria change to almost exclusive LED lighting.

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



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

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

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Fighting in laboratory mice has long been a discouraging issue for animal research personnel. Separation of animals is often employed

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

cell composition and activation status to that of a control mouse.

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



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