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Abstracts of Scientific Presentations

2020 AALAS Virtual National Meeting

Poster Sessions

PS1 Withdrawn

PS2 The Influence of Daytime LED Light Exposure on Fatty Acid and Protein Levels in the Major Metabolic Tissues of Mice

RT Dauchy,[1,*] LM Dupepe,[2] GL Dobek,[2] AT Pierce,[2] GB Voros,[2] SM Hill,[1] VP Belancio,[1] BB Kiely,[1] EM Dauchy,[3] AA Allen,[2] DE Blask,[1]

[1]Department of Structural and Cellular Biology, Tulane University, New Orleans, LA; [2]Department of Comparative Medicine, Tulane University, New Orleans, LA; [3]Department of Medicine, Louisiana State University Health Sciences Center , New Orleans, LA

Light, an extrinsic environmental factor, profoundly influences animal circadian, neuroendocrine, and neurobehavioral regulation. Appropriate laboratory animal facility lighting and lighting protocols, as outlined in the Guide, are essential for maintaining animal health and wellbeing and ensuring the credible outcome of scientific studies. Institutions worldwide are rapidly transitioning to blue-enriched (465 – 485 nm) light-emitting diode (LED) technology, due mainly to improved efficiency and long-term cost savings. Our previous studies demonstrated a positive influence on neuroendocrine and neurobehavioral parameters in rats exposed to blue-enriched LED light at daytime (bLAD). In this GLAS-supported investigation using 3 common mouse strains (C3H, C57/BL/6, and BALB/c) we tested the hypothesis that bLAD, compared with cool white fluorescent (CWF) lighting, optimizes homeostatic regulation of tissue fat and protein levels associated with a healthy phenotype. We exposed male and female mice (n = 120/strain) for 12-wk in an AAALAC-accredited facility to either bLAD (experimental) or standard broad-spectrum (300–700 nm) CWF light (control) on a common lighting regimen: 12L (68.8 ± 5.2 lux [within cage]; lights on 0600 h):12D (0 lux). Results showed significantly higher ($P < 0.001$) dietary and water intake, and body growth rates in both male and female C3H mice (male > female), but not in either C57BL/6 or BALB/c mice, maintained under CWF versus bLAD light. Mean of combined total fatty acid content of the major metabolic tissues (adipose > testes > ovary > skeletal muscle > gut > kidney > liver > brain > lung > heart), normally increasing with age, was significantly lower by 39.5 ± 1.3% ($P < 0.001$) only in C3H mice (male > female) maintained under bLAD versus CWF light. Conversely, mean of combined tissue protein levels (C3H > C57BL/6 > BALB/c), decreasing with age, were significantly higher by 23.5 ± 0.5% ($P < 0.001$) only in C3H mice (male > female) maintained under bLAD versus CWF light (skeletal muscle > liver > lung > heart > kidney > brain > fat > gut > testes > ovary). These data argue that exposure of C3H mice to bLAD, compared with CWF light, positively impacts parameters associated with the promotion of animal health and wellbeing.

PS3 Exposure to Animal Facility Light at Night Disrupts Circadian Rhythms of Metabolism and Physiology in Nude Mice

RT Dauchy,[1,*] LM Dupepe,[2] GL Dobek,[2] GB Voros,[2] AT Pierce,[2] BB Kiely,[1] SM Hill,[1] M Anbalagan,[1] DE Blask,[1]

[1]Department of Structural and Cellular Biology, Tulane University, New Orleans, LA; [2]Department of Comparative Medicine, Tulane University, New Orleans, LA

Controlled lighting in animal facilities, as outlined in the *Guide*, is a concern to both biomedical scientists and animal care personnel. Light exposure of sufficient intensity, wavelength, and duration at a given time of day significantly influences temporal coordination of circadian rhythms of neuroendocrine, metabolic, and physiologic parameters associated with the promotion of animal health and wellbeing and thus may influence scientific outcomes. Our previous studies demonstrated that animal facility light at night (LAN) contamination with as little as 0.20 lux suppressed production of the nighttime circadian neurohormone melatonin and induced chronobiologic disruptions in rat metabolism and physiology. In this GLAS-supported investigation, we examined the hypothesis that LAN suppression of the nocturnal melatonin signal in male and female nude mice (Crl:NU(NCR)*Foxn1*$^{(nu)}$; n = 36/group), commonly used in cancer and metabolic studies, alters dietary and water intake, body weights, as well as normal circadian rhythms of arterial plasma total fatty acid (TFA), neurohormones (melatonin, corticosterone, insulin, and leptin), glucose and lactate levels, pO_2 and pCO_2. Mice were maintained for 12 weeks on either a control 12L(155.2 ± 6.3 lux; 63.3 ± 2.6 mW/cm2 light phase intensity [within cage]):12 (0 lux; 0 mW/cm2) or experimental (LAN) 12L:12LAN (0.20 ± 0.02 lux; 0.08 ± 0.01 µW/cm2 dark phase intensity) light/dark cycle (lights on 0600 hrs) under an IACUC-approved protocol in an AAALAC-accredited facility. Plasma melatonin levels in controls were high in the dark phase (183.8 ± 12.8 pg/mL) and low (2.5 ± 0.3 pg/mL) in the light phase and in LAN-exposed mice throughout the 24-h period. Dietary and water intake, body growth rates, as well as normal circadian rhythms in levels of arterial plasma TFA, corticosterone, insulin, leptin, glucose and lactate levels, pO2 and pCO2, were significantly elevated ($P < 0.001$) in LAN-exposed mice, compared to control mice, by over 3.2%, 12.1%, 12.5%, 5.9%, 32.3%, 19.1%, 20.4%, 15.5%, 15.9%, 54.8%, and 62.1%, respectively. These findings show in vivo that integrated circadian rhythms of mouse neuroendocrine, metabolic, and physiologic parameters can be disrupted by LAN, as sometimes occurs in laboratory animal facilities.

PS4 Pathogenicity of 2 *Corynebacterium bovis* Isolates in NSG Mice

K Szilagyi,[1,*] J Liu,1 AK Bloom,[1] M Granitar,[1] GE Sandusky,[1] R Livingston,[2]

[1]LARC, Indiana University School of Medicine, Indianapolis, IN; [2]IDEXX BioAnalytics, Columbia, MO

Corynebacterium bovis infection is a well-known and prevalent cause of scaly skin disease and wasting disease in athymic nude mouse colonies and can also cause severe disease in haired immunocompromised mouse strains. Besides causing systemic disease affecting animal welfare, *C. bovis* has been documented to affect the haired immunodeficient mouse ability to take up neoplastic cells and thus affect oncology research outcomes, a field in which they are the primary animal model. *C. bovis* is also unable to be cleared fully by available antibiotic treatments and is thought to be easily spread within animal housing rooms. Two colony forming unit sizes (CFU; large and small) have been described for *C. bovis*, but to this date no publications have described the pathogenicity differences between these 2 isolates. In this study, we illuminate the pathogenicity differences between large and small CFU isolates, originally cultured from clinical cases, in NOD.Cg-*Prkdcscid*$Il2rg^{tm1Wjl}$/

SzJ (NSG™) female mice. At approximately 3.5 mo of age, groups of 10 mice (5 per cage) were inoculated with saline (negative control), large CFU, small CFU, small and large CFU low dose, or small and large CFU high dose inoculums of *C. bovis*. Cage and pelt swabs were sampled every 7 d for a *C. bovis* PCR test, and assessed for clinical signs, until 42 to 43 d post inoculation. By 35 to 43 d post inoculation, all *C. bovis* inoculated cages reported positive PCR test results. All *C. bovis* inoculated cages, except for the mice inoculated with the large CFU isolate, reported clinical signs and had significantly higher histopathology scores than negative control mice. Negative control mice, housed in the same room as the inoculated mice, remained free of clinical signs and remained PCR negative for the duration of the study. In summary, in this study, the small CFU isolate appears to be responsible for the clinical signs associated with *C. bovis* infection. With strict husbandry procedures and engineering controls, it is also possible to house *C. bovis* infected mice in the same animal housing room as *C. bovis*-free mice, and maintain *C. bovis*-free mice.

PS5 Evaluation of Ankylosing Enthesopathy in C57BL/6J Male Mice

BL Kick,[1,*] L Anderson,[2] Z Bichler,[2] R Doty,[1] J Wotton,[3]

[1]Comparative Medicine and Quality, The Jackson Laboratory, Bar Harbor, ME; [2]Neurobehavioral Phenotyping Core, The Jackson Laboratory, Bar Harbor, ME; [3]Center for Biometric Analysis, The Jackson Laboratory, Bar Harbor, ME

Ankylosing enthesopathy affects the tarsal joint of aged male mice on a C57BL background. This joint disease begins with inflammation at enthesis sites, leading to proliferation of cartilage and connective tissue, and eventually results in ankylosis of the joint. While ankylosing enthesopathy has been described more thoroughly in the B10.BR strain, no current literature describes this disease occurring in C57BL/6J mice. Importantly, behavioral phenotype resulting from these joint changes have yet to be evaluated. To assess potential phenotypic sequela, this study evaluated frailty assessment, home cage wheel running, dynamic weight bearing, and von Frey test in affected (n= 30) and unaffected mice (n= 68) in the age range of 45–55 wk. Overall, mice with ankylosing enthesopathy had significantly higher frailty scores ($P < 0.05$) and weighed less ($P < 0.001$) compared to unaffected mice. Affected mice had greater overall touch sensitivity ($P < 0.05$) and they placed more weight on their unaffected limb ($P < 0.01$) compared to unaffected mice. Lastly, affected mice ran for a shorter length of time ($P < 0.01$) but ran a greater distance during their run ($P < 0.05$), compared to unaffected mice. These findings highlight the importance of identifying spontaneously occurring conditions in mouse colonies, and characterizing their phenotypic effects, as they may significantly impact research programs or specific study goals.

PS6 Development and Characterization of the Ultra-immunodeficient NOD.CB17-*Prkdc*^{scid} *IL2rg*^{tm1}/Bcgen (B-NDG) Mouse Model

J Naden,[1,*] J Xiang,[2]

[1]Veterinary Science, Research and Support, Envigo RMS Inc, Indianapolis, IN; [2]Biocytogen, Yizhuang, China

The "B-NDG" (NOD.CB17-*Prkdc*^{scid} *IL2rg*^{tm1}/Bcgen) mouse was designed and generated by Biocytogen. *IL2rg* (common gamma) gene was deleted in NOD-*scid* mice resulting in a mouse that lacks mature B cells, T cells, NK cells, and has a deficiency in cytokine signaling. This level of immunodeficiency makes this model ideal for engraftment with human immune cells and human tumor cells or tissues. Herein we describe the development and characterization of the B-NDG model, which includes growth curve, complete blood count, serum chemistry, flow cytometry, tumor growth, and humanization. A cohort of B-NDG mice (n = 50 males and 50 females) were weighed weekly from 3 to 9 wk of age. A cohort of 6 male B-NDG mice were analyzed to determine baseline levels for complete blood cell count and serum chemistry. Splenocytes of C57BL/6, NOD-*scid*, and B-NDG mice were isolated and examined for total T cells, B cells, NK cells, CD4+ T cells, CD8+ T cells, and regulatory T cells using flow cytometry. Data showed complete lack of all T cells, B cell,s and NK cells in the B-NDG model. Two different methods of humanization were examined in the B-NDG model. Human peripheral blood mononuclear cells (hPBMCs) were injected at 5x106 into the caudal vein of 3 B-NDG mice. After 24 d, the hCD45+ and mCD45+ cells were examined and mice had 30-80% hCD45+ cells. Of those hCD45+ cells, over 99% were identified as T cells. The second humanization method used was injection of 1x105 human CD34+ hematopoietic stem cells after irradiation. The percentage of human CD45+ cell in the peripheral blood was over 20% by 8 wk post injection in over 70% engrafted mice. At 16 wk post injection, of the total number of human lymphocytes in the humanized mice, 60% were B cells, 2% were NK cells and over 10% were T cells. The percentage of human T cells increases steadily with the extended observation window. We have demonstrated that B-NDG mice have several features that translate many benefits as compared to other immunodeficient models. This model has demonstrated better tumor growth than the NOD-*scid* and C. B17 *scid* mice for cell lines as well as PDX lines. We have also shown that this model is extremely useful for studies that require humanization of an immunodeficient mouse model.

PS7 Withdrawn

PS8 From Vivarium to Hospital: Safeguarding Animals and People in the Face of COVID-19

LE Riddle,* KD Bean, CV Rose, CM Bell

Veterinary Support Group, US Army Institute of Surgical Research, San Antonio, TX

The occurrence of pandemics in the last several decades has sharply highlighted the need for robust and coordinated plans for animal research facilities, as the welfare of both the animals and the human personnel caring for them must be maintained to the fullest extent possible. Specifically, the pandemic caused by SARS-CoV-2 has greatly impacted animal research facilities worldwide in a myriad of ways. The United States Army Institute of Surgical Research (USAISR) Vivarium in San Antonio, Texas is in a unique situation in terms of emergency response to pandemics due to being designated as a backup mass casualty (MASCAL) site to Brooke Army Medical Center (BAMC). At the start of the pandemic, BAMC needed innovative, effectual plans to alleviate a potentially high number of severe COVID-19 cases in the surrounding area. As part of this mitigation strategy, USAISR staff was tasked with converting the vivarium for human use to potentially receive patients, including the set-up of intensive care unit (ICU) units. The animals were safely moved to an off-site location to start the conversion process. Areas of the vivarium were then assigned to fulfill specific hospital needs, and additional equipment, such as ventilators and anesthesia machines, were accrued. Personnel were brought in to assist with clearing out rooms and moving equipment, while still observing critical social distancing measures. Because the vivarium was not designed for human use, some facility challenges were identified and addressed to still ensure mission success. After the completion of over 600 man hours of work by dedicated personnel, the 120,000 square foot animal facility was converted into a human hospital capable of supporting 72 patients, including 32 ICU beds. We have updated our disaster plan and facility SOPs to facilitate the execution of this scenario should the need arise again in order to save both human and animal lives.

PS9 A Collaborative Solution for Animal Care Technician Recruitment

S Ogeka,[1,2,*] L Muca,[1,2] M Morrison,[1,2] K Krueger[1,2]

[1]Animal Resources, Boston Children's Hospital, Boston, MA;

[2]Charles River Laboratories, Wilmington, , United States Minor Outlying Islands

Recruiting animal care technicians (ACT) has become increasingly difficult. The position of requires candidates to have a high school diploma (or equivalent) and have the ability to communicate (speak, read, and write) in English. The Animal Resources department (ARCH) works closely with Human Resources (HR) to recruit and hire candidates. Despite many meetings and brainstorm sessions, there has been an ongoing struggle to improve and reinvent recruitment strategies. The central theme for these discussions was, where do we find people who are interested in lab animal husbandry and how we do effectively and appropriately advertise for the position? HR made the suggestion to partner with Jewish Vocational Services (JVS), which is a workforce development organization in New England, and develop a 12-wk training program within the department. JVS would recruit potential candidates based on the ACT job description, and for 6 wk the trainees would attend JVS-managed classroom time for half of the day where departmental terminology and SOPs would be reviewed in English. The remainder of their time each day would be spent on the work floor with the ARCH supervisor of training learning the responsibilities of an ACT. The final 6 wk of the program would consist of full-time training in the workplace with various members of the department. Multiple rounds of this program have been completed, and this method of recruitment has proven to be successful with 10/12 candidates hired. We have been able to train and evaluate the candidates over a period of 12 wk prior to hiring decisions. As an additional benefit, we find these trainees developed a genuine interest and dedication in the lab animal field, and overall their work performance and attendance has been at or above standard expectations. If your institution needs assistance with candidate recruitment, contact your department's HR staff to see if there are any programs already in place where the institution partners with a workforce development center. You may also find workforce development centers in your community, state, and/or with local colleges.

PS10 Rapid Buildup of Intracage Ammonia (NH3) in Small Volume Mouse IVC Cages: A Husbandry Challenge

MA Eskandarani, O Kalliokoski, J Hau*

Department of Experimental Medicine, University of Copenhagen, Copenhagen, Denmark

Ammonia is a health hazard for both animals and humans. Human health and safety regulations often operate with 25 ppm as maximum level tolerated for workers. However, there are no official guidelines established for maximum acceptable NH3-levels inside rodent cages, but literature suggests that we should aim to keep levels below 50 ppm. We investigated if our routine 2-wk cage changing interval for individually ventilated mouse cages was sufficient to keep ammonia levels below 50 ppm. Furthermore, we investigated if varying levels of bedding (aspen wood shavings) had an effect on intracage ammonia levels. All mice were housed in individually ventilated cages of either Type II (501 cm2 area, volume 6 L) or Type III (820 cm2 area, volume 11 L). Ammonia build-up in the mouse cages was measured with a handheld photoionization detection (PID) sensor. By day 14 after cage change of type II cages (n = 321), all cages containing fewer than 3 mice had NH3-levels below 50 ppm. Seventy-seven percent of trio breeding cages had ammonia levels above 25 ppm and 51% had levels above 50 ppm. Seventy-eight percent of cages housing 4-6 adult mice each had levels above 25 ppm and 61% had levels above 50 ppm. There was no significant effect of varying amounts (halving the amount of bedding, or increasing to 150% of the reference volume) of bedding on intracage ammonia levels. By day 14 after cage change of type III cages, all cages were measured at < 50 ppm NH3. Only 27% of the type III cages reached a final NH3 level exceeding 25 ppm, and this was found for only 6 cages (14%) housing less than 5 mice. In conclusion, type II cages housing either more than 3 mice reached high (> 50 ppm) NH3 levels between weeks 1 and 2 after cage change. Consequently, cage change frequency should be no longer than 1 wk. Type III cages can be kept on a 14-d cage change schedule, although there is room for improvement also for these cages. Increasing the absorbent bedding could not be used as a tool to extend the acceptable cage change schedule in our setup. Decreasing cage volume for mice–a trend observed in the EU–to reduce the cage foot print and house more animals per square footage of vivarium comes at the cost of surprisingly rapid buildup of intracage NH3. This, in turn, necessitates more frequent cage changes for small cages thus increasing variable costs and labor costs significantly.

PS11 Space Is at a Premium: A Real-time Graphical Tool for Cage Space Allocation Planning

D Hanson*, H Manning, M Terrasi

Animal Resource Facility, Dana Farber Cancer Institute, Boston, MA

Facility managers often struggle with determining future rodent cage allocations due to the inherent challenges involved with understanding current space utilization and past trends. Cage space allocation involves promises, requests, utilization, and limited capacity. To address this, custom graphical web-based tools were created to display future and trended cage space allocation information which integrated data pulled from the existing census management software. Only select facility managers have access to this system, which resides behind the institutional firewall. On an on-demand basis, users instantaneously see graphical data both current and trended over time (no notifications are sent from the system to PIs.). The first tool, "room utilization by building," displays rodent room capacity, total PI allocations for each room (promise), and current census (utilization). Graphs are layered, allowing the viewer to promptly address concerns (e.g. current census numbers are greater than what was allocated) and opportunities (e.g. under-utilization). The second tool, "room level allocation," shows PI allocation and census totals. These 2 tools alert a manager that a PI's designated usage level has come within a specified percentage of their allocation (percent utilization). A third tool, "trended data," shows allocations (promise), requests, and census (utilization) by room or by room plus PI. This tool trends data over several years, although only the past 1.5 y of data is used for projections. Reviewing trended data allows users to determine if PIs have historically used their allocated cage amounts. This graphical tool allows multiple users to easily digest real-time data selected from a variety of different search parameters, all without concern for the original data. Managers can assign cage space allocations for new PIs or change allocations when PIs depart (upon notification by the IACUC). Since implementation, use of this system has eliminated the need for manually populated spreadsheets.

PS12 Forays into Farrowing Sow: Extra-large Swine in a Modest Facility

A Hicks-Nelson*, S Baker

Comparative Medicine, Stanford University, San Jose, CA

Transitioning housing facilities to accommodate large breed pregnant sows can present unique challenges that don't occur in housing gilts and boar. Gravid sows are larger and less mobile than our historically housed swine, and poorly navigate our elevated flooring rooms. Multiparous swine were also accustomed to a farrowing crate, but our IACUC determined such a crate inappropriate for long-term housing. The cardiovascular lab working with the sows also wanted accurate birth information and adequate space to evaluate postoperative behavior of the piglets and exercise tolerance. Our facility faced the challenge of housing multiparous gravid sows for the first time and prolonged piglet management. We theorized that we could promote uncomplicated farrowing and rearing without the use of a farrowing crate by video monitoring sows and minimizing intrusion during rearing. We created a husbandry and housing regime divided into a farrowing room and rearing room. New

housing differed from existing elevated flooring pens in the facility in that chain link fencing was utilized, an adjustable half wall was erected, a post-farrowing room was adapted for pig use, and we arranged a video camera network and adopted new veterinary and staff procedures for swine and personnel safety. We concluded that gravid swine could be housed without the use of farrowing crates but piglet mortality was increased at all stages of rearing independent of but also exacerbated by room intrusion. We recommend investing in farrowing flooring or traction mats to promote gravid sow mobility. Using video cameras, it wasn't necessary to enter rooms during the piglet rearing post-operatively to evaluate exercise tolerance in post-operative piglets, and video of interaction with the straw and littermates in a large room was sufficient. We further describe methods for staff education and animal handling for acute and prolonged studies with gravid sow.

PS13 Two Case Management Lessons Learned from Gas-Bubble Disease in Xenopus laevis: Practical Problem-Solving in an Aquatic Facility

NJ Fabian,[1,*] S Muthupalani,[1] NY Chan,[2] O Paugois,[3] C Bresilla,[3] CS McCoy,[1] OL Franco Mahecha,[1] JG Fox,1 D Annamalai,[1]

[1]Division of Comparative Medicine, Massachusetts Institute of Technology (MIT), Cambridge, MA; [2]Broad Institute, Cambridge, MA; [3]Whitehead Institute for Biomedical Research, Cambridge, MA

Gas-bubble disease (GBD), caused by exposure to water supersaturated with dissolved gases, is an important noninfectious disease in aquatic species. Routine monitoring and maintenance of recirculating systems in aquatic facilities are necessary to minimize the risk of GBD. Piping defects are often the culprit, but many failures in aquatic life support system components may lead to this condition. This report describes the identification and remediation of 2 GBD incidents in separate recirculating systems in our facility housing adult African clawed frogs (*Xenopus laevis*). In both cases, water ammonia, nitrite, pH, conductivity, and temperature parameters were within normal range. Clinical findings in affected frogs such as red and ulcerated skin, interdigital webbing gas bubbles, and abnormal buoyancy, as well as histopathological results, were consistent with GBD. *Aeromonas* spp., *Bacteroides uniformis*, and *E. coli* were cultured from skin lesions collected at necropsy. Diagnosis of GBD in both cases was based on a history of a compromised recirculating water system and pathognomonic lesions in affected frogs. The first case involved a faulty pump trap gasket that was identified and replaced 2 d later; no clinical abnormalities were noted in the colony. However, 3 cohoused frogs presented with GBD signs and required euthanasia 5 d after gasket replacement, suggesting that GBD can manifest even after repair of the recirculating water system. The second case involved 7 frogs with signs of GBD and suspected secondary red leg syndrome over a 2-wk period. Affected frogs were euthanized. Remaining frogs were placed in static tanks with aeration and daily water changes to prevent further morbidity and mortality and to allow assessment of the water system. Several water valves supplying individual tanks were noted to have a reduction in fluid pressure due to a defect within the valve and were drawing air into the water line (i.e., Venturi effect), which was thought to be the source of gas supersaturation of the water. To resolve the issue, all valves were replaced. These cases illustrate that routine maintenance of all parts of recirculating water systems and ongoing surveillance after repair are essential components in successful aquatic facility management.

PS14 Enrichment Innovation: Human Team Building Activity Benefits Nonhuman Primates

B Culp,[1,2,*]

[1]Research, Virscio, New Haven, CT; 2Research, St. Kitts Biomedical Research Foundation, Basseterre, Saint Kitts and Nevis

Ninety seconds to create a crossfunctional team. Two minutes to select parts. Twenty-five minutes to create a nonhuman primate enrichment toy. Go! Team building exercises too frequently are forced-fun activities that employees meet with eye rolls, often failing to produce lasting tangible results. With the meaningful goal of developing a new nonhuman primate enrichment device, our entire organizational chart, from CEO to housekeeping, became instantly motivated to participate. Requirements were simple: 1) form a team of any size, 2) include members across all departments, with members from both local and international business locations, and 3) define team roles, including an "accountant" to provide a per unit cost, a "social media expert" for documentation of the process, and a "marketing agent" to pitch the benefits of the enrichment device. We provided a limited choice of resources from which the teams could select from a display table full of random parts and a sparse selection of tools for construction. The limited selection of tools was intentional, compelling negotiation between teams for the use of these resources. A "Shark Tank"-style management panel collectively decided which team had the most beneficial primate enrichment device, at the best unit cost. In addition to winner's bragging rights, prizes were awarded only to the first place team. Additionally, the winning enrichment device was actually put into use throughout the facility, and the African green monkey, our sole research species, readily engaged with the new toy. The humans were engaged, and the nonhuman primates were engaged. As an enthusiastic exercise that is truly a win-win, this team-building strategy can be employed at other organizations to encourage a positive work environment and reward employee innovation.

PS15 Challenges of Capturing, Transporting, Housing, and Safely Handling Wild Caught Juvenile Alligators: Husbandry Considerations and Adaptations For Successful Research

RK Banks*

Office of Research, UCF, Orlando, FL

The successful acquisition, transport, housing and handling of farm raised and wild caught juvenile alligators across multiple states and jurisdictions requires planning and coordination. Various challenges are involved in adapting traditional techniques, lab animal processes, and standard operating procedures in preparing a conventional animal facility and staff to safely acquire, transport, and handle juvenile alligators. Different federal and state requirements exist regarding acquiring, possessing, and transporting a protected species across multiple state lines. Transporting the animals hundreds of miles across multiple states required steps to ensure the animals would not do harm to themselves or to staff. Holding tanks, covers, and adequate enrichment devices were developed to provide opportunities for subject animals be able to exhibit and practice normal hiding and basking behaviors mimicking their native environment. Additionally, unique handling techniques were developed and used to safely acquire blood samples, measurements, weights, and daily and weekly monitoring. Unique methods to identify individual animals and group housed animals within holding tanks and methods were employed to easily distinguish the study animals' state of origin. This unique species' behavior, daily interactions, and weekly feedings necessitated sanitization processes and management techniques development, including primary and secondary forms of euthanasia. The behaviors of this unique reptile species required adapting LAS skills and husbandry techniques for the successful completion of this important PhD candidate research project.

PS16 Reusable Surgical Masks in the Face of Supply Shortages

K Brannick,* MA Robinson, C Janssen, T Gomez

CLAMC, UTHealth, Houston, TX

Shortages of personal protective equipment (PPE) during the COVID-19 pandemic presents challenges within laboratory animal facilities. The FDA and CDC provided guidance on PPE conservation and surgical mask reuse, however little scientific guidance exists on

appropriate methods for achieving safe reuse. Similarly, the AVMA advocated the critical role veterinarians play in PPE conservation and collaborations were encouraged to share best practices on reusing disposable masks and determining viability of cloth masks as an acceptable substitute. To address these concerns, we sought to identify if our current disposable surgical masks are usable after general wear and resterilization through a standard autoclave cycle. Additionally, we sought to determine if cloth masks are suitable as an alternative to surgical masks during aseptic procedures. All mask types showed an immediate reduction in oropharyngeal bacterial secretions of the wearer compared to no mask (surgical 0.4 +/- 0.36 SEM; $P < 0.0001$; autoclaved 0, +/- 0 SEM, $P < 0.0001$; cloth 0, +/- 0 SEM, $P < 0.0001$; no mask 7.2+/- 2.37 SEM). No significance was identified between mask type ($P = 0.92$). These results supported modifications to our standard operating procedures to allow cloth masks during aseptic procedures resulting in conservation of critical PPE while simultaneously maintaining standard of care.

PS17 Chapparvovirus/Mouse Kidney Parvovirus Eradication by Cross-foster Rederivation

P Sharp,* I Faseeh, K Greca, A Frost, G Manship

Animal Resources Centre, Murdoch, Australia

In 2018 Australian and US researchers published their findings on a novel parvovirus which they termed Mouse Kidney Parvovirus (MKPV). They determined the virus was responsible for intranuclear renal tubule inclusion bodies observed in mouse lines over several decades, particularly immunocompromised lines.Our facility completed extensive PCR testing and found that MKPV was absent in all our production lines and sporadically present in our custom strains barrier area which consists of imported mice lines bred, maintained, curated, and supplied to researchers. The production line and custom strains barriers are physically separate areas with separate staff. Although parvovirus transmission is predominantly fecal-oral, there are other possible transmission routes, including through gametes, as reported in other parvoviruses. Because of the possibility of gamete transmission, our facility chose to pursue a cross-foster rederivation trial using naturally infected MKPV positive mice from the custom strains barrier facility. Mouse pups were removed from the dam within 24 h after birth, rolled in tissue paper sprayed with disinfectant before transferring to clean Swiss outbred foster mothers. Cross-fostered pups were tested for MKPV at 10 wk of age by fecal PCR and then at 20 wk of age by both serology and fecal PCR. Since February 2020, 7 litters from various mouse strains have been cross-fostered and the results thus far indicates 100% success in riding the lines of MKPV. Therefore our results demonstrate cross-fostering, with strict sanitation procedures, is an effective MKPV eradication strategy for naturally infected colonies.

PS18 Public Outreach at Work: Kids Allowed!

AT Pierce*

Comparative Medicine, Tulane University, New Orleans, LA

Public outreach by laboratory animal science (LAS) professionals can increase public support and awareness of the ethical use of animals in biomedical research. Career Day is a type of public outreach that is commonly done at high schools, where students listen to the professionals speak about their jobs. While this is an effective method of introducing students to various careers, they do not get to experience it. A summer public outreach program was created to give students hands-on work experience in the LAS field. A student must have approval from the institution, school, and student's guardian before entering the program. The activities done by the student depends on the interest of the student. Additional activities may be given as the student's skill level or interest changes. The possible activities include veterinary case rounds, facility management, clean cage production, sentinel program, inventory, and attending a college lecture and laboratory. During the summer of 2019 5 high school students entered the program. Two of the students had special needs and received accommodations during the program. At the conclusion of the program each student received a lab coat as a keepsake. A survey was sent to the students and their guardians and they all gave positive feedback. The inaugural summer public outreach program was a success and will be offered annually.

PS19 Modern Teaching Methods Increase Employee Success

KL Brackett,* A Schile

JMCRS Finance and Operations, The Jackson Laboratory, Bar Harbor, ME

At many institutions with animal caretakers, there is a need to conduct on-the-job training. In addition to the procedural and technical hands-on trainings required for job-related tasks, sometimes it is necessary to deliver content for the sake of knowledge. Often, this type of instruction is done through lecture or textbooks and student-employees are expected to simply memorize and regurgitate the information on multiple choice exams. However, learning through this style is simply not possible for some, and the disadvantage is more pronounced in courses with culturally and socioeconomically diverse students. Genetics courses for employees at our institution have used this traditional style for many years. On an average year, 25% of students would withdraw from the courses, and another 25% would fail upon completion. We knew there had to be a better way to support our employees in their career advancement. We turned to biology education research, an extremely active field with an abundance of publications, workshops, and conferences on best practices in teaching and learning in biology. The body of work produced by this field has a clear message: to reach more students, more diverse teaching methods must be used; learning is not one size fits all. We hypothesized that application of modernized pedagogical approaches to traditional courses would increase success and overall employee retention. Multiple best practices in teaching were implemented simultaneously for courses in 2019-2020: development of learning goals, active learning (writing prompts, independent problem solving, partner work, group work, anonymous live polling, acting out processes, creating models, drawing, and discussions), use of multimedia, social media engagement, instructor as the facilitator, explicitly building connects between content, peer-learning, effective feedback, mentorship and standards based grading. These methods in combination led to a 5% withdraw rate and 8% failure rate during the academic year. These practices had a clear and dramatic effect on employee success within a single year. We implore other workforce educators to use these proven methods in the development of their employees.

PS20 Reimagining Training and 3Rs through Extended Reality

E Crooker,* S Baran

Novartis Institute for Biomedical Research, Cambridge, MA

As the world becomes more technologically advanced and socially distant, it is pertinent that every industry looks deeper into the potential of extended reality (XR). Considering the importance of animal welfare, training, and the 3Rs, we studied the impact this technology could have on our industry. Recent advancements in XR present an opportunity to increase efficiency and effectiveness of processes within drug development journey, reduce animal use, boost training procedures, and address animal wellbeing concerns. In collaboration with a spatial computing company we trained on the use of a mixed reality headset and XML programming to create mixed reality, a subgroup of XR, trainings. Mixed reality training on the use of an anesthesia machine on a mouse model was completed by in vivo staff. This training program allows the user to store data for technique certification purposes, whether that be a picture or video recording of trainee performing the procedure. This aspect allows the trainer to view the work the trainee has completed and allow for granting of certifications. The most important facet of

the project is that the trainer does not have to physically be with the trainee during a training, giving more time back to the training services team. This approach has been a success and has led to the creation of more trainings, as well as a number of new "super users" to create their own trainings for different teams within the department. We reduced animal use numbers by no longer using live animals for each training. We are able to refine our trainings by reviewing the steps prior to project finalization to establish that we are within compliance of animal use and to ensure the animals do not experience pain or distress while simultaneously conveying important information to the trainee. The virtual reality training saves trainer time and thus gives more time to focus on other projects. This project has gained significantly more traction with the aspect of social distancing and reducing the amount of people in close quarters.

PS21 Evaluation of Various Handling Methods and Frequencies to Minimize Stress in Rats

J Kurpinski,[2,*] T Plachta,[3] D Cooper,[1] J Kylie,[1]

[1]Veterinary Services, Charles River Laboratories, Mattawan, MI; [2]Infusion, Charles River Laboratories, Mattawan, MI; [3]Study Directors, Charles River Laboratories, Mattawan, MI

Rats are known to be a relatively docile species that enjoys human interaction, but occasionally they will attempt to bite their human handlers. While this may be interpreted as an aggressive behavior, it is likely to be a fear or stress response. We assessed several different handling techniques and handling frequencies using 140 Sprague-Dawley rats that were singly or socially housed and handled either daily or weekly for 12 wk. Rats were handled in a standard manner (grasping around the body) with varying frequencies, or were handled using 1 of 4 alternative handling techniques. A modified Functional Observation Battery (FOB) Test and Interaction Test was used to assess stress. Rats handled using alternative methods showed resistance to being removed from the cage during FOB examinations compared to all standard handling methods; daily handling appeared to be the least stressful of these methods for both sexes (M: n = 4/10; F: n = 2/10) and handling with a bite glove or being picked up using a PVC tube appeared to be the most stressful methods (M: n = 10/10, 8/10; F: n = 9/10, n = 10/10, respectively). Increases in hypersensitivity and vocalization were noted in animals handled with a bite glove (M: n = 3/10, 8/10, respectively; F: n = 4/10, 6/10, respectively) or a PVC tunnel (M: n = 4/10, 6/10; F: n = 4/10, 5/10); females that were tickled prior to handling also showed increases in these parameters (n=5/10, 6/10) although this was not noted in males (n = 2/10, 2/10). Rats handled with a soft, absorbent paper towel had fewer of these observations noted than all other alternative handling methods (M: n = 2/10 and 4/10; F: n = 0/10 and 0/10). This suggests that while pair housing and daily handling is optimal to reduce fear, tickling can be used as a preventive measure for fear-aggressive male rats. A soft, absorbent paper towel may also be used at any time for handling fear-aggressive rats, whereas the use of a bite glove or PVC tube may increase stress in these animals.

PS22 Identifying Risk Factors of Self-directed Abnormal Behavior in Rhesus Macaques (*Macaca Mulatta*)

C Widmaier,* K Baker

Unit of Behavioral Management, Tulane National Primate Research Center, Covington, LA

Some nonhuman primates in captive settings can develop abnormal behaviors, which may include self-directed behaviors such as eye-poking (pressing of finger or hand to eye) and floating limb (elevation of a limb for no apparent reason). These behaviors are indicative of poor welfare. Furthermore, while noninjurious, eye-poking (EP) and floating limb (FL) may be precursors to the development of self-injurious behavior (SIB). This study explored the relationship between EP/FL and sex, rearing history, single housing history, and temperament, as well as the number of anesthetic events per month. Forty-two rhesus macaques aged 2–18 years at the time of the initial observation of these behaviors were selected for study who had previously been observed to perform EP/FL. Data consisted of 358 10-min observations, during which all events of either behavior were recorded. There were no significant effects of sex, rearing history, single housing history, or temperament on levels of EP/FL. However, individuals were more likely to perform EP/FL during months that they were anesthetized and accessed from their cage. This finding mirrors the lifetime incidence of stressful events as a risk factor, but suggests a more direct relationship between the behaviors and these events in the short-term. While overall sex differences were not detected, females showed greater elevations in EP/FL following anesthetization than males. Thirteen of the 42 subjects also had a history of SIB. Interestingly, EP/FL was not triggered by recent anesthetic events among the subset of individuals who also displayed SIB. This finding may relate to greater tenure in single housing among these subjects, and also may hint at altered coping mechanisms in rhesus macaques with a history of SIB. Findings of this study suggest that evaluations of interventions for EP/FL must take into account confounds that may be introduced by recent anesthetic events.

PS23 Withdrawn

PS24 Treadmill Training of Laboratory Swine

J Kim,[1,*] R Eder,[1] J Camacho,[2] C Nguyen[1]

[1]Cardiovascular Research Center, Massachusetts General Hospital, Boston, MA; [2]Center for Comparative Medicine, Masschusetts General Hospital, Boston, MA

Applications of exercise are important for cardiovascular research, and can be enriching for laboratory swine at our institution. Twelve male Yucatans, ranging in age from 2–6 mos were trained to run on a treadmill at speeds up to 3 miles per hour and up to 45 min for 5 d/wk, for 12 wk. Swine were housed in compatible pairs and trained with operant conditioning strategies paired with food access, followed by reward. Animals are first taught basic commands and acclimated to human-animal socialization, as well as a temperature and heart rate monitor, used to monitor cardiovascular output and gauge comfort. Treadmill use starts slow before increasing in time and speed. Treadmill sessions generally consist of a warm-up of 1.5 miles per hour for 10–15 min, and increased 0.1–0.2 miles per hour every 510 min, before a rapid pace of 3 miles per hour for 5 min before concluding with a cool down period at 1.5 miles per hour for 2 min. Training was conducted in consultation with veterinary and behavioral staff to ensure that animals willingly cooperate with treadmill use, time, and speed. Exercise regimens were customized to the individual animal using preferred treats, operant tools, and enrichment. Trained pigs showed motivation and enthusiasm with time spent on the treadmill. At the start of each session, animals eagerly greeted study staff and made their way to the front of the treadmill without prompting. During sessions, study staff observed tail wagging and positive vocalizations. At session completion, swine returned to their home pens eager to engage with enrichment. As an unintended outcome of treadmill training, husbandry staff reported that the animals were easier to interact with during routine care and handling. The work to train laboratory swine to run on a treadmill proved successful for study goals, for husbandry handling and as an enriching experience overall for laboratory swine.

PS25 Is a Sentinel Program Good for the Detection of Murine Chapparvovirus?

RK Dhawan,[1,*] ML Wunderlich,[1] S Jennings,[1] D Nicholson,[1] B Tedesco,[1] S Dayani,[2] KS Henderson[2]

[1]BioAssay Services, Charles River Laboratories, Wilmington, MA; [2]Charles River Laboratories, Wilmington, MA

Murine Chapparvovirus (MuCPV aka MKPV) has been linked with the development of kidney disease and renal failure specifically in immunodeficient animals. In a time-course study immunocompetent (CD-1) mice were used as sentinels and received soiled bedding weekly from CD-1 or immunodeficient (Nu/Nu) mice chronically shedding MuCPV. Three cages with 4 animals per cage for each donor strain were housed in microisolators. All CD-1 sentinels were confirmed MuCPV negative by fecal and urine PCR at beginning (week 0) of the study. Fecal pellets and urine samples were individually collected weekly from each recipient mouse over a 22-wk period. Cage swabs were also taken from each bedding donor and recipient cage to verify MuCPV PCR positive or negative status. MuCPV was detected in fecal pellets starting week 1 from sentinel CD-1 mice that received the immunodeficient donor bedding while detection in urine samples did not occur until week 7. Detection of MuCPV in the CD-1 donor bedding group occurred sporadically in fecal pellets starting at week 1 and not until week 12 in urine samples. The difference in the virus infection rate and copy numbers can be attributed to the viral load in donor bedding source. The immunodeficient bedding donor mice had much higher viral copy numbers in their feces and urine samples versus the CD-1 donor mice. This further confirms that the immunodeficient bedding recipient group was exposed to a much higher MuCPV infectious dose from the initiation of the study. This experiment confirms efficacy of using bedding sentinels for immunodeficient colonies where MuCPV detection in sentinel feces and urine can occur as early as week 1 and week 7, respectively. MuCPV detection was inconsistent in feces and urine until after week 12 in bedding sentinels representing immunocompetent donors. This makes the use of bedding sentinels questionable for monitoring of immunocompetent colonies and may need to be held for 4-6 months for efficient detection.

PS26 Indicators of Postoperative Pain in Syrian Hamsters (*Mesocricetus auratus*)

AM Edmunson,[1,*] FD Duke Boynton,[1] AK Rendahl,[2] A Merley,[1] N Koewler,[1] ML Dunbar,[3,1] CP Sivula[1]

[1]Research Animal Resources, University of Minnesota, Minneapolis, MN; [2]College of Veterinary Medicine, University of Minnesota, Saint Paul, MN; [3]Animal Resource Center, University of Texas Southwestern Medical Center, Dallas, TX

Despite their use in research, little is known about the evaluation of pain in Syrian hamsters (*Mesocricetus auratus*). This study investigated whether behavior frequency, grimace scale, the treat-take-test proxy indicator, body weight, water consumption, and coat appearance would be affected by a laparotomy, and therefore, could be used to recognize postoperative pain in a research setting. Nineteen Syrian hamsters of LVG stock were allocated to 1 of 3 intervention groups that underwent either a laparotomy under isoflurane anesthesia without analgesia, anesthesia only, or no intervention. An ethogram was constructed and used to record pain, active, or passive behavior frequency using in-person and remote video recording observation methods. The Syrian Hamster Grimace Scale (SHGS) was developed for the evaluation of facial features (action units) before and after the surgery. The treat-take-test assessed whether surgery would affect the animals' motivation to take a high-value food item from a handler. The hypothesis was that behavior frequency, grimace scale, treat-take-test score, body weight, water consumption, and coat appearance would change from baseline after surgery, but not in the control groups. Hamsters in the surgery group showed a modest change in pain and passive behavior frequency from baseline on the day of surgery, as well as 2 d and 3 d after surgery. This difference was not seen in the anesthesia-only and control groups. The SHGS score increased 46–595% after surgery from baseline in 3 of 9 animals studied. Overall, observing 1 or more pain behaviors to identify animals in the surgery group was highly specific (>95%) but poorly sensitive (</=50%). Using a SHGS score of 1.25 or higher to identify painful animals had a specificity of 94.4% and a sensitivity of 27.8%. The treat-take-test scores, body weight, water consumption, and coat appearance did not change from baseline. The findings of this study indicate that the methods assessed were not effective for identifying Syrian hamsters experiencing postoperative pain. More research is needed on clinically relevant strategies to analyze pain in Syrian hamsters.

PS27 Anti-G-CSF Antibody Does Not Prevent Bone Marrow B Cell Loss in Murine Norovirus Infected Stat1-/- Mice

D Eldridge*, K Mears, C Hsu

Comparative Medicine, University of Washington, Seattle, WA

Murine norovirus (MNV) is highly prevalent in laboratory mice and can infect macrophages, dendritic cells, T cells, and B cells. MNV infection in *Stat1*$^{-/-}$ mice (129S6/SvEv-*Stat1*^{tm1Rds}) causes a significant depletion of B cell populations in the bone marrow (BM). Concurrent with B cell depletion, MNV-infected Stat1-/- mice have a significant increase in serum granulocyte colony stimulating factor (G-CSF), as well as BM granulocytes and macrophages, suggesting that granulopoiesis may play a role in B cell losses observed after infection. We previously reported that anti-G-CSF antibody treatment for 7 days in MNV infected *Stat1*$^{-/-}$ mice partially prevented the depletion of BM B cells seen after infection, although these results were not statistically significant. In this study, we hypothesized that a longer treatment course of anti-G-CSF antibody (14 days would significantly rescue BM B cell losses by preventing increases in BM granulocytes and macrophages. To test this hypothesis, 5- to 9-week-old female *Stat1*$^{-/-}$ mice (n = 5) were infected with MNV and administered anti-G-CSF antibody (10 ug/mouse) daily via intraperitoneal (IP) injection. Control groups consisted of MNV infected (n = 5) and uninfected (n = 5) mice administered isotype IgG control antibody (10 µg/mouse) daily by IP injection. At 14 days post-infection, BM was evaluated by flow cytometry. As expected, MNV infected IgG treated control mice had significantly decreased ($P < 0.05$) BM B cells and significantly ($P < 0.05$) increased granulocytes and macrophages compared to uninfected controls. MNV infected mice treated with anti-G-CSF showed a significant decrease in the number of BM granulocytes ($P = 0.0008$) and macrophages ($P = 0.02$) compared to MNV infected IgG treated controls. Surprisingly, anti-G-CSF treated mice still had a significant loss of all BM B cell subsets ($P < 0.05$), and were similar to the B cell losses seen in MNV infected IgG treated controls. Therefore, contrary to what was previously thought based on treatment with anti-G-CSF antibody for 7 days, our current findings after 14 days of treatment indicate that B cell losses during MNV infection are not dependent on increased granulocytes or macrophages in the BM, but rather are caused by another indirect mechanism that warrants further investigation.

PS28 Impact of Differing Gut Microbiota Transfer Methods on Model Phenotypes

K Gustafson,[1,2,*] A Ericsson,[1,2] CL Franklin,[1,2]

[1]Veterinary Pathobiology, University of Missouri, Columbia, MO; [2]Comparative Medicine Program, University of Missouri, Columbia, MO

The gut microbiome (GM) plays an essential role in the modulation of various disease processes as well as animal model phenotypes of disease. Assessing its role in model phenotypes requires the ability to define and efficiently manipulate (transfer) the GM. Our current study sought to assess the efficiency of 3 common microbiome transfer methods and whether these differing methods resulted in changes in the phenotype of acute dextran sodium sulfate (DSS) colitis. C57BL/6 mice from 2 separate vendors known to have different GM richness were used as gut microbiome recipients to evaluate the gut microbiome transfer efficiency of embryo transfer, cross fostering, and cohousing. Each cohort contained 24 mice, 12 males and 12 females. Feces were collected at 3, 7, and 9 wk of age for metagenomic analysis. Mice were treated with a 7-d administration of DSS, 25mg/ml in free choice water, starting at 7 wk of age. Body

weights were collected from 7–9 wk of age during and following administration of DSS, and colon lengths collected at necropsy at 9 wk of age. Endpoints for this study included mice 9 wk of age, or mice that lost 20% or greater of their week 7 pre-DSS weight during DSS treatment. Results indicated that recipients of a high richness GM via both cross-fostering and cohousing had significantly longer colon lengths at necropsy ($P < 0.001$), and consistently less weight loss during treatment with DSS compared with mice that were recipients of a low richness GM. Interestingly, mice receiving a high richness microbiome via cohousing had a 96% survival rate at 9 wk of age, while mice receiving a low richness microbiome via cohousing had an 8% survival rate at 9 wk of age, while no such differences were observed in the cross-fostered groups. Determining the reasons for these discrepancies awaits further study, and highlights the complex interplay between recipient GM richness, substrain, and method of transfer. This research will provide the scientific community with resources necessary to determine the best method for transferring GM for their research.

PS29 Evaluation of Goblet Cell-associated Antigen Passages and Tolerogenic Dendritic Cells in the Cystic Fibrosis Mouse Intestine

SM Young,[1,*] R Woode,[2] NM Walker,[3] L Clarke,[2]

[1]Office of Animal Research , University of Missouri, Columbia, MO; [2]Biomedical Sciences-Dalton Cardiovascular Research Center, University of Missouri, Columbia, MO; [3]Surgery-Administration, University of Missouri, Columbia, MO

Greater than 70,000 individuals worldwide are living with the monogenetic disease cystic fibrosis (CF). The development of dysbiosis and chronic intestinal inflammation is a poorly understood common occurrence in CF patients. Prior research utilizing intestinal organoids (mini-guts) cultured from Cftr knockout mice has shown that goblet cells in the CF mouse intestine demonstrate defective clearance of mucin granules and abnormal mucus retention. Goblet cell-associated antigen passages (GAPs) provide delivery of intraluminal antigens to antigen-presenting dendritic cells in the submucosa, an important step in the development of tolerogenic dendritic and regulatory T cell activation. We hypothesized that mucus plugging of goblet cells in the CF intestine leads to defective GAP function and decreased production of tolerogenic dendritic cells. To test this hypothesis, Cftr KO-WT sex-matched littermate pairs (n = 2) maintained on a commercially available oral nutritional supplementation were anesthetized with ketamine/xylazine for a laparotomy to inject a luminal fluorescent dextran dye into the mid-jejunum. After 30 min, the mice were euthanized with CO_2, and the intestine was collected for immunofluorescent staining to evaluate GAP formation. In the WT, the dextran dye was observed within the goblet cells outlined by CK18 immunofluorescence, a goblet cell marker, with punctate dextran dye in the submucosa, indicative of dendritic cell uptake. In contrast, the Cftr KO mice showed dye only on the luminal surface of goblet cells, indicating defective GAP formation/function. To evaluate the population of tolerogenic dendritic cells, intestinal segments from Cftr KO-WT sex-matched littermate pairs (3-female and 2-male pairs) were collected for FACS sorting of submucosal CD103+ (tolerogenic) and CD103- (inflammatory associated) dendritic cells. The WT mice were found to have significantly higher percentage of CD103+ tolerogenic dendritic cells compared to the CF mice (WT:20.5+/-2, CF:9.2+/-3, $P < 0.006$). A trend towards an increase in CD103- dendritic cells were seen in the CF mice compared to WT littermates. In summary, the CF mice were found to have defective intraluminal antigen transfer through the GAP pathway and a significant decrease in tolerogenic dendritic cells.

PS30 Bile Acid Composition Contributes to Metabolic Improvements after Sleeve Gastrectomy in Mice

J Tu[1,3,*] Y Liu,[2] L Ding,[2] E Zhang,[2] Z Fang,[2] L Jin,[2] W Huang,[2,3]
[1]Division of Comparative Medicine/Diabetes Complications and Metabolism, City of Hope National Medical Center, Duarte, CA; [2]Diabetes Complications and Metabolism, City of Hope National Medical Center, Duarte, CA; [3]Irell & Manella Graduate School of Biological Sciences, City of Hope National Medical Center, Duarte, CA

Obesity and type 2 diabetes (T2D) can be surgically managed by vertical sleeve gastrectomy (VSG), one of the most frequently performed bariatric surgeries. Although VSG can effectively reverse obesity and T2D, the underlying mechanism of how VSG does so is still largely unknown. Besides metabolic improvements, we found that VSG downregulated a bile acid synthesis enzyme in the liver named CYP8B1. To investigate whether or not CYP8B1 mediates the metabolic effects of VSG, we performed VSG and sham surgery on wildtype mice and mice that overexpressed CYP8B1 (Tg) after high-fat diet feeding for about 10 wk (n = 4–5 per group, C57BL/6 males, 6–7 wk old). The results show that although VSG induced weight loss and improvement in glucose tolerance in wildtype mice, VSG did not improve the same parameters in Tg mice. Along with the downregulation of CYP8B1 after VSG, the serum bile acid composition in wildtype mice was altered significantly between the sham and VSG groups. Because changes in gut microbiota community are associated with changes in bile acid compositions, and vice versa, we performed 16s rRNA analysis on the fecal samples from the mice. The analysis revealed that while the gut microbial diversity shifted significantly between wildtype mice in the sham and VSG groups, such shift was not observed between the gut microbial communities of the Tg mice in the sham and VSG groups. In conclusion, our study suggests that CYP8B1 could be an important mediator downstream of VSG by altering bile acid and gut microbiota profiles. Future studies will include strategies to define the interaction between gut microbiota and bile acid in the context of VSG.

PS31 Evaluation of Various Individually Ventilated Cage Systems Based on Mouse Reproductive Performance and Husbandry and Environmental Parameters

MG Stover,* JS Villano

Johns Hopkins University, Baltimore, MD

Various individually ventilated caging (IVC) systems are marketed for improving the health and management of mouse colonies. The purpose of this study was to compare mouse reproductive performance and husbandry and environmental parameters among 3 high-density IVC rack systems (RS1-3), which were placed in their own designated rooms. Three breeding trios each of Swiss Webster (CFW) and BALB/c mice were placed in each room (n = 36 females, n = 18 males). Measured reproductive indices for 2 breeding cycles included time to parturition, interbirth intervals, litter size, and average pup weight at 7 d (P7) and 21 d (P21, weaning) post-parturition. Over 18 wk, husbandry personnel used scoring systems to evaluate RS daily to every other week based on cage dirtiness, need for spot changing, and ease of cage changeout, daily health checks, and cage wash processing. Macroenvironmental parameters (temperature, humidity, noise, total particulate matter) were measured weekly over the same period. Microenvironmental parameters (temperature, humidity, NH3, CO2, O2) of 2 cages each of male and female CFW mice (4 mice/cage) per RS were measured at 6 timepoints during a 2-wk cage changeout cycle. RS1 had significantly smaller litter sizes of CFW mice (mean=5) when compared to RS3 (mean = 10). Average time to parturition (25, 33, 33 d for RS1-3, respectively), P7 pup weight (4.9, 5.2, 5.1 grams for RS1-3, respectively), P21 pup weight (10.2, 10.4, 11.1 grams for RS1-3, respectively), and interbirth intervals (45, 38, 36 days for RS1-3, respectively) were not significantly different. RS1 scored significantly easier to change and process through the cage wash; RS2 scored significantly easier to do health checks on; and RS3 had significantly lower room noise levels (mean = 46 ± SD = 5 dBA). Although cage humidity for all RS fell within an acceptable range, RS3 cages had significantly higher average humidity levels (59%) when compared to both RS1 (44%) and RS2 (46%), especially at 8 and 12 d after cage

change. In conclusion, each system had at least 1 advantage over the other 2 when comparing mouse reproductive performance and husbandry and environmental parameters. Therefore, various factors need to be carefully considered when choosing an IVC system for mice.

PS32 The Influence of Daytime LED Light Exposure on Sprague Dawley Rats

AA Allen,[1,*] AT Pierce,[1] RT Dauchy,[2] GB Voros,[1] GL Dobek,[1]

[1]Comparative Medicine, Tulane University, New Orleans, LA; [2]Structural & Cellular Biology, Tulane University, New Orleans, LA

Light can significantly influence regulation of circadian rhythms of physiology and metabolism in laboratory animals. Light-emitting diode (LED) light is a rapidly emerging technology, yet little is understood regarding its impact on animal circadian, neuroendocrine, and neurobehavioral responses. Previous studies from our laboratory demonstrated that daytime exposure to LED light enriched in the blue-appearing portion (465–480 nm) of the visible spectrum (bLAD), compared to broad spectrum (300–740 nm) cool white fluorescent (CWF) light, enhances these responses in mice. The effects were mediated via photic innervation in part of the rod/cone system of the primary optic tract (POT; visual system), but more so by innervation of the intrinsically photosensitive retinal ganglion cells (ipRGCs) of the retinohypothalmic tract (RHT; non-visual system). We hypothesize that exposure of male and female Sprague Dawley rats to bLAD, compared to CWF light, innervates the POT and RHT systems. Control animals (n = 72) were maintained on a standard IVC system under CWF light, whereas experimental animals (n = 72) were housed on a light-controlled IVC system under bLAD light. Both groups were housed for 30 d under a common lighting regimen 12L (68.8 ± 5.2 lux; 168.6 ± 12.8 µW/cm2):12D (0 lux), and were assessed for photic innervation of both the POT and RHT systems. Results revealed that photon flux, the number of photons per second per unit area (cm2/s), across the retina of rats maintained under bLAD versus CWF lighting was nearly identical at 1.32E+15 and 1.35E+15, respectively, as well radiometric (476 – 484 µW/cm2) and photopic illuminance (1534 – 1721 lux) measures of light intensity. In contrast, retinal photopigment stimulation of the visual POT rod and cone system was enhanced significantly ($P < 0.001$) by nearly 20% in bLAD, compared to CWF light. Stimulation of the non-visual RHT (ipRGC) system was elevated by nearly 30% ($P < 0.001$) in rats maintained under bLAD, compared to CWF lighting environment. These data show that daytime exposure of Sprague Dawley rats to bLAD, compared to CWF light, positively enhances both the POT visual and RHT non-visual optic systems influencing the circadian regulation of neuroendocrine, metabolic, and physiological parameters.

PS33 PCR Testing of Media Placed in Soiled Bedding as a Method for Mouse Colony Health Surveillance

W Hanson,* K Taylor, DK Taylor

Division of Animal Resources, Emory University, Atlanta, GA

Rodent colony health surveillance has traditionally been accomplished by testing sentinel animals exposed to soiled bedding from colony animals. Recently, sampling exhaust plenums on ventilated caging systems followed by PCR analysis has emerged as a promising method. However, environmental testing at the rack level is not effective for all ventilated rack designs. In this study, we aimed to determine whether media placed in soiled bedding is effective in detecting three adventitious agents: mouse norovirus (MNV), *Helicobacter* spp., and fur mites. Soiled bedding was collected from agent-positive colony animals and distributed to traditional sentinel mouse cages and mouse-free experimental cages every 1–2 weeks for static and ventilated cages, respectively. Experimental cages contained 10 flocked swabs ('passive swabs') and 1 piece of filter media. After 90 days, fresh feces, pelage swabs, and blood were collected from the sentinel cages; passive swabs and filter media were collected from the experimental cages. Concurrently, 10 additional flocked swabs ('active swabs') were stirred through the cumulated soiled bedding of each experimental cage. Sentinel mice were positive for MNV and *Helicobacter* spp. but negative for fur mites by pelage swab PCR. For the experimental cages, all samples were positive for *Helicobacter* spp. and fur mites in both caging types. For MNV, passive swabs were most effective at detection (100%) followed by active swabs (80–100%) and filter media (60–80%). These findings suggest that testing of media placed in pooled soiled bedding samples is feasible and more effective than our traditional sentinel methods for colony health surveillance. In cases where sampling at the rack level is ineffective, this approach may be a valuable option for reducing animal use.

PS34 Differences in Strain Susceptibility and Shedding of Murine Chapparvovirus in CD-1, C57BL/6, and NSG Mice

ML Kain,[3,*] S Monette,[1] RJ Ricart Arbona,[4] KS Henderson,[2] RK Dhawan,[2] M Wunderlich,[2] NS Lipman,[4]

[1]Laboratory of Comparative Pathology, MSK, WCM, The Rockefeller University, New York, NY; [2]Charles River Laboratories, Wilmington, MA; [3]Tri-Institutional Training Program in Laboratory Animal Medicine and Science, New York, NY; [4]Center of Comparative Medicine and Pathology, MSK & WCM, New York, NY

Murine Chapparvovirus (MuCPV), the etiological agent responsible for a long-recognized inclusion body nephropathy syndrome in mice, has been identified in laboratory mice in several countries with an estimated prevalence of 9%. This virus can cause significant renal pathology, morbidity, and mortality in immunodeficient strains, and subclinical infection in immunocompetent mice. We investigated viral infectivity and shedding in CD-1, C57BL/6 (B6), and NOD scid gamma (NSG) mice. Four viral doses (ranging from 1.16 x 103- 1.16 x 106 viral copies/uL) were used to infect 8 mice per strain (2 mice per dose) via oronasal inoculation. A persistent infection with prolonged shedding was found in all 3 strains, detected by quantitative PCR. CD-1 mice shed virus earliest (4 weeks post-inoculation) and at high numbers (>1 x 107 viral copies/ul) in both the urine and feces. B6 mice shed virus later (7 wk post-inoculation), with lower average viral numbers (up to 1 x 106 viral copies/uL). Peak shedding was observed 11-14 wk after inoculation of CD-1 and B6 mice, after which MuCPV levels gradually decline until 6 mo after infection (last time point assessed). There was a 3–4 wk delay in the onset of shedding in the NSG mice (10 wk post-inoculation), despite the NSG mice showing more severe histopathological lesions at the time of first shedding. NSG mice continue to shed virus in high numbers (>1x 107 viral copies/uL) in the urine for 6 mo post-inoculation. For all strains, we found consistent urinary, but not fecal shedding, even during peak infection. There were clear differences in strain susceptibility, with the CD-1 outbred stock requiring the lowest initial viral dose (1.16 x 103 viral copies/uL) for productive infection. These results have practical applications for the design of effective screening programs to detect this virus in laboratory colonies.

PS35 Pulmonary Delivery of Test Articles by Intratracheal Nebulization or Liquid Instillation in Small Animal Models: implications for Covid19 Therapeutic Testing

A Werts,* T Yellowhair, P Kuehl

Lovelace Biomedical, Albuquerque , NM

COVID19 has increased global interest in methods to deliver test articles (TAs) (e.g. AAVs, siRNAs, proteins, small molecules, etc.) locally to the lung parenchyma. Ferrets are attractive preclinical models for this purpose as these animals are permissive to SARS-CoV-2 infection, and they have both similar pulmonary physiology and clinical signs to humans when infected by multiple respiratory viruses. Nose-only inhalation exposures are a standard method

for TA delivery in ferrets and other small animal species; however, researchers are often interested in less complex methods of TA lung delivery, such as intratracheal (IT) administration. Here we ask whether nebulization of a TA offers advantages over simple liquid delivery when both are delivered intratracheally. Using simulated and in vivo models, we tested the delivery and distribution of a reporter TA (i.e. 2% Evans blue dye in saline) administered through a clinical syringe-activated IT nebulizer, with or without the nebulizer tip in place. The simulated trachea model comprised an endotracheal tube; the in vivo model comprised anesthetized ferrets (n = 10). Dye distribution was photographically recorded. Simulated trachea tests showed that confining the nebulizer tip in a small space results in significant rainout and liquid generation. In vivo tests demonstrated inconsistent, patchy delivery to the pulmonary parenchyma, independent of device modification status and position of the animal at dosing. In conclusion, 1) nebulization of a TA in the simulated and in vivo ferret trachea resulted in precipitation of the TA and ultimate delivery as a liquid; 2) IT delivery of dye by nebulization or liquid resulted in similar pulmonary distribution in the lungs of ferrets; and 3) compared to IT delivery, external nebulization – as in the standard nose-only exposure system – likely results in more consistent and reliable TA distribution in the lungs of ferrets and other small animal models.

PS36–38 Withdrawn

PS39 Development of a COVID-19 Multiplex for Serological Screening of Nonhuman Primate Colonies

RK Dhawan,* M Wunderlich, L Campbell

Charles River Laboratories, Wilmington, MA

In the fall of 2019, a novel beta coronavirus was detected in humans in Wuhan, China. The infection spread globally causing a pandemic in the human population. This novel virus is most closely related to severe acute respiratory syndrome (SARS-CoV aka SARS-CoV1), which spread globally in 2002–2004, and has been formally named as SARS-CoV2 or more commonly referred to as COVID19. A new high throughput COVID-Plex serology assay has been developed that utilizes 6-antigen coupled beads, 2 specific for detecting SARS-CoV2 antibodies and 1 antigen for each of the 4 human seasonal coronavirus strains (229E, NL63, HKU1 and OC43). Recombinant SARS-CoV2 proteins for the full length spike (S1 + S2 subunits) and nucleoprotein (NP) as well as full length spike proteins for 229E, NL63, HKU1 and OC43 were coupled to Luminex magnetic beads. Samples were interpreted as COVID19 antibody positive if both SARS-CoV2 beads (spike and NP) scored above the assay cutoffs. Sensitivity of the COVID-Plex assay was assessed by using 50 positive human sera with 47/50 scoring positive for COVID antibodies. Three negative samples either scored only on the alpha coronavirus seasonal strain beads or completely negative on all 6 coupled beads (COVID as well as the 4 seasonal spike protein coupled beads). These samples were also confirmed negative by a commercial COVID19 spike protein ELISA. Specificity of the assay was tested by screening pre-2019 human and macaque samples with 0/8 and 0/24 positive samples, respectively. Recently collected sera in 2020 from rhesus and cynomolgus macaque colonies (n=322) representing several different institutions resulted in only one positive finding resulting in 99.7% assay specificity. These studies confirm that COVID-Plex, a blood based test using serum/plasma is sensitive and specific for screening of COVID19 (SARS-CoV2) antibodies in NHPs. Also the COVID-Plex can be performed in a user-friendly and high throughput format while screening for other infectious SPF agents using the same sample.

PS40 Comparative Genomic Analysis of Novel *Helicobacter pylori* Strains Isolated from Domestic Cats with Gastritis Reveals a Unique Genetic Profile that May Contribute to Persistent Colonization and Pathogenicity in Nonhuman Hosts

A Mannion,* Z Shen, JG Fox

DCM, MIT, Cambridge, MA

While *Helicobacter pylori* (*Hp*) is primarily a human-specific pathogen, natural infection has been detected in domestic, commercially available cats with chronic gastritis. Experimental inoculation of these *Hp* strains isolated from cats recapitulated gastritis in cats and mice. Factors that influence host specificity for *Hp* infection are under active investigation; the aim of this study was to perform comparative genomic analyses of *Hp* strains from domestic cats to identify features that may influence *Hp* colonization and pathogenicity in nonhuman hosts. Genomes from 4 representative *Hp* strains isolated from the naturally colonized cats and a mouse-passaged *Hp* cat strain were compared against 257 *Hp* genomes from human, macaque, mouse, and gerbil natural or experimental hosts. Pan-genome phylogeny showed the *Hp* cat genomes formed a distinct clade that neighbored strains B38, 29CaP, and G-Mx-2006-583 isolated from human patients with gastritis and gastric cancer and was distant to clades containing other non-human *Hp* strains. Average nucleotide identity showed that the *Hp* cat genomes were nearly identical (>99.7%) to each other and were distinct from other strains (~95%). The pan-genome for the *Hp* cat genomes contained 1142 core and 519 accessory genes.Twenty-eight genes were unique to cat isolates, but were hypothetical annotations. Many genes were truncated, elongated, or fragmented due to mutations, especially in polynucleotide repeats regions, which may modify or inactivate gene products. Methyltransferase genes were often disrupted and may alter DNA modifications that influence DNA-protein interactions, such as gene transcription. Outer membrane genes that act as adhesion and immune modulating virulence factors as well as transporter and metabolic genes were also disrupted. Vacuolating cytotoxin subtype s1-il/i2-m2 was present in the *Hp* cat genomes, but these strains did not cause vacuolation to gastric epithelial cells in vitro, indicating gene inactivity. The cag pathogenicity island, a type IV secretion system that promotes gastric cancer, was absent in the *Hp* cat genomes. Results from this study suggest *Hp* strains from cats have a unique genetic profile that may be responsible for feline *Hp* strains to persistently colonize and cause gastritis in non-human hosts.

PS41 Comparison of the Fecal Bacterial Microbiota of Rhesus Macaques (*Macaca Mulatta*) by Housing Type and Health Status

NR Compo,[1,2,*] L Mieles Rodriguez,[2] D Gomez,[3]

[1]SoBran BioSciences, Inc, Norfolk, VA; [2]Caribbean Primate Research Center, Sabana Seca, Puerto Rico; [3]University of Guelph, Guelph, , Canada

An ongoing clinical challenge to nearly every primate facility in North America is chronic idiopathic diarrhea. Despite extensive study, little is known regarding the pathogenesis of CID; however, wild macaques appear resistant to it, a trend we observe in our free-ranging population. The gastrointestinal microbiota has been shown to have a significant role in the pathogenesis of disease and in maintaining normal health and development of the gut. In humans, chronic diarrhea due to *Clostridium difficile* infection is associated with alteration of the gut microbiota, which shows lower bacterial diversity compared to that of healthy humans. The goals of the current study were to describe and compare the fecal bacterial microbiota of corralled healthy and corralled CID macaques and healthy, free-ranging animals. After meeting specific criteria for study inclusion, fecal samples were collected from healthy (HS; n = 30) and CID (n = 27) corralled rhesus macaques and from healthy macaques in our free-ranging colony, Cayo Santiago (CS; n = 43). An animal was not considered if it had received antibiotics in the preceding 90 d (60 for CID animals). Bacterial DNA was extracted and the V4 region of the 16S rRNA gene was sequenced and compared to known databases. The relative abundance (RA) of Proteobacteria and *Lactobacillus* was higher and lower, respectively, in CID animals than HS animals (P = 0.001 and 0.042, respectively), but there were

otherwise few differences between these 2 groups in RA or alpha- and beta-diversities. Similarly, principal coordinate analysis (PCoA) showed that fecal bacterial composition clustered by housing site, but not health status. Healthy animals from CS were differentially enriched with members of phylum Firmicutes and class Bacilli (LDA scores >4.5), generally associated with a 'healthy' gut in humans, while CID animals were enriched with Proteobacteria (LDA score = 4.3), which has been associated with dysbiosis in various species. These results indicate that both environment and health status influence the gut flora, though the former likely has a stronger impact. Our results offer preliminary data for potential clinical interventions, such as probiotics and fecal transplants.

PS42 The Use of Gnotobiotic Piglets as a Model for Human Norovirus (HuNoV) Infection and Recombinant PIV5 Vector Vaccine Efficacy

Z Chen,[1] Y Ma,[2] J Risalvato,[*1] Z Li,[1] H Wei,[1] J Zengel,[1] M Lu,[2] A Li,[2] X Liang,[2] E DiCaprio,[2,3] R Jennings,[2] A Niehaus,[4] J Li,[2] B He,[1]

[1]Department of Infectious Disease, College of Veterinary Medicine, University of Georgia, Athens, GA; [2]Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH; [3]Department of Food Science and Technology, University of California Davis, Davis, CA; [4]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH

Human norovirus (HuNoV) is the leading causative agent of acute nonbacterial gastroenteritis worldwide. The CDC estimates 900,000 pediatric visits and 64,000 hospitalizations due to HuNoV infection occur annually in the US. Unfortunately, in developing nations HuNoV causes approximately 200,000 deaths of children under 5. Currently, there is no vaccine available for HuNoV. Parainfluenza virus 5 (PIV5) has promise for use in recombinant vector vaccine development. We hypothesized that a recombinant PIV5 expressing VP1 major capsid protein of a human NoV GII.4 strain (GenBank FJ537136) would be protective against challenge in gnotobiotic piglets. Due to their developmental and immunological similarity to the human gastrointestinal system, gnotobiotic piglets were selected as an infection and vaccination model. Twenty-seven gnotobiotic piglets were delivered into a sterile environment via Cesarean section from a specific-pathogen-free gravid sow and housed in germfree isolation units. The PIV5-VP1 vaccine was administered intranasally to mixed-sex 2-d-old gnotobiotic piglets. No clinical signs of infection were noted. Blood and fecal samples for evaluating VP1-specific IgG and IgA (respectively) were collected weekly post-vaccination. PIV5-VP1 generated robust HuNoV-specific IgG and IgA responses in the gnotobiotic piglets. At 3 wk post-vaccination, piglets were orally challenged with 108 genomic RNA copies of HuNoV GII.4 strain 765 (GenBank JX126912.1). After challenge, rectal swabs were collected, and diarrhea scores recorded daily. Fecal samples were also used to determine viral shedding. All piglets were sacrificed at day 5 post-challenge, and intestinal segments (duodenum, jejunum, ileum, and colon) collected for gross histopathology. PIV5-VP1 immunized piglets were protected against HuNoV challenge: immunized animals had minimal HuNoV antigen detected in the small intestine and a 1.8 log reduction in HuNoV RNA shedding in feces after infection compared to unvaccinated control piglets. Gnotobiotic piglets are a potentially effective model for HuNoV infection, and PIV5-VP1 shows promise as an effective vaccine candidate against HuNoV.

PS43 Comparison of Laboratory Disinfectants in their Effect on the Mouse Gut Microbiota

JD Sciurba,[*1] GE Chlipala,[2] SJ Green,[3] MA Delaney,[4] JD Fortman,[1] JE Purcell,[1]

[1]Biologic Resources Laboratory, University of Illinois at Chicago, Chicago, IL; [2]Research Informatics Core, Research Resources Center, University of Illinois at Chicago, Chicago, IL; [3]Genome Research Core, Research Resources Center, University of Illinois at Chicago, Chicago, IL; [4]Zoological Pathology Program, College of Veterinary Medicine, University of Illinois, Brookfield, IL

The mouse model has proven to be an invaluable tool for investigating the complex biologic mechanisms in which the gut microbiota (GM) interacts with organ system function throughout the body. Biomedical research studies using mice have demonstrated great potential in the ability of GM to alter phenotype. Therefore, factors that result in unanticipated alterations in GM can be confounding variables. Seemingly innocuous changes in husbandry, including differences in diet or housing environment, may have significant effects on mouse GM. One such common husbandry practice to consider is the application of disinfectant chemicals to gloved hands for aseptic handling of specific pathogen-free laboratory mice. The effect of exposing mice to disinfectants used in this manner is not known. The purpose of this study was to compare 4 different disinfectants in their potential to alter the mouse GM. Eight-wk-old female C57BL/6 mice were exposed daily for 27 consecutive d to either sterile water, 70% ethanol, or 1 of 3 commercially available disinfectant products which use chlorine dioxide, potassium peroxymonosulfate, or accelerated hydrogen peroxide (n = 9 mice/ group). Disinfectant or water was applied at a volume which would be reasonably transferred through standard mouse handling procedures. Throughout the course of these daily exposures, fecal pellets were collected and immediately flash frozen in liquid nitrogen every 7 d, until day 28, at which point mice were euthanized for collection of ceca. DNA extractions were performed on all cecal and fecal samples, and subsequently genomic fragments of microbial 16S ribosomal RNA genes were PCR amplified for deep microbiome amplicon sequencing. Microbial alpha and beta diversity was examined across disinfectant groups, and it was found that the 3 commercial disinfectants had the strongest effect on the microbial community. This included significant decreases in overall diversity compared with baseline fecal samples and significant dissimilarity compared both to baseline fecal samples, as well as compared to the water control group for cecal and fecal samples at day 28. This research suggests that choice of laboratory disinfectant should be done with consideration of the potential to impact GM.

PS44 Effects of Cisapride, Buprenorphine, and Their Combination on Gastrointestinal Transit in New Zealand White Rabbits

ER Feldman,[*1] B Singh,[1] N Mishkin,[1,2] E Lachenauer,[1] M Martin-Flores,[3] EK Daugherity[1]

[1]Center for Animal Resources and Education, Cornell University, Ithaca, NY; [2]College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO; [3]Department of Clinical Sciences, Cornell University, Ithaca, NY

Opioids are worthy of consideration for postoperative pain management in rabbits due to their effective analgesic properties. However, this class of drugs has undesirable effects which warrant caution in this species. Opioids are known to significantly decrease gastrointestinal (GI) motility, reduce fecal output, and delay GI transit times; thus, increasing the risk of a more serious condition known as GI stasis or ileus. The risk of ileus discourages the use of opioids in rabbits, directly impacting animal welfare. Gastroprokinetic agents such as cisapride are effective in promoting gastric emptying in many species, but it is not known whether this effect occurs in rabbits. This study assessed efficacy of cisapride in rabbits by measuring GI transit times, fecal output, daily body weights, and food and water intake when administered as a single agent, and in combination with buprenorphine. Ten healthy New Zealand White (NZW) rabbits were studied in a crossover, randomized design, and received 4 treatments: vehicle and buprenorphine (0.03 mg/kg SC), cisapride (0.5 mg/kg oral) and saline, cisapride and buprenorphine, or a vehicle and saline control every eight hours for 2 d. Rabbits were fed a pelleted diet during the study period. Rabbits were anesthetized with isoflurane and administered radio-opaque, barium-filled spheres via orogastric

tube. Feces was assessed via radiography for detection of the barium-spheres to determine GI transit time. GI transit time, food and water intake, fecal output, and body weight were analyzed using a linear mixed effects model. GI transit time was significantly longer for buprenorphine groups than control groups, regardless of receiving cisapride treatment. Fecal output and food and water intake were lower for buprenorphine groups than control groups. Cisapride did not significantly alter GI transit, fecal output, or food and water intake. Additionally, treatment group did not significantly affect body weight. In conclusion, treatment with three times daily buprenorphine increased GI transit time, and decreased fecal output, and food and water consumption. Co-administration with cisapride did not ameliorate negative side effects, and administration of cisapride alone did not appear to affect GI motility.

PS45 The Use of Midazolam as an Appetite Stimulant and Anxiolytic in the Common Marmoset (*Callithrix jacchus*)

JA Herrod,[*1] J Avelino,[3,1] K Schonvisky,[1] J Lynch,[2] EK Hutchinson,[1] JM Izzi,[1]

[1]Molecular and Comparative Pathobiology, Johns Hopkins University, Baltimore, MD; [2]Biomedical Engineering - Neuroengineering, Johns Hopkins University, BALTIMORE, MD; [3]College of Veterinary Medicine, NC State University, Raleigh, NC

The common marmoset, *Callithrix jacchus*, is an important translational model with increasing popularity and maintaining the health and welfare of these animals in the laboratory environment is imperative. Due to their small body size and high metabolic rate, food avoidance secondary to disease or stress can quickly lead to marked weight loss or other adverse clinical effects, including impaired wound healing, weakened immune function, and catabolism. Successful treatment of anorexia in this species can be challenging and, though pharmacologic treatment of anorexia in other species is common, there is currently no evidence supporting the use of any drug as an appetite stimulant in marmosets. In this study, we investigated the use of the benzodiazepine midazolam as an appetite stimulant and anxiolytic in the common marmoset. We hypothesized that midazolam would increase appetite in both normal and induced-anxiety states. Three female and 3 male healthy adult marmosets were divided into 3 treatment groups and a placebo group in a cross-over study design. Midazolam (1mg/kg, 2mg/kg, 2.5mg/kg) and a commercially available flavored syrup placebo were administered orally in the home cage. Animals were offered feed and total food intake (TFI) and latency to eat (LTE) were recorded over a 1-h period and compared to baseline data. The study was repeated administering midazolam (2.5mg/kg) and placebo in a transport cage (induced-anxiety state). Results indicated that oral midazolam did not significantly increase TFI or significantly reduce LTE in either normal (Friedman test; TFI: P = 0.5, LTE: $P = 0.4$) or anxious states (Wilcoxon test; TFI: $P = 0.4$, LTE: $P = > 0.9$). Still, data show that higher doses of midazolam did increase appetite and reduce time to eat in some animals, which is comparable to the use of appetite stimulants in other species where individual response is variable and dose-dependent. In conclusion, midazolam should be considered as a potential adjunct therapy for anorexia in common marmosets, and further investigation into the dosage and use in unhealthy marmosets is warranted.

PS46 Pharmacokinetics of Single-Dose Intramuscular Buprenorphine in Common Marmosets (*Callithrix jacchus*)

NJ Fabian,[*1] DE Moody,[2] WB Fang,[2] JG Fox,[1] MA Burns,[1]

[1]Division of Comparative Medicine, Massachusetts Institute of Technology (MIT), Cambridge, MA; [2]Center for Human Toxicology, University of Utah, Salt Lake City, UT

Common marmosets (*Callithrix jacchus*) are used in many areas of biomedical research such as neuroscience and the development of transgenic non-human primate research models. Laboratory marmosets routinely receive buprenorphine intramuscularly (IM) or subcutaneously after surgical procedures. Although buprenorphine is the most frequently used opioid analgesic in marmosets, there is limited information in the literature supporting current dosing regimens used for this species. The purpose of this study was to determine the pharmacokinetic (PK) profile of single-dose buprenorphine HCl administered IM at 0.01 mg/kg in 6 adult marmosets (1.8–12.8 y old; 2 males, 4 females). Blood was collected at time points 0.25, 0.5, 1, 2, 4, 6, 8, 16, 20 and 24 h from non-sedated animals following a hybrid sparse-serial sampling design. Time points were split between 3 phases with 2-wk washout periods to accommodate the limited blood volume of marmosets. Plasma concentrations of buprenorphine were measured using liquid chromatography–electrospray ionization–tandem mass spectrometry. Non-compartmental pharmacokinetic analysis was performed. The maximum plasma concentration of buprenorphine (2.57 ± 0.95 ng/mL) occurred at 17.4 ± 6 min. Area under the curve was 2.4 ± 0.84 ng x h/ml, with a terminal elimination half-life of 1.48 ± 0.5 h and clearance of 4.19 ± 1.3 L/h/kg. The last quantifiable concentration was at 5 ± 1.67 h. These data suggest that marmosets should be re-dosed approximately every 4-6 hours when using buprenorphine at 0.01 mg/kg IM to maintain analgesia based on a theoretical therapeutic plasma concentration threshold of 0.1 ng/mL. Importantly, these results indicate that common dosing regimens (e.g., every 8-12 h) could be suboptimal, as plasma levels of buprenorphine may drop below the therapeutic threshold prior to redosing and thus provide inadequate analgesia. No adverse or clinical side effects were observed that could be attributed to study manipulations. Future PK studies involving buprenorphine at additional doses, frequencies, routes of administration, and formulations as well as pharmacodynamic studies are recommended to validate pain management practices for C. jacchus in the laboratory animal setting.

PS47 Reference Intervals for Total T4 and Free T4 in Cynomolgus Macaques (*Macaca fascicularis*) and Rhesus Macaques (*Macaca mulatta*)

JD Sciurba,[*1] JM Hayes,[2] SM Nouraie,[3] HM Wilson,[4] LC Halliday,[1] JD Fortman,[1]

[1]Biologic Resources Laboratory, University of Illinois at Chicago, Chicago, IL; [2]Pathology Services Unit, Wisconsin National Primate Research Center, University of Wisconsin-Madison, Madison, WI; [3]Division of Pulmonary, Allergy and Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA; [4]College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO

Thyroid diseases, associated with either increased or decreased concentrations of circulating thyroid hormones, are prevalent in both human and veterinary patient populations. Hypothyroidism in particular is a differential diagnosis for many medical problems as the disease presents with nonspecific clinical signs which can include lethargy, weight gain, cold intolerance, and dermatologic manifestations such as alopecia. Alopecia is a frequently reported problem in captive nonhuman primates (NHP) in which hypothyroidism has been considered to be a differential diagnosis. Thyroid function test results in NHPs using total T4 (TT4) and free T4 (FT4) assays have been difficult to interpret without accurate reference intervals (RIs) for comparison, and therefore it is possible that hypothyroidism is underdiagnosed in these species as a consequence. The objective of this study was to establish RIs for TT4 and FT4 in healthy populations of cynomolgus macaques (n=133, age range 2.6–24.7 y) and rhesus macaques (n=172, age range 0.8–31.0 years). Serum samples were collected longitudinally across a 14-y period during routine anesthetic events in clinically healthy animals, and TT4 and FT4 concentrations were measured using a commercially available immunoassay analyzer. The RIs established for TT4 and FT4 were 5.07–14.94 ug/dL and 0.48–1.17 ng/dL for cynomolgus macaques, and 3.89–14.66 ug/dL and 0.36–1.12 ng/dL

for rhesus macaques. Within these larger species groups, significant differences in thyroid hormone concentrations were found between Indian and Chinese origin rhesus, and between Mauritian and other origin cynomolgus. In addition, juvenile and subadult rhesus exhibited significantly elevated FT4 and TT4 concentrations. Individual RIs were established for these subgroups which displayed sufficiently different thyroid hormone concentrations. These results will allow for a more thorough diagnostic evaluation in cynomolgus and rhesus macaques with clinical signs consistent with thyroid disease and will ultimately be a refinement in animal welfare.

PS48 Effects of Pair Housing on Behavior, Cortisol, and Clinical Outcomes after Intrafacility Transfer and Acclimation in Rhesus Macaques (*Macaca mulatta*)

MN Jackson,*[1] M Truelove,[2] K Williams,[2] J Chen,[3] RH Moore,[3] JS Wood,[1] JK Cohen,[1] M Bloomsmith,[2]

[1]Animal Resources, Yerkes National Primate Research Center, Atlanta, GA; [2]Behavioral Management, Yerkes National Primate Research Center, Atlanta, GA; [3]Biostatistics Collaboration Core, Emory University, Atlanta, GA

Pair housing of rhesus macaques increases affiliative interactions, exploration, and other species-specific behaviors amid active research. Social buffering is also a benefit of compatible pair housing, in which individuals temper stress-induced reactions to environmental changes. However, it is not well reported whether these benefits are maintained after shipment to a new facility and throughout domestic quarantine, 2 major stressors that can lead to behavioral and clinical abnormalities. We evaluated the effects of pair housing on targeted behaviors, cortisol levels, and clinical outcomes immediately following an intrafacility transfer and throughout a 4-wk acclimation period in 40 male rhesus macaques. Behavioral assessments comprised 320 h of one-zero sampling data on single- and pair-housed subjects during the acclimation period. Fecal samples were collected weekly for fecal cortisol measures, while incidence of diarrhea and weights were recorded throughout. Pair-housed animals showed a significantly lower prevalence of some targeted behaviors as shown by a mixed effect model including hunched posture ($P = 0.003$), hair plucking ($P = 0.005$), self-clasping ($P < 0.001$), whole body stereotypies ($P = 0.037$), other stereotyped behaviors ($P = 0.017$), and fecal-directed behaviors ($P = 0.016$). No significant difference in cortisol levels were found between pair- and single-housed animals when evaluated by mixed effect model ($P > 0.05$). Rates of diarrhea were low in both subject groups. An additional retrospective analysis of 120 previously transferred juvenile male and female rhesus macaques balanced for housing type, included evaluation of clinical abnormalities and weight fluctuations. Of the 18 monkeys with diarrhea in this group, 67% were single-housed. Via t-test, pair-housed animals gained significantly more weight than single-housed subjects ($P < 0.05$; $t = 2.328$), although both groups remained within the bounds of an institutional nomogram. This suggests paired monkeys were more robust for transitioning onto active study assignments. Findings from these analyses demonstrate the beneficial effects of pair housing and active social buffering in the face of intrafacility transfer and acclimation stress for adolescent rhesus macaques.

PS49 Comparisons of Intervertebral Disc Metabolic Responses between Chondrodystrophic and Non-chondrodystrophic Dogs

NN Lee,*[1,2] JS Kramer,[2] AM Stoker,[2] JL Cook,[2]

[1]Comparative Medicine Program, University of Missouri, Columbia, MO; [2]Thompson Laboratory for Regenerative Orthopaedics, University of Missouri, Columbia, MO

Intervertebral disc disease (IVDD) is strongly associated with spinal pain and disability, Inflammatory, and degradative mechanisms are associated with IVDD; however, specific pathways resulting in symptomatic disease have not been fully elucidated. Chondrodystrophic (CD) dogs, such as dachshunds and beagles, develop spontaneous IVDD with acute clinical signs and can serve as a translational model for human IVD degeneration and disease. IVDD in non-chondrodystrophic (NCD) dogs is most commonly recognized as a chronic condition in performance and working dogs. While disc degeneration is a feature in IVDD for both CD and NCD dogs, mechanistic metabolic responses have not been well characterized and compared for application to veterinary medicine or translational modeling. For this study it was hypothesized that CD IVDs would have higher basal and cytokine-stimulated inflammatory and degradative biomarker production compared to NCD IVDs. With ACUC approval, IVDs were collected from NCD (adult Mongrel dogs; n = 17) and CD (adult Beagles, n = 6) euthanized for reasons unrelated to this study. Cervical and lumbar IVDs were collected aseptically and annulus fibrosus (AF) and nucleus pulposus (NP) explants were created from isolated IVDs using a dermal biopsy punch. Explants were separated into control and cytokine-stimulated (10 ng/mL rcIL-1β) groups. Explants were cultured for 3 d and media were tested for inflammatory and degradative biomarkers using commercially available assays. Significant ($P < 0.05$) differences between groups were determined using Mann–Whitney test. Basal release of MMP-3, and cytokine-stimulated release of CXCL1, MMP-1, and MMP-3, were significantly higher for CD AF compared to NCD AF. Basal release of IL-8, MCP-1, MMP-2, and MMP-3, and cytokine-stimulated release of IL-6, IL-8, MCP-1, MMP-1, and MMP-3, were significantly higher in CD NP compared to NCD NP. Collectively, these data suggest that AF and NP from CD dogs produce a higher number of inflammatory and degenerative biomarkers compared to tissues from NCD dogs. Ongoing translational studies will be aimed at further elucidation of these important similarities and differences between the two canine models to better understand and address IVDD mechanisms in dogs and humans.

PS50 Withdrawn

PS51 Propofol as an Effective Means of Euthanasia for Adult Zebrafish

A Davis*, DK Chu, JP Garner, SA Felt

Comparative Medicine, Stanford University, Palo Alto, CA

Despite the popularity of the zebrafish (*Danio rerio*) as a model organism in biomedical research, the most frequently used methods of euthanasia for these small fish (MS222 and ice bath) have multiple shortcomings with regard to animal welfare and human safety. Propofol has been known to cause rapid induction of anesthesia for many species including zebrafish, but its efficacy as a euthanasia agent for zebrafish has not been tested. Fifty-eight adult zebrafish were used in this project, with 8 to 9 fish per treatment group. Each fish was individually placed in a small tank containing water from their home tank plus ice, 250ppm MS222, 600ppm lidocaine hydrochloride, 100ppm propofol, or 150ppm propofol for a period of 20 or 30 min. Display of aversive behaviors (erratic swimming, piping at the surface), time to loss of righting reflex, time to cessation of opercular movement, time to loss of tank tap response, and time to recovery after removal to clean tank water were recorded. Propofol at both concentrations induced loss of righting reflex and opercular movement more quickly than MS222 or lidocaine, without display of aversive behaviors as seen with ice bath exposure. However, for fish euthanized with propofol, exposure duration was a significant predictor of recovery from anesthesia whereas dose was not. Of the animals exposed to propofol for 20 min, 3 animals (1 of 8 at 100ppm, 2 of 8 at 150ppm) experienced deep anesthesia with loss of all measured responses but recovered within 30 min after being returned to a clean tank. A 30-min exposure to propfol at either concentration was sufficient for euthanasia of all animals tested. These findings suggest that exposure to propofol for a duration of at least 30 min may be an effective and humane means of euthanasia for adult zebrafish.

PS52 Optimizing Food Accessibility During Zebrafish Rearing Improves Growth, Survival, and Breeding Performance

TA Collins,*[1] S Cabrera,[1] E Teets,[2] JL Shaffer,[2] BW Blaser,[2]

[1]University Laboratory Animal Resources, The Ohio State University, Columbus, OH; [2]Division of Hematology, The Ohio State University Comprehensive Cancer Center, Columbus, OH

Zebrafish (*Danio rerio*) are the second most common animal used in biomedical research. Setting nutritional and feeding standards for larval and juvenile zebrafish that maximize growth and survival, and enhance reproductive success is a challenge. We hypothesized that by increasing nutrient availability through continuous delivery of live food or high-nutrient density pelleted food, larval zebrafish will experience faster growth, better survival and better reproductive success. Gemma Micro 75 pelleted diet and live type L rotifers (*Brachionus plicatilis*) were compared in 3 feeding regimens: bolus feeding of live diet (BL), continuous feeding of live diet (CL), and pelleted diet (PD). Each was administered to zebrafish (n = 13-21/tank) from 9 to 30 days post-fertilization (dpf). At 38 dpf, fork length was greater for PD and CL groups compared to BL ($P = 0.001$ and $P = 0.0009$, respectively, compared to BL). At 113 dpf, fork length was significantly greater in the PD group compared to BL ($P = 0.03$). Sexual maturity was assessed by external morphology by 3 independent observers and confirmed in breeding trials using validated breeding stock. Fish in the PD and CL groups were sexually mature by morphology at 55 dpf and 52 dpf respectively, while the BL feeding group was sexually mature at 118 dpf. The sex distribution was 67% male for the PD group and 69% male for the CL group. The age of successful spawning in more than 50% of breeding pairs was 93 dpf for the PD and CL groups and 118 dpf in the BL group. Our data suggest that the PD and CL feeding methods promote faster growth and decrease the age at sexual maturity while also maintaining a useful sex distribution. Pelleted diets also offer a clear advantage in labor cost and therefore represent an attractive option for zebrafish nursery care.

PS53 Sudden Clinical Decline in an Experimentally Naïve Yorkshire Pig

AL Carlson,*[2] S Kitz,[1] C Cheleuitte,[3] A Michel,[4]

[1]Fellowship in Comparative & Genomic Pathology, Memorial Sloan Kettering Cancer Center, Weill Cornell Medical College, and the Rockefeller University, New York, NY; [2]Tri-Institutional Training Program in Laboratory Animal Medicine and Science, Memorial Sloan Kettering Cancer Center, Weill Cornell Medical College, and the Rockefeller University, New York, NY; [3]Center of Comparative Medicine and Pathology, Memorial Sloan Kettering Cancer Center and Weill Cornell Medical College, New York, NY; [4]Laboratory of Comparative Pathology, Memorial Sloan Kettering Cancer Center, Weill Cornell Medical College, and the Rockefeller University, New York, NY

A 2.5-mo-old, 30 kg, female, experimentally naïve Yorkshire pig presented with acute onset inappetence and lethargy. Physical exam (PE) and complete blood count (CBC), serum chemistry, rectal culture, and fecal flotation for endoparasites were unremarkable during quarantine 8 d earlier. On exam, hyporexia and mild lethargy were noted. Four hours later, the pig vomited twice and refused treats. Repeat PE revealed dull mentation, pale pink mucous membranes, and pain on abdominal palpation. Differential diagnoses included a gastrointestinal foreign body, a gastrointestinal ulcer, gastritis, or another primary gastrointestinal (GI) disease. Abdominal ultrasound demonstrated a small amount of free fluid at the caudal border of the liver. Blood was collected for CBC and serum chemistry, but results were not immediately available. The animal was treated with maropitant (1 mg/kg SC), famotidine (0.5 mg/kg SC), meloxicam (0.1 mg/kg SC), and 0.9% saline (150 mL SC). The animal's clinical status declined and due to the poor prognosis, euthanasia was elected. CBC and serum chemistry received after euthanasia revealed a nonregenerative anemia, thrombocytopenia, neutropenia, hypoalbuminemia, and hyperglobulinemia. On gross necropsy, there was tricavitary effusion and marked expansion of the tricuspid valve leaflets by variably sized, tan, granular, semi-firm, nodules. Necro-suppurative endocarditis and myocarditis with intralesional gram-positive bacteria were observed microscopically. Culture of the vegetative lesions identified *Streptococcus suis*. The wide spectrum of clinical manifestations of *S. suis* infection in pigs, as well as the pathogenesis, management, and zoonotic potential of this highly prevalent pathogen will be discussed.

PS54 Fluffy, White Growth on a Captive Freshwater Cichlid

M Reichert,* N Koewler

Research Animal Resources, University of Minnesota, Minneapolis, MN

A ~5-y-old, male Jack Dempsey cichlid (*Rocio octofasciata*) single-housed in an investigator-managed housing area (IMHA) was examined for a white, cottony-mass on his right flank. The fish was lethargic with a decreased appetite at the time of examination. All water parameters (water hardness, pH, temperature) were unchanged from the months prior to the condition. A scale scrape from the fish's right flank and a water sample were taken, but no inciting cause was identified in either sample. The differential diagnosis list included ICH (*Ichthyophthirius multifilis*), fin rot (*Flacobacterium* spp), and water mold (*Saprolegnia* spp). After consultation with a veterinarian trained in aquatic diseases, a presumptive diagnosis of water mold was made based on clinical presentation. Water molds are ubiquitous in water and soil, typically feeding on decaying organic matter and only causing pathology in injured or immunocompromised fish. Treatment with 5g/L of aquarium salt was started and after 2 wk of treatment, the fish became brighter and his appetite returned. During recovery, more in-depth discussions were had with the lab regarding husbandry practices and it was revealed that deionized water with no mineral additives was being used in the tank. The water hardness of the tank was 50 ppm, but published water hardness for freshwater cichlids ranges from 100–200 ppm. The water mold outbreak was therefore thought to be secondary to husbandry-related stresses caused by the inappropriate water hardness. Continued treatment of the water with a cichlid-specific commercial water hardener was recommended and no further health issues occurred. Species-specific husbandry values should always be sought in aquatic cases, even if there have been no recent changes in water quality.

PS55 Neurologic Signs in a Zebra Finch (*Taeniopygia guttata*)

RJ Floyd,*[1] S Monette,[1,2] LL Diaz,[3]

[1]Tri-Institutional Training Program in Laboratory Animal Medicine and Science, New York City, NY; [2] Center of Comparative Medicine and Pathology, Memorial Sloan Kettering & Weill Cornel Medicine, New York City, NY; [3] Comparative Bioscience Center, The Rockefeller University, New York City, NY

An approximately 4-y-old, experimentally naïve, male zebra finch (*Taeniopygia guttata*), used for breeding, presented with bilateral paresis, followed a few days later with a left sided head tilt, consistent head bob, and difficulty flying and perching. All birds in the room had recently been treated with a 5-d course of amprolium after isosporosis was diagnosed in several birds on routine sem-annual examination. Differential diagnoses considered included lesions of the central nervous system or adjacent structures, including bacterial, viral or protozoal encephalitis, osteomyelitis or abscess (caused by *Chlamydophila psittaci*, *Mycobacterium avium*, *M. genavense*, *Salmonella typhimurium*, avian paramyxovirus type 1, avian polyomavirus, avian influenza virus, *Sarcocystis* sp., *Toxoplasma gondii*), neoplasia (primary and metastatic), toxicity, (lead, zinc, or amprolium) concussive head trauma (resulting in damage to the vestibulocochlear nerve, nuclei, or cerebellum),

infarction, hypovitaminosis E or selenium deficiency (resulting in encephalomalacia, hyperlipidemia), and otitis media/interna. The bird was euthanized and submitted for a complete necropsy. A poorly demarcated and invasive neoplasm composed of atypical spindle to polygonal cells lining spaces filled with red blood cells consistent with a hemangiosarcoma, appearing to originate from the skull, with extension into the adjacent neural parenchyma was identified histologically and was the likely cause of the neurologic presentation. Hemangiosarcomas are malignant tumors originating from vascular endothelium and are rarely reported in passerines. Histologic findings, differential diagnoses, and prevalence in avian species will be discussed.

PS56 Bump on the Rump in a Bunny

A Skorupski,* IL Bergin, PA Lester

Unit for Lab Animal Medicine, University of Michigan, Ann Arbor, MI

A 2-d-old female New Zealand White kit (*Oryctolagus cuniculus*) presented with a small caudal, dorsal midline dermal vesicle that was alopecic and filled with serosanguinous fluid. The kit was quiet, alert, and responsive with no other lesions. Differential diagnosis included trauma, localized skin infection, dermoid cyst, or spinal dysraphism (including spina bifida). Fluid could not be obtained via fine needle aspirate. The kit was monitored and continued to grow without deviation from her littermates for 2 wk. At 2.5-wk-old, the kit presented with left hindlimb paralysis and right hindlimb paresis. The vesicular lesion had become scarified. Euthanasia was elected since neurologic or musculoskeletal signs were not expected adverse consequences. Gross necropsy findings showed a membranous sac-like protrusion from the dorsal L4 vertebra extending toward the dermis and continuous with the raised, alopecic lesion on the caudal dorsum. Histologically, the lesion showed thickened meninges extending through a dorsal opening of the L3-L4 vertebrae and continuing through the overlaying epaxial muscles into the subcutis. The adjacent spinal segment was partially protruding through the defect and was asymmetric with decreased neurons and decreased distinction between the grey and white matter. The skin adjacent to the vesicle showed aggregates of spindloid mesenchymal cells extending to the dorsal epithelial surface accompanied by mild inflammation and fibrosis. The lesion was consistent with myelomeningocele, a congenital form of spina bifida resulting from failure of neural tube closure and manifesting as protrusion of the meninges and spinal cord through a vertebral defect. Myelomeningocele symptoms in humans range from none to progressive locomotor difficulties. Myelomeningoceles can be caused by genetic or nutritional factors, including folic acid deficiency. They have been experimentally induced in rabbit models, however this case appears to be a spontaneous occurrence with the condition exhibiting the progressive neurological presentation.

PS57 Ulcerative Glossitis and Mortality in Neonatal Muntjac Deer (*Muntiacus reevesi*)

E Houston,*[1] J Helbling,[1] M Burton,[1] E McNulty,[2] JD Ayers,[1] LV Kendall,[1] C Mathiason,[2]

[1]Department of Laboratory Animal Resources, Colorado State University, Fort Collins, CO; [2]Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO

Reeve's muntjac is a small cervid used in prion research. In 2019, our captive breeding colony experienced 2 instances of neonatal mortality secondary to glossal ulceration. Three fawns were born to the 4 females in the breeding colony. Fawns were housed individually with their dam postpartum on wood shavings. A mix of timothy and alfalfa hay was provided ad libitum and deer chow given daily. The enclosure included multiple huts, feed and water bowls, and enrichment. Fawns were weighed daily. The dam of fawn 1 had a history of chronic diarrhea managed with probiotics. At 20-d-old, weight loss was observed in fawn 1. The fawn remained bright and was observed nursing. Physical exam revealed ptyalism and halitosis leading to a sedated oral exam. Multifocal ulcerations were observed on the caudal aspect of the tongue. The fawn was started on flunixin, ceftiofur, and oxytetracycline. He was administered multiple supplemental feedings daily. Subcutaneous fluids, butorphanol, vitamin B12, dextrose, and supplemental herbivore diet were given. At 27-d-old, fawn 1 was found dead. Two mo later, fawn 2 was born. At 17-d-old, this fawn experienced weight loss, ptyalism, lethargy, and halitosis. The fawn was started on milk replacer and tulathromycin. Sedation was administered for exam, ultrasound and blood collection. Hay stems and shavings were observed in a similar ulcerative lesion on the tongue. Complete blood count and chemistry revealed elevated fibrinogen, toxic neutrophils, and azotemia. Fawn 2 was euthanized at 18-d-old due to poor prognosis. Necropsy revealed extensive ulceration of the distal tongue and buccal mucosa. Histology revealed villous blunting in the intestines and Clostridium perfringens was cultured, though no diarrhea was observed. Immunohistochemistry for bovine viral diarrhea virus was negative. Culture of the glossal lesions grew *Bibersteinia trehalosi*, a pathogen not previously associated with ulcerative disease, but commonly found in the nasopharynx and tonsils of healthy ovids and bovids. *B. trehalosi* has been described to cause pneumonia and septicemia in cervids during periods of stress, and can be spread through ingestion of feed, water, or materials contaminated by oral or nasal secretions. Due to the stemmy forage and shavings found in the mouth of affected neonates at necropsy, changes in hay and bedding were made for a fourth neonate in 2019 and 2 fawns in 2020, that survived past weaning. Necrotizing ulcerative glossitis and increased neonatal mortality associated with *Bibersteinia trehalosi* in Reeve's muntjac may be avoided through altering husbandry protocols in the postpartum period.

PS58 Hematochezia in a Rhesus Macaque (*Macaca mulatta*)

L Birdwell,[1,*] R Stammen,[1] S Kirejczyk,[2] J Jenkins,[1]

[1]Division of Animal Resources, Yerkes National Primate Research Center, Emory University, Atlanta, GA; [2]Division of Pathology, Yerkes National Primate Research Center, Emory University, Atlanta, GA

A 26-y-old, indoor-housed, male rhesus macaque (*Macaca mulatta*) was examined for a large amount of frank blood in his stool. Physical exam revealed multiple firm areas of GI on abdominal palpation. A complete blood count was unremarkable. Blood chemistry revealed a mildly elevated creatine kinase. Differential diagnoses included infectious enteritis, diverticulitis, and gastrointestinal neoplasia. Antibiotic therapy was initiated and the hematochezia appeared to resolve. Two wk later, reoccurrence of the hematochezia led to a second physical exam. A grade II/VI heart murmur was auscultated at that time. An abdominal ultrasound exam revealed no obvious abnormalities. Again, chemistry revealed only an elevated CK and additional bloodwork was unremarkable. Treatment at that time included antacids and hematinics. A barium series study was performed with radiograph images taken at 0, 0.5, 1, 6, 24, 29 and 48 h post barium administration. The series initially revealed delayed gastric emptying. At 48 h, barium contrast remained present in the colon and large intestines. Based on the patient's overall clinical condition, a poor prognosis was assigned. The patient was euthanized and a necropsy was performed. On necropsy, a well demarcated, 3 cm-diameter, round, dark red, soft intramural mass was found 6 cm proximal to the anus at the colorectal junction. The mass was solid with a necrotic center and extensive mucosal ulceration. Histologic examination revealed a well-demarcated, well-vascularized tumor arising from and expanding the tunica muscularis. Cells of the tumor were described as fibrillar, or spindle-shaped, with elongated nuclei. The neoplastic cells were diffusely and strongly immunopositive for CD117 (c-kit) immunohistochemistry, consistent with a diagnosis of gastrointestinal stromal tumor (GIST). GISTs are rarely identified in the rhesus macaque based on published literature. In general, tumors

of the GI tract usually affect older animals and accurate diagnosis may be difficult without histology. Colorectal tumors should always be included in the differentials for a geriatric macaque with hematochezia.

PS59 Subcutaneous Mass in a Brown-headed Cowbird (*Molothrus ater*)

EM David,* KL Gardiner, AK Brice

University Laboratory Animal Resources , University of Pennsylvania, Philadelphia, PA

An experimentally naïve, adult female wild-caught brown-headed cowbird (*Molothrus ater*) was reported for a mass observed under the right wing. The bird had been quarantined for 2 wk prior to entry into the animal facility and housed indoors for 1 mo per IACUC-approved protocol. During observation, the bird was bright and alert with good body condition and normal flight. On physical exam, there was an approximately 1.0 cm diameter, subcutaneous, firm, tan axillary mass. Differentials for the mass included cyst, abscess, neoplasia, and granuloma. Due to the natural behavior study objectives, capture and/or sedation for diagnostics was not initially performed. Veterinary staff elected to monitor the bird through weekly cageside observations. After 5 mo, the mass increased in size and began to affect right wing range of motion. The bird was hand-caught and a physical exam was performed, which revealed multiple coalescing 0.5–2.0 cm diameter tan masses of the neck and axilla bilaterally. Due to the progression of the disease process, as well as a concern for a potential infectious etiology, the bird was euthanized and submitted for postmortem examination. Grossly, there were multiple firm, yellow, granular subcutaneous masses on the ventral and dorsal thorax. Microscopically, the masses were composed of expansile cysts lined by stratified, squamous epithelium and filled with abundant keratin and mites admixed with bacterial cocci. There was a diffuse, mild to moderate, lymphohistiocytic and heterophilic, dermal infiltrate. The clinical, gross and microscopic presentations are consistent with Harpirhynchus quasimodo, the hunchback mite, infestation, as described in the literature. This cowbird likely was infected in the wild, and there was no evidence of spread to other animals in the colony. This diagnosis changed the intake procedure to include an in-hand physical exam performed by a veterinarian prior to entry indoors. All birds previously received topical pyrethrin treatment for mites, however if another *Harpirhynchus* case were identified, veterinary staff would additionally treat with ivermectin. This case demonstrates the importance of a broad differential list, including parasitic disease, for a subcutaneous mass in a wild-caught cowbird.

PS60 Constipation and Weight Loss in a Rhesus Macaque (*Macaca mulatta*)

E Cho,* PC Smith, S Wilson, JL Asher, K Killoran, CJ Zeiss

Comparative Medicine, Yale University, New Haven, CT

A 22-y-old diabetic female rhesus macaque (*Macaca mulatta*) presented with chronic constipation and intermittent anorexia over the course of a year. These episodes appeared to be cyclic in nature, with each lasting 3–5 d. Upon physical exam, a 7 x 3 cm firm but compressible mass was palpated in her mid-abdomen, CBC was characteristic of a stress leukogram (leukocytosis with neutrophilia and lymphopenia), and serum biochemistry revealed a mild hypertriglyceridemia. Radiography indicated the presence of a mass in her cranioventral abdomen, likely distinct from the colon. This mass appeared to produce a focal impaction, but did not appear to obstruct the small bowel or the majority of the colon. Ultrasonic examination revealed a distended hypomotile distal colon, presumably filled with fecal matter, and a motile proximal GI tract. Abdominal CT indicated the presence of a soft tissue mass in the upper right quadrant. The colon was dilated for most of its length with the exception of the terminal colon and rectum. FDG PET was within normal limits, showing no signal indicative of a proliferative mass. Therapeutic efforts were largely palliative and consisted of lactulose, dietary fiber supplements, enemas, and subcutaneous fluids. Due to her poor response to therapy and declining condition she was euthanized and a necropsy was performed. Gross findings included approximately 20 mL of serous fluid in the peritoneal cavity. Marked fibrosis and adhesions were present throughout the peritoneum and viscera, most severely associated with the distal jejunum, which was markedly dilated. Histological findings include peritoneal fibroplasia, fibrosis, and endometriosis that increased in density as it proliferated along the jejunum distally. Microscopic endometriotic glandular foci became more evident throughout the mesentery and on the peritoneal surface. Pathology results supported a diagnosis of functional bowel obstruction due to diffuse fibrosing endometriosis.

PS61 Abdominal Pain and Distention in a Ferret

SD Alaniz*

Department of Comparative Medicine, Oregon Health and Science University, Portland, OR

A 1-yr-old, 1kg, male, castrated, pair-housed ferret with a head cap and multiple previous craniotomies was reported for being lethargic and inappetent. While the ferret's head cap was being cleaned, the lab member also observed that the ferret was lethargic, refused to drink, and was retching. On exam, the ferret was hypothermic with an internal temperature of 35.5°C, dehydrated with tacky mucus membranes, and bloated and painful in the cranial abdomen. Supportive therapy was instituted by warming him up on a water heating pad covered with a forced air warming blanket, providing analgesia (0.12 mg buprenorphine SQ), and starting to rehydrate with 20 mL warmed NaCl subcutaneously. Diagnostic fluoroscopy was performed, which revealed there was a large air filled cranial abdominal structure noted on the right side of the abdomen, with air filled small intestines with increased peristalsis. Euthanasia was elected and performed by an IP injection of a commercial euthanasia solution and intracardiac perfusion under a deep plane of anesthesia. On necropsy, the ferret was diagnosed with gastric dilatation-volvulus (GDV), after observing that the stomach was severely congested, distended with air, and rotated ~120°. The spleen was malpositioned along with the stomach, enlarged, and severely congested with dark purple to black coloration. GDV is a rare condition in ferrets and should be considered in any ferret with an acutely painful and distended abdomen. Acute therapy is necessary for survival, needing immediate gastric decompression and repositioning, and +/- splenectomy based on vascular compromise.

PS62 Ocular Lesions in a New Zealand White Rabbit

LF Mosca*, J Snyder

Department of Comparative Medicine, University of Washington, Seattle, WA

A 4-mo-old male New Zealand rabbit presented for hyporexia, acute diarrhea, dehydration, and weight loss. As a part of a study to model atherosclerosis, this animal underwent a bilateral jugular vein graft surgery 2 mo prior, and a subsequent atheroprotective gene transfer therapy procedure 1 mo prior to presentation. In order to develop arterial lesions that resemble human atherosclerosis, this rabbit had also been on a 0.3% commercial high-cholesterol pellet diet for 2 mo. The rabbit was submitted for necropsy at the planned experimental endpoint to further investigate the clinicals signs. Lesions at necropsy included mild edematous enteritis and typhlitis peritoneal effusion, pericardial effusion, and hepatic lipidosis. Multifocal, white-to-tan granular specks ranging from 1–2 mm were observed in the irises bilaterally. The kidneys and adrenal glands were diffusely pale and enlarged. Surfaces of the pulmonary outflow tract and ascending aorta contained white-to-tan streaks presumed to be mild, chronic, multifocal cholesterol

deposition. Differential diagnoses for gastrointestinal lesions included dysbiosis due to Clostridia spp., *E. coli*, or other pathogens causing rabbit enterocolitis. Differentials for the ocular abnormalities included xanthomatosis, anterior uveitis, and intraocular neoplasia. Diagnostics performed included aerobic and anaerobic culture and histopathology. Culture of cecal contents yielded a diagnosis of enteropathogenic *E. coli* although polymicrobial contribution could not be ruled out. On histologic examination, macrophage foam cells, which play a key role in the development of atherosclerosis, were identified in arteries of the heart and lungs and in the adrenal glands. In the eye, there was macrophage foam cell accumulation secondary to excessive deposition of lipids within the sclera, ciliary body, and iris. Infiltration by these lipid-laden macrophages, also known as xanthoma cells, is well-characterized in rabbits receiving high-fat commercial diets. Based on the clinical and pathologic findings, the iridial xanthomatosis observed in this rabbit was likely secondary to the 0.3% high-cholesterol diet.

PS63 Hindlimb Lameness in a Pregnant Common Marmoset (*Callithrix jacchus*)

OL Franco Mahecha*, JL Haupt, HR Holcombe, RM Kramer, M Jamiel, JG Fox

Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA

A 2.3-y-old, pair-housed, primiparous marmoset was presented with non-weight bearing lameness of the right hind limb (RH) approximately 1 wk before the estimated due date. On physical exam, there were no palpable abnormalities of the RH and radiographs showed no significant findings. After ruling out a possible fracture, we considered soft tissue injury or nerve paresis as possible causes of the lameness. Abdominal ultrasound was performed confirming 3 viable feti with normal fetal development. The skulls of 2 feti were positioned very close to the birth canal. The female was maintained under observation and administered analgesics. Minimal improvement was noted the following week, but the animal remained active. Approximately 6 d after presenting with lameness, the animal started showing progressive signs of discomfort and decreased ambulation. Bloodwork showed a mild elevation in creatinine and mild hypocalcemia. Fluid therapy, calcium supplementation, and acupuncture were instituted. As the RH lameness did not improve and the marmoset was due for parturition, a cesarean section was performed. Three viable infants were delivered, and the dam recovered uneventfully. Analgesics were given pre- and post- surgery and the animal was monitored closely for pain and discomfort. Limited improvement in the RH lameness/disuse was noted for the first 3 wk. At reevaluation 3 wk after surgery there was evident disuse atrophy of the RH muscular mass. Slow recovery was noted during the following weeks, and normal ambulation was observed by 6 wk after surgery. Gradual improvement in RH muscle mass was noted with complete recovery 11 wk following the initial presentation of nonweight bearing lameness. With the lack of significant abnormalities on palpation and radiographs in conjunction with gradual improvement following delivery, nerve compression syndrome was thought to be the cause of lameness in this animal. Compression of the lateral femoral cutaneous, sciatic, and obturator nerves, and mononeuropathies such as lumbosacral plexopathy or lumbar radiculopathy have been reported in pregnant women as cause of lameness during the third trimester. These conditions are associated to numbness and paresthesias in the anterolateral thigh. We suspect that a fetal skull close to the birth canal was compressing one of these local nerves inducing neuralgia and lameness in the dam. Unexpected events during pregnancy can be properly managed by close monitoring and prompt clinical/surgical intervention to support the wellbeing and safe recovery of periparturient marmosets.

PS64 Sustained Release Buprenorphine Effectively Attenuates Thermal Hypersensitivity in an Incisional Model of Postoperative Pain in Neonatal Rats (*Rattus norvegicus*)

A Blaney*

Comparative Medicine, Stanford University, Los Altos, CA

Despite the need for safe and effective postoperative analgesia in neonates, research regarding pain management in neonatal rodents is relatively limited. Here, we investigate whether sustained release buprenorphine effectively attenuates thermal hypersensitivity in a neonatal rat model of incisional pain. Male and female postnatal day 3 Sprague Dawley rat pups (n = 34) were randomly assigned to 1 of 4 treatment groups: 1) saline (control), 0.1 mL, once SC; 2) buprenorphine HCl (Bup), 0.05 mg/kg, once SC; 3) low-dose sustained release buprenorphine (low-SR), 0.5 mg/kg, once SC; 4) high-dose sustained release buprenorphine (high-SR), 1 mg/kg, once SC. Pups were anesthetized with sevoflurane and a 5 mm long skin incision was made over the left lateral thigh. The underlying muscle was dissected and closed using surgical glue. Thermal hypersensitivity testing was performed at 24 hr prior to surgery and subsequently at 1, 4, 8, 24, and 48 h postsurgery using an infrared diode laser. Thermal latency was prolonged at 1 h postsurgery in the Bup group, while it remained prolonged through the entire postsurgical period in both low SR and high SR groups. This data suggests that a single dose of low SR (0.5 mg/kg) or high SR (1 mg/kg) effectively attenuates heat hypersensitivity for at least 8 h in neonatal rat pups.

PS65 Evaluation of Acute Regurgitation in an Olive Baboon (*Papio anubis*)

S Lane,* D Marchi, W Williams

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

A 7-y-old female Olive Baboon (*Papio anubis*) housed in a small group (n = 6) in an indoor corral was evaluated for 2-wk duration, intermittent regurgitation following ingestion of small food items. She was sedated for medical evaluation and workup. Physical exam, vital signs, and CBC and serum chemistry values were unremarkable. The animal had a BCS of 2/5 and lost 1.5kg in the previous 6 mo despite being mid-gestation in her first pregnancy. Abdominal ultrasound did not reveal significant abnormal findings. Standard radiography revealed abnormal GI pattern and a barium contrast series was elected. This revealed the stomach with rotated position, delayed gastric emptying, and delayed GI transit. Differentials included constipation, delayed gastric emptying, and incomplete bowel obstruction. Animal was treated medically with Lactulose, mineral oil, and apple juice to promote GI passage. After 2 wk, the animal was seen vomiting again with decrease intake of large food items. We repeated the barium series under full anesthesia. Differential diagnoses now included partial obstruction, intraabdominal mass, and adhesions. Given the poor response to medical management and new, more severe differentials, abdominal exploratory surgery was elected. Surgery revealed approximately a 3″ x 3″ section in the right cranial abdomen of adhesions attaching sections of duodenum, jejunum, ileum, ascending and transverse colon to the body wall. Adhesions were broken down manually, freeing all segments save for a single, vascular adhesion attaching the duodenum to the lateral peritoneal wall. The remainder of the abdominal organs were evaluated and found to be within normal limits. Animal recovered single-housed and was managed postoperatively with multimodal analgesia, gastro protectants, prokinetics, and bland diet. Within 2 wk of surgery, the animal was eating and drinking within normal limits, defecating within normal limits, and showing no signs of discomfort. In conclusion, this case is the first case at our institution of a successfully managed abdominal explore in a pregnant adult baboon.

PS66 Planning and Managing Postoperative Care for Spanish Cross Goats Receiving Total Knee Replacement

D Marchi,[1,*]S Lane,[1] C Rasbach,[1] E Clary,[2] W Williams,[1]

[1]Division of Comparative Medicine, The Univerisy Of Oklahoma Health Science Center, Oklahoma City, OK; [2]Oklahoma State University, STILLWATER, OK

Managing an animal after major surgery can be a challenging feat. Orthopedic procedures often add further complexity as they are generally quite invasive, lengthy in duration, intense in nociceptive stimulus, and prone to significant complications that include hemorrhage, infection, implant failure, and ambulatory dysfunction. In a recent pilot study, our division took on full clinical and research care responsibilities for several Spanish cross goats receiving a unilateral total knee replacement (TKR) device. We convey useful information to other researchers contemplating use of goats in TKR research since little exists in the literature about postoperative management of orthopedic procedures in goats. In developing our postoperative regimen, the chief goals were to maximize animal comfort, function, and quality of life while limiting the risk of major complications. Preparations included repurposing facility resources and ordering special equipment to facilitate animal handling and protection of the operated limb. Intra- and postoperative drug regimens were developed with particular concern to deliver good analgesia that could facilitate rehabilitative care and early limb usage. We evaluated animal progress and well-being by assessing mentation, TPR, pain level, digestive function (weight, appetite, rumen contractions, feed intake, fecal output), and limb status (usage, range of motion, muscular volume). Implant status was further assessed with serial radiography. Management evolved through the postoperative weeks with changes in pain medications, housing arrangements and intra-species social interactions. A team-oriented approach was critical as veterinarians, veterinary technicians, animal care staff, and the animal behaviorist together reviewed and evaluated animal care and progress during recovery. Our planning, periodic reviews, and adjustments have led to a successful recovery process and given us a standardized platform for future studies.

PS67 Development of a Novel Clinical Scoring System for *Corynebacterium bovis* Infections for NSG Mice

CA Manuel,[2,*] EC Pearson,[1] U Pugazhenthi,[3] M Fink,[2] L Habenicht,[2] DL Fong,[2] JK Leszczynski,[2] MJ Schurr,[4]

[1]Center for Animal Resources and Education, Cornell University, Ithaca, NY; [2]Office of Laboratory Animal Resources, University of Colorado Anschutz Medical Campus, Aurora, CO; [3]Division of Endocrinology, Metabolism and Diabetes, University of Colorado Anschutz Medical Campus, Aurora, CO; [4]Department of Immunology and Microbiology, University of Colorado Anschutz Medical Campus, Aurora, CO

The assessment of *Corynebacterium bovis* infected mice starts at the time of overt clinical signs. This delay provides little information on the incubation time until clinical signs develop. The goal of this study was to characterize the complete clinical progression of *C. bovis* infection and develop a clinical scoring system for NSG (NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ) mice. NSG mice were selected because they are common and are similar to several other mutant strains of haired, immunodeficient mice. Naïve, 7-wk-old, female, NSG mice (n = 30) were exposed to both soiled bedding and NSG mice with C. bovis clinical signs, or maintained as negative controls (n = 6). Mice were monitored weekly for 8 wk for signs and progression of disease. All exposed mice were confirmed *C. bovis* positive by qPCR after inoculation. We identified signs which present in phased progression that include periocular and ear pinnae inflammation, decreased hair-coat quality, and increased grooming activity. The primary benefit of these signs are that they can be observed and scored by cageside observation. Scores of 4 and 17 represent clinically normal and peak clinical severity, respectively. Onset of clinical signs occurred at 2.6 ± 0.6 wk postinoculation (PI) with a significant increase in clinical score beginning at 3 wk PI ($P \leq 0.05$). Conjunctivitis and blepharitis were observed first, followed by ear hyperemia, decrease in hair-coat quality, and finally an increase in grooming activity. Hunched posture (4.6 ± 1.0 wk), repetitive head shaking (5.9 ± 0.8 wk), and increased water consumption were all apparent by 6 wk PI. A significant decrease in body weight was observed in mice with clinical signs at 7 wk PI ($P \leq 0.05$). Symptoms steadily increased during week's 3-7 PI, and did not decrease in severity with a mean score of 16.7 ± 0.5 by wk 8 PI. Control mice remained *C. bovis* negative by qPCR with a mean clinical score of 4.02 ± 0.07 for the study. The clinical course of *C. bovis* infection in NSG mice is very different from reported clinical signs in athymic nude mice. Unlike in nude mice, clinical *C. bovis* in NSG mice does not spontaneously resolve. Further investigation is needed into the pathogenesis of infection that contribute to *C. bovis* clinical disease.

PS68 Outbreak of Enteropathogenic *E. Coli* and Diarrhea in a Colony of New Zealand White Rabbits

D Eldridge*, B Iritani

Comparative Medicine, University of Washington, Seattle, WA

Thirteen singly housed New Zealand White rabbits out of a colony of 89 rabbits presented over the course of 1 mo with varying degrees of inappetence, diarrhea, and dehydration (14.6% incidence). CBCs, serum chemistries, and fecal cultures were submitted on the initial 2 cases and metronidazole benzoate 32 mg/kg was administered orally BID. Bloodwork revealed severe azotemia, and despite treatment, both presenting rabbits continued to decline and were euthanized. Typhloenteritis with serosal hemorrhages and swollen, pale kidneys were noted on gross necropsy. Fecal cultures revealed heavy growth of *E. coli*, which were subsequently sent to Penn State *E. Coli* Reference Center for further characterization using a pathogenic *E. coli* PCR screen and serotyping. Samples came back positive for the *Attaching and Effacing Intimin* (*EAE*) gene and negative for toxin genes, and O:128 H:2 serotype. Microscopic histology revealed necrohemorrhagic typhlocolitis with adherent bacilli, consistent with enteropathogenic *E. coli* (EPEC). In an effort to knock down levels of pathogenic *E. coli*, all remaining colony rabbits were dosed with a single subcutaneous injection of 10mg/kg enrofloxacin and were transferred to newly cleaned racks and cages. Additional strict control measures were simultaneously implemented, which included isolation and restricted handling of sick rabbits, changing gloves/gowns between each rabbit handled, frequent sanitization of pellet scoops and hay tongs, daily pan liner changes, and weekly cage and rack changes. Eight of the 13 cases of diarrhea were promptly started on a 10-d course of enrofloxacin, which resulted in improvement within 48 h. Two cases resolved spontaneously, and 1 case presented 1 d prior to planned endpoint. All new rabbit arrivals were treated with a single dose of enrofloxacin on arrival. Over the course of the following 6 mo, the incidence of diarrhea was reduced significantly to 0-2 cases per month. These results suggest that prophylactic treatment of *E. coli* with a single injection of enrofloxacin, combined with strict management procedures, may provide an effective control measure for colonies with endemic EPEC.

PS69 Management of Distal Tibia and Fibula Fracture Using External Coaptation in a Common Marmoset (*Callithrix jacchus*)

AJ McLuckie,[1,*] RA Ober,[2] R Donocoff,[3] CL Winnicker,[1]

[1]Institute of Comparative Medicine, Columbia University, New York, NY; [2]Northwestern University, Chicago, IL; [3]Gene Therapy Program, University of Pennsylvania, Philadelphia, PA

A naïve nulliparous 3.5-y-old female common marmoset (*Callithrix jacchus*) was observed to be nonweight bearing on the left hindlimb during routine rounds. Sedated examination revealed a palpable fracture proximal to the ankle, with an overlying 4mm skin wound medially. Radiographs revealed displaced spiral fractures of the distal tibia and fibula. Fractured bone was visible through the skin wound with mild soft tissue bruising surrounding, indicating a

type I open fracture injury. Under sedation, the wound was clipped, sterilely prepped, and flushed. The fracture ends were reduced using digital pressure, a nonadherent dressing was placed over the skin wound, and a Robert-Jones bandage was applied. Sustained-release buprenorphine was administered, plus a 10-d course of a nonsteroidal anti-inflammatory, and a 5-wk course of antibiotics, was initiated. Recovery was uneventful and the marmoset was able to ambulate in the home cage while bandaged. Cageside observation was performed at least 3 times a day for the first 3 wk, including visualization of toes, with bandage changes every 4–5 d. A soft fibrous callus was palpable 10 d postfracture. At 3 wk, the skin wound was closed with no evidence of infection, and the fracture site was stable on gentle digital manipulation. On radiographs, there was mineralization evident at the fracture ends, but no bony callus. Bandaging was decreased to once weekly, skin dressing was discontinued, and repeat radiographs performed every 2 wk. After 7 wk postfracture there was concern that atrophic nonunion was occurring. Consultation with orthopedic surgeons revealed subtle signs of callus progression however, and it was noted that nonunions are usually not diagnosed until 6–8 mo postfracture. A bony callus was observed on radiographs at 11 wk. Maturation of the callus and increased range-of-motion in the knee were evident by 13 wk, and bandaging was discontinued. At this time, the marmoset was weight bearing and using toes for grasping. No further lameness has been observed to date, and after 6 mo, radiographs showed significant bone remodeling. The results of this case management suggest that external coaptation can be successfully used to manage hindlimb fractures in marmosets.

PS70 Evaluation of a Continuous Glucose Monitoring System for Use in Diabetic Cynomolgus Macaques (*Macaca fascicularis*)

S Oh*, LC Halliday

Biologic resources Laboratory, University of Illinois, Chicago, IL

Cynomolgus macaques are a common nonhuman primate (NHP) research model for studying diabetes mellitus. In order to manage diabetic NHPs, serial blood glucose concentration monitoring is used to determine the type of insulin, dosage, and frequency of administration necessary to maintain appropriate blood glucose concentration. Blood glucose concentration is usually monitored by repeated capillary venipuncture of the tail or toe pads with a portable blood glucose meter (PBGM). These procedures require manipulation of the animal, which may result in stress-induced hyperglycemia, discomfort, and possible tissue damage with multiple punctures. This pilot study describes the clinical use of a continuous glucose monitoring system (CGMS) that is monitored remotely by Bluetooth as an alternative method to the PBGM. The CGMS was placed on the dorsum of 3 cynomolgus macaques (diabetic (n = 1), and normoglycemic (n = 2)), and glucose concentration was recorded continuously for 7 d. Blood glucose was also measured using a PBGM in the morning and afternoon, prior to administration of insulin to the diabetic animal. Glucose values from each measuring device were averaged over the 7-d period and compared. The application and use of the CGMS was easy and well tolerated by all NHPs. Morning and afternoon glucose values for PBGM/CGMS in the diabetic NHP were 127 ± 26 / 173 ± 70 mg/ml and 303 ± 70 / 377 ± 28 mg/mL. The values in the nondiabetic NHPs were 34 ±20 / 94.5 ± 56.5 mg/ml and 44.4 ± 14 / 95 ± 32 mg/mL. Though glucose values for the CGMS were higher than PBGM, there was a good correlation between the PBGM and CGMS measurements. This study indicates that real time CGMS is reliable for monitoring blood glucose remotely and continuously in conscious, unrestrained NHP with minimal labor and is appropriate for diabetic patient management.

PS71 Characterization of Gastric *Helicobacter* spp in a Colony of Research Macaques

HR Holcombe,[1,*]R Marini,[1] M Patterson,[1] S Muthupalani,[1] Y Feng,[1] AG Swennes,[2] R Ducore,[3] M Whary,[1] Z Shen,[1] JG Fox,[1]

[1]Division of Comparative Medicine, MIT, Cambridge, MA; [2]Center for Comparative Medicine, Baylor College of Medicine, Houston, TX; [3]Oregon National Primate Research Center, Portland, OR

Helicobacter pylori was identified in nonhuman primates in 1993, and has since been described in rhesus, cynomolgus, and pig-tailed macaques. Non-*H. pylori* helicobacters, primarily *H. suis*, have also been isolated from stomachs of macaques. *H. suis* is now recognized to have zoonotic potential and, like *H. pylori*, can cause gastritis and MALT lymphoma in humans. We evaluated the colonization of *H. suis* and *H. pylori* in 21 rhesus macaques (7 females, 14 males; mean age 15.6 years) from a colony used in neuroscience research. Gastric samples were obtained by gastric endoscopic biopsy, necropsy, or both. PCR was used to determine helicobacter status. Additional diagnostic tests included helicobacter culture, fluorescence in-situ hybridization (FISH), histological analysis, and serology. The index case was a 15-y-old, singly housed, male rhesus macaque evaluated for chronic daily vomiting. Gastric endoscopy revealed 4 punctate ulcers, 1 body and 3 antral, each 1–2 mm in size. Biopsy samples were submitted for diagnostic testing indicated above. Histological findings included multifocal, moderate, chronic lymphoplasmacytic gastritis with intraglandular and luminal spiral bacteria. PCR results were positive for helicobacter at the genus level. The animal was treated with enrofloxacin (5mg/kg), ampicillin (25mg/kg), and famotidine (0.5mg/kg) administered intramuscularly, twice daily for 4 wk. Posttreatment biopsies were negative for *Helicobacter* spp. and the inflammation score of the gastric body improved from moderate to mild. Nineteen of the remaining 20 animals were positive by PCR for *H. suis*; 5 of these were also PCR positive for *H. pylori*. Five of the *H. suis*-positive animals had clinical signs of disease. Seventeen animals were positive for large spiral organisms by FISH. Serological analysis demonstrated a 70% sensitivity and 75% specificity in ELISAs using H. suis antigen, and 100% sensitivity and 36.4% specificity for ELISAs using H. pylori antigen indicating that serology is not sufficient for helicobacter diagnosis. Our results indicate that *H. suis* is prevalent in captive rhesus macaques. The role of *Helicobacter* spp in causing clinical disease attributed to gastritis and peptic ulcer disease requires further investigation.

Poster Sessions

P1 A Pilot Program Using Positive Reinforcement and Target Training in Ported Yucatan Pigs

JL Zorge,[1,*] G Arnold,[2] W Gately,[3] S Perrotta,[1]

[1]Veterinary Services, Charles River Laboratories, Shrewsbury, MA; [2]Animal Care, Charles River, Shrewsbury, MA; [3]In Vivo, Charles River, Shrewsbury, MA

Sling restraint is a common restraint method used for pigs. The sling restraints approved for use at our facility have a weight limit of 50 kg. For studies requiring animals over 50 kg, sedation was required to perform procedures. In preparation for a study requiring 60 animals over 50 kg, a program for target training with positive reinforcement was developed. The animals were trained to follow and connect with a target which resulted in a food reward. The following milestones were built into the training program: connecting to the target equals reward, safely exiting the home cage, walking onto a scale, and entering a transport cage for sample collection. The target was presented twice daily for short intervals using high-value food. Most pigs reached all milestones within 21 d; however, 1 pig required additional target training. Target training provided mental stimulation and engagement to the pigs and provided positive human contact. Performing procedures was easier and faster, the need for sedation was eliminated, stress minimized and the risk of injuries to staff and animals reduced. The pilot's success led to the expansion of the program to include all pigs entering the facility, regardless of body weight.

P2 Rehoming of Research Animals: Program Implementation, Development, and Benefits

A Anguiano-Rosales*

Vet Resources, Covance, Indianapolis, IN

Developing and implementing an animal rehoming program from proposal to fully established program requires planning and wide stakeholder engagement in a global contract research organization. It involves a range of activities, including development of proposals, organizing meetings, and writing detailed training plans and procedures. Following a step-by-step approach is key to the successful implementation of such a program. A few of the key components needed to successfully create a rehoming program are securing senior leadership, IACUC and veterinary support, developing a detailed animal acclimation and socialization plan, establishing written procedures and policies, and identifying a dedicated team to sustain the program. In a short amount of time our program has evolved and expanded to include the rehoming of cats and non-naïve dogs. Since implementing the rehoming program at this company 2 years ago, over 40 dogs and 7 cats have been rehomed. All animals quickly acclimated to their new environment, experiencing only minimal issues with housebreaking which resolved over time. Following an animal's growth and progress from laboratory to home has generated immense joy among staff, positively impacting the lives of both humans and animals.

P3 Initiative to Promote the 3Rs

LM Stanislawczyk,[1,*] MC Kundu[2]

[1]Veterinary Sciences / Animal Welfare Assurance, Bristol-Myers Squibb, New Brunswick, NJ; [2]Veterinary Sciences, Bristol-Myers Squibb, New Brunswick, NJ

There is an unwritten awareness and perception to the significance of applying the 3Rs within the biomedical community. The theory is discernable, but the understanding and implementation may not be as straight forward in reality.Researchers want to apply the 3Rs to their study designs but applying these principles may require additional resources and training. There may be innovative methods that could benefit multiple areas of research, however the silos within an institution between various departments or sites, diminishes the opportunity for collaboration and implementation of 3Rs opportunities. The veterinary sciences department kicked off a 3Rs initiative by creating a small team with the goal of increasing awareness of the 3Rs efforts and opportunities in our scientific community. The initial team (referred to as the 3Rs Strategy Team) was comprised of a few staff members who defined objectives for the team and determined the need for additional team members to comprise a global team for the organization. The 3Rs Strategy Team solicited volunteers comprised of representatives from various research departments/sites, IACUCs, technical operations, and the Global Continuous Innovation Team (GCIT). The 3Rs Strategy Team defined the responsibilities and objectives of the members of the global team which was referred to as the Global 3Rs Team (G3T). The main objective was to initiate an annual 3Rs award. Through several brainstorming meetings, the submission questions were identified and criteria for judging and award process determined. In order to further augment the prestige of the award the team decided to hold a ceremony where team members could present the awards in front of an audience and submissions could be presented. In addition, a People's Choice Award was selected after each team presented their submission through the use of a voting app. The ceremony not only increased awareness and exposure of the innovations and opportunities, but it allowed broad communication of the important work being conducted, caused an exponential increase in personnel morale across every level of personnel working in in the vivaria and an opportunity for people to make connections and discuss future collaborations. The feedback was overwhelmingly positive.

P4 Improving the Quality of Continuing Reviews: One IACUC's Journey

ML Budda,* AL Burke

Office of Animal Welfare Assurance, University of Oklahoma Health Sciences Center, Oklahoma City, OK

Determining the best means to conduct continuing reviews of approved animal activities is an important decision for IACUCs. For years our IACUC performed these reviews with a renewal form tool. Briefly, investigators submitted renewal forms to the committee annually for each approved animal protocol, and a designated member reviewed them. Substantial confusion existed among committee members and investigators regarding the standards in which these renewals should be reviewed. The IACUC therefore desired a new approach to its continuing review process. In 2018, the committee retired its annual renewal form and replaced it with a postapproval monitoring process. Personnel from the Animal Welfare Assurance office were trained on the new process and tasked to conduct postapproval monitoring at routine intervals based on the animal species and funding sources listed in each protocol. The new process was composed of 3 key actions: read the approved protocol, observe procedures, and check laboratory records. Findings from the postapproval monitoring of each protocol were then reviewed by the IACUC. Information from the calendar years before (2017) and after (2019) this change were compared to assess the interventional impact. On average, continuing reviews in 2017 numbered 16.4/month, while in 2019 it numbered 9.5/month. Time spent conducting continuing reviews increased from an average of 12 hours/month in 2017 to an average of 28.5 hours/month in 2019. Notably, the new process produced evidence of how animal activities were being performed, whereas the old process depended on self-reporting by investigators. The new process also facilitated more communication between investigators and the IACUC. Overall, the committee concluded that the greater investiture required of the new postapproval monitoring process was worthwhile because it adhered more closely to the goal of confirming animal activities were conducted in a manner consistent with IACUC-approved protocols, policies, and guidelines.

P5 Lavender Essential Oil Infusion Can Help Reduce Pacing in Rhesus Monkeys (***Macaca mulatta***)

B BoWell*

Neurobiology, Duke University, Durham, NC

While there are few studies providing evidence for overall effectiveness of lavender essential oil for anxiety in humans, many people claim they benefit from it. There have also been claims in the primate research community that infusing lavender essential oil has made their primates seem calmer. Our facility uses weekly treat feeders to reduce pacing, presumed to be a sign of stress, in our primates. Given that some of our animals are overweight, we looked to see if lavender essential oil can reduce pacing. Four animals were chosen to participate in this study. Two are individually housed and 2 are paired. Data was collected Tuesdays through Thursdays to reduce the variable of rack changes. After the primates had an acclimation period to the observer in the room, observations were noted using focal sampling for a period of 30 min each for baseline, lavender, and 1-wk post-lavender sessions. This data collection occurred over a 3-wk period. In week 1 we analyzed baseline responses prior to exposure of lavender. Week 2 consisted of 30 min of lavender infusion and then observations immediately after exposure. In week 3 we recorded pacing behavior without infusion to determine whether pacing increased again. The results were positive in that all animals averaged decreased the amount of pacing from 44% during baseline to 27% during the week of essential oil infusion. The week after essential oil infusion, on average the 2 paired animals had an increase in pacing, while the 2 individually housed animals continued to decrease. As a result of this study, we will be able to use infused lavender essential oil as a form of scent enrichment to help reduce anxiety in our primates.

P6 Validation of Mouse Jugular Venipuncture for Serial Blood Collection

CR Deugan, NC Masterson*

Training, Covance, Greenfield, IN

The number of blood collection methods available to obtain larger (i.e. greater than 40µL) samples of whole blood from mice are limited. Each method comes with its own pros and cons, whether via retroorbital sinus, submandibular, tail vein, or cardiac puncture. Jugular venipuncture has the potential to be a viable option for large volume serial sampling from a single animal when microsampling is not an option. Jugular venipuncture can be used to reduce the number of animals for bioavailability sampling, as well as refining the use of these animals (e.g. anesthesia, prolonged recovery times, terminal procedures). Three male mice per strain (CD-1, Athymic Nude, and C57BL/6), weighing approximately 25g, were given an intravenous injection of antipyrine, which is a known agent with a predictable bioavailability curve and a short half-life, followed by blood sampling (100µL), and detailed observations occurring throughout the 24-h period following dosing. Detailed observations were performed in conjunction with the 5 blood sampling time points (0.083, 2, 4, 8, and 24 h post dose) to capture any abnormalities that may have occurred due to the sampling technique, volume collected, or any other factors. We established that blood can be collected at multiple time points, and that the sampling method duplicated historical observations relative to how this compound absorbs, distributes, metabolizes, and excretes, thus sufficiently validating this method as a viable option for large volume serial sample collection in mice.

P7 Five-year Outcomes after Implementation of a Critical Incident Reporting System

SJ Bischoff*, D Trietschel, A Enkelmann

Animal Welfare, University Hospital Jena, Jena, , Germany

From today's perspective and the current state of science, the use of animals for experimental purposes cannot be completely discontinued. However, negative experiences from these experiments are often not referred to in publications or are lost. The Critical Incident Reporting System in Laboratory Animal Science (CIRS-LAS) was implemented in 2015 and is the world's first database for critical incidents in laboratory animal science. The CIRS-LAS.de database provides any person involved in laboratory animal science the ability to write an anonymous report about critical incidents during animal experiments.5 years after the launch of CIRS-LAS.de, 150 people from Europe are already registered users of the CIRS-LAS database and more than 50 critical incidents have already been entered. Reported incidents were mainly observed in the fields of laboratory animal husbandry (>25%), followed by reported incidents, which occurred in experiments: orthopedics (24%), anesthesiology (20%), and cardiovascular research (13%). Less frequently, cases were reported in experimental fields for regulatory purposes (7%) or breeding of genetically modified animals (4%). The objective of CIRS-LAS is an open dialogue about failures which can help to avoid them in the future and to improve lab animal safety. CIRS-LAS provides an online platform for an open-minded and active failure management and supports transparent exchange of negative experiences and possible solutions to improve animal welfare. Five years after the start of CIRS-LAS.de database use is steadily increasing. This demonstrates researchers have a strong awareness regarding the responsible handling of the laboratory animals. It is time to foster lab animal science transparency. It is time to talk about critical incidents. It is time to join CIRS-LAS.de.

P8 Patency of Jugular and Femoral Vein Catheters Attached to Transcutaneous Buttons in Sprague Dawley Rats with Weekly Maintenance

V Karicheti,[2,*] L Luciano,[1] SG Camaya,[1] M Koubaitary,[1] MC Pledger,[1] TR Gleason,[3] A Evans,[3] SE Gledhill,[3] PM Sparks,[3]

[1]RMS Surgery, Charles River Laboratories, Raleigh, NC; [2]RMS Product Portfolio Acceleration, Charles River Laboratories, Raleigh, NC; [3]Infusion Toxicology, Safety Assessment, Charles River Laboratories, Ashland, OH

Vascular access in conscious rats for repeated blood sampling in pharmacokinetic studies is achieved using chronically implanted jugular vein catheters (JVCs) or femoral vein catheters (FVCs). One factor that affects patency is the catheter maintenance schedule. We conducted a study to determine the duration of blood collection patency and infusion only patency of JVCs and FVCs attached to transcutaneous buttons with weekly catheter maintenance up to 9 wk. Forty adult CD® rats (Crl:CD(SD)), 20 males at 200–225 grams and 20 females at 175–200 grams, were allocated into 4 groups of 10 each. Rats were anesthetized and a polyurethane catheter was inserted into the right jugular or left femoral vein. The catheter was connected to a transcutaneous button in the interscapular region. The catheter and transcutaneous button were locked using heparinized (500 IU/mL) 50% dextrose solution. Animals were shipped to another site for patency checks to mimic a standard customer order. At the study site, rats were socially housed in polycarbonate cages using a commercially available paper bedding and maintained at 21 ± 2°C with relative humidity of 30–70% and a 12:12 hour dark:light cycle. Feed and water were provided ad libitum. Animal health was evaluated weekly with body weights and detailed physicals as well as catheter patency checks. For patency checks, animals were manually restrained and catheters accessed using a button adaptor injector. The catheter was aspirated to confirm the ability to withdraw blood. The catheter was considered fully patent if withdrawal of blood was successful. The catheter and transcutaneous buttons were relocked using heparinized (20 IU/mL) saline after patency checks. Both JVC and FVC catheters were 100% patent up to 2 wk post-surgery in all the animals. FVC catheter patency rates maintained at 80% up to 7 wk post-surgery. However, JVC catheter patency decreased to 60% in males and 50% in females. At conclusion of the study day 63–64, patency rates varied from 20% to 70% between groups. However, 90% of FVC and 70% of JVC were fully patent for infusion. This data suggests that bidirectional catheter patency for blood collection is longer in FVC compared to JVC attached to transcutaneous buttons with weekly catheter maintenance.

P9 Carprofen Delivery via Water-based Gel

J Finlay,[1,*] L Saganek,[1] J Palmer,[3] TW Adamson,[2] R Ermel,[1]

[1]Center for Comparative Medicine, City of Hope, Duarte, CA; [2]The Salk Institute, La Jolla, CA; [3]ClearH2O, Portland, ME

Pain management for research animals is a scientific and moral imperative. Appropriate use and selection of an analgesic minimizes the confounding variables of pain in a study and mitigates the discomfort experienced by the animal. There are various types of analgesics and treatment modalities available for pain management with each having their benefits and drawbacks. One class of analgesic that is frequently used in biomedical research is the nonsteroidal antiinflammatory, the most common being the COX inhibitor carprofen. Carprofen is widely used in an injectable formulation, though this route requires daily restraint and injection leading to additional distress/discomfort on the recovering animal as well as spikes in blood plasma levels. Additionally, there is increased risk of skipped injections and added labor costs. Until late 2019 a commercial diet gel formulation containing carprofen was available that assisted in overcoming some of the negative aspects of injectable carprofen. Unfortunately, this product was removed from the market due to regulatory hurdles. Having experienced great analgesic success and researcher compliance with this product we sought to replicate the oral delivery of carprofen using a sucralose-sweetened gel. To this end, a process for mixing and storing the in-house

carprofen gel was tested with the intent of determining homogeneity of the mixing process. Sterility and stability following the addition of carprofen to gel cups, were analyzed over time (2 cups/time point/storage method). It was determined that a combination of mixing modes (hand shaking and vortexing) produced a homogeneous mixture of gel and carprofen. Following inoculation of the carprofen (1.25 mg in 60 ul saline) into the sealed gel cup (56 mL) the sterility of the inside of the cup was maintained for up to 6 mo. High performance liquid chromatography results demonstrated that the carprofen distribution was homogeneous and remained stable at therapeutic concentrations for up to 3 and 6 mo for room temperature and refrigerated samples, respectively. In summary, this research demonstrates the relative ease with which an in-house gel-based delivery of carprofen can be achieved while maintaining sterility and stability of the analgesic carprofen.

P10 Success of a Nonhuman Primate Training Core

S Combs,2,*A Dillard,3 KR Lambert,1

[1]Animal Resources Program, Wake Forest School of Medicine, Winston Salem, NC; [2]Preclinical Translational Services/Comparative Medicine, Wake Forest School of Medicine, Winston Salem, NC; [3]Clinical Translational Science Institute, Wake Forest School of Medicine, Winston Salem, NC

The Nonhuman Primate (NHP) Training Core was developed as a supplement to the training program for all individuals working with the animal species housed at our institution. The goals are to improve animal care and welfare, employee knowledge and skill set, transparency and collaboration, research outcomes, and compliance for the entire institution. The NHP Training Core is a free training service that promotes best practices in animal care, while providing support for technicians and investigators that need assistance. The staff that benefit most from the NHP Training Core could include those that are new to the institution, working with a new species, or intending to use new methodologies to collect data. The NHP Training Core is comprised of several individuals within the institution that have extensive experience working with NHP. Areas of training include, but are not limited to, anesthesia, aseptic technique, emergency situations (such as CPR), laboratory organization/set up, collar/pole and chair training, awake bleeding, NHP sedation, blood collection and NHP safety. A survey was provided to staff and faculty that took part in a training opportunity provided by the NHP Training Core to better understand areas of possible improvement. The majority of responses were positive, with 85% of survey participants find the material to be relevant to their role, 77% felt the training helped to meet their job responsibilities, 96% felt the trainer's knowledge was sufficient for the material, and 85% felt comfortable contacting the trainers again for additional training if necessary. Based on the positive comments from staff and faculty, the NHP Training Core is a meaningful addition to our staff-training program.

P11 Harmonizing Recommendation for Enhancing the Care and Wellbeing of Research Pigs across Global Sites

E Moreau,[1,*] T Bryant,[2] M Wilkinson,[1] PV Turner,[1]

[1]Global Animal Welfare & Training, Charles River Laboratories, Wilmington, MA; [2]Ocular and Neuroscience, Charles River Laboratories, Senneville, , Canada

Pigs, both conventional and mini-pig breeds, are common laboratory animals and yet there are few specific guidelines that relate to working with pigs in a biomedical research environment. Using the lunar designation of 'Year of the Pig' in 2019, our corporate animal welfare group initiated a project to bring together individuals (behavior champions, toxicologists, veterinarians, and technicians) from across sites in North America and Europe working with research pigs to establish a set of best practices for enhancing research pig care and welfare. Seven subcommittees were formed to consider components that are critical elements in behavioral management programs for research pigs; recommendations for optimizing pig housing; recommendations for refining euthanasia of pigs; methods to refine husbandry, restraint and transportation for pigs; optimizing blood collection practices for pigs; recommendations for rehoming pigs following the conclusion of studies; and determining areas for which no clear practices or recommendations exist to enhance pig well-being (i.e., potential areas for further research). Subcommittees met electronically and in person at a specific pig 3Rs workshop to discuss current practices and experiences, constraints imposed by research needs, as well as information in peer-reviewed literature for multiple mini-pig breeds as well as conventional pigs. Despite the challenges of working across multiple countries with different regulatory environments, a final consensus report was prepared with 12 general recommendations for improving pig care and welfare. Although no site had yet to implement all the recommendations suggested, it was agreed that this provided a good future roadmap and vison for refining research pig care and welfare at all sites. This report was distributed to senior management at sites working with pigs and implementation discussions are in progress.

P12 Development of Standardized Assessment Tools for Evaluation of Animal Care and Use Programs at Contract Research Organizations

HN Burr,[1,*]A Zuvich,[5] A Lange,[2] W West,[3] NA Bratcher,[4]

[1]Bristol Myers Squibb, New Brunswick, NJ; [2]Novartis, East Hanover, NJ; [3]Boehringer-Ingelheim, Ridgefield, CT; [4]AbbVie, North Chicago, IL; [5]Incyte, Wilmington, DE

The mission of the 3Rs Translational and Predictive Sciences Leadership Group (3Rs TPS LG) of the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ Consortium) is to promote sharing and integration of science and technology to advance the 3Rs in the discovery and development of new medicines, vaccines, medical devices, and health care products for humans and animals. The 3Rs TPS LG is dedicated to identifying opportunities to share practices in a precompetitive way and formed the CRO Outreach Working Group (WG) in 2013, comprised of IQ member companies and partner contract research organizations (CROs), to further advance the 3Rs. The WG identified that CROs routinely undergo assessment of their animal care and use program, with each sponsor-specific evaluation requiring considerable time investment by the CRO. To minimize duplicative effort while also bringing 3Rs into the forefront, the WG developed both a comprehensive and a low-risk tool to assess animal care and use programs at partner CROs based on risk. The Comprehensive Assessment Tool explores all programmatic areas, including accreditation status, regulatory compliance, veterinary care, IACUC, 3Rs, facilities, housing and husbandry, and occupational health and safety. The Low-Risk Assessment Tool is an abbreviated questionnaire recommended for use when risk is considered minimal. Both programmatic assessment tools were piloted by IQ member companies for approximately 2 y and refined prior to final release in 2019. Now publicly available (iqconsortium.org), the assessment tools are a resource to assist both sponsors and CROs in optimizing information sharing and facilitating comprehensive and consistent programmatic assessment. As additional sponsors adapt and consistently use this resource, we anticipate a more efficient programmatic assessment process that highlights a common commitment to the 3Rs. Data will be shared highlighting current usage of these tools and identified process improvements.

P13 Training and Low Stress Handling Procedures to Reduce Fear during Restraint for Blood Collection in Sprague Dawley Rats

C Moody, E Moreau, PV Turner*

Global Animal Welfare and Training, Charles River Laboratories, Senneville, , Canada

Laboratory animal handling and restraint procedures may induce behavioral and physiological changes that negatively impact an animal's welfare, detract from the the human-animal bond, and, ultimately, may bias study data. Thus, it is important to habituate, desensitize, and counter-condition animals to people and procedures that may be perceived negatively. This study examined the consistent use of training procedures (habituation, counter-conditioning with food) and low stress handling techniques with a soft handling mat to reduce negative responses during full-body restraint for blood collection. Pair-housed 5-wk-old Sprague Dawley rats (n = 48), were randomly assigned by cage to 1 of 3 groups: 1) low handling (15 s gentle handling with mat, 3 times/week; n=8 cages), 2) moderate handling (45 s gentle handling with mat, 3 times/week n = 8 cages), and control (no handling sessions; n = 8 cages). Handling sessions involved slow and gentle interactions with a soft fleece mat, and gradually increasing amounts of restraint throughout the 2-wk period. At the end of the training period, rats underwent elevated plus maze (EPM) testing and full-body restraint for blood sampling with blood glucose levels analyzed by glucometer. EPM outcomes assessed included percentage of time spent in the closed, and open arms, and were analyzed using ANOVA with cage included in the model. Blood glucose levels were assessed using a mixed linear regression model with cage as a random effect. As well, presence of feces and/or urine during restraint was analyzed using a Fisher's Exact test. During restraint for blood collection, more control rats defecated and/or urinated during restraint compared to both the low and moderate handling groups ($P = 0.009$). No other significant results were found. Our results suggest that nonhandled rats responded more negatively to restraint and blood collection. There were no differences noted between the moderate and low handling groups, suggesting short periods of handling may be sufficient to achieve this effect. Overall, the results indicate that consistent pre-study gentle handling and training paired with a soft handling mat and food treats, may reduce negative responses during later restraint for blood collection in rats.

P14 Evaluating In-cage Resources for Mice: Nest Materials and a Cardboard Cage Semi-divider for Improving CD-1 and C57BL/6 Mouse Welfare

C Moody,[1] E Paterson,[2] PV Turner,[1,2,*]

[1]Global Animal Welfare and Training, Charles River Laboratories, Senneville, , Canada; [2]Dept of Pathobiology, University of Guelph, Guelph, , Canada

Providing meaningful in-cage resources is important to allow mice the opportunity to perform highly motivated behaviors, such as nest building, as well as providing choice in their environment and increased psychological and physical stimulation. This study examined the use of brown paper pucks and cardboard cage semi-dividers as manipulable resources for mice. Male C57BL/6 and CD-1 mice were randomly assigned (3 per cage) to 1 of 4 groups: 1) 1 tissue (control; CD-1 n = 30 mice, C57BL/6 n = 30 mice), 2) tissue + brown paper puck (CD-1 n = 30, C57BL/6 n = 27), 3) divider + tissue (CD-1 n = 24, C57BL/6 n = 27), 4) divider + tissue + brown paper puck (CD-1 n = 30, C57BL/6 n = 27). Nest structure and cage-divider use were evaluated and replaced at weekly cage change for 2 wk. Analyses occurred at the cage level, examining the presence/absence of: divider collapse, divider material used to construct the nest, and nest placement beside the divider. Fisher's Exact tests were used to analyze strain and treatment differences. Results showed that CD-1 mice were more likely to destroy and collapse the dividers than C57BL/6 cages for both week 1 ($P < 0.0001$) and week 2 ($P < 0.0001$). As well, CD-1 mice were more likely to incorporate parts of the divider as nesting material compared to C57BL/6 mice during both weeks ($P < 0.0001$). In contrast, C57BL/6 mice were more likely to build their nests within a divided part of the cage compared to CD-1 cages during both weeks ($P < 0.0001$). Overall, our results show strong strain differences in the way mice used the nest material and dividers. CD-1 male mice preferred to collapse and rip up the dividers, whereas C57BL/6 cages maintained divider shape and built nests within the divided areas. Overall, providing a cardboard semi-cage divider with a tissue and nest puck may improve home environment choice in male C57BL/6 and CD-1 mice.

P15 The Blues of Teaching Retroorbital Injections: A Refinement to Using Dye as a Validation Technique in Mice

JL Ludwig*

Division of Animal Resources, University of Illinois, Urbana, IL

Retroorbital injection is a common route for intravenous (IV) injection in rodents. IV injection of Evan's Blue dye causes the mouse's extremities to turn blue. Many training programs use Evan's Blue dye when teaching retroorbital injection to verify successful distribution into the bloodstream. However, this is routinely done as a terminal procedure. With intent to refine this training technique, we performed a pilot study that would determine if dye could be used as a survival tool without detriment to the well-being of the mouse. We allocated mice into 3 groups that consisted of 6 mice of equally distributed CD-1 and ICR strains to evaluate this concept with repeated retroorbital injection with 1 of 3 compounds: 1) 2% Evan's blue dye, 2) FD&C blue No 1 dye, and 3) 0.9% saline (control). FD&C blue No 1 dye is a brilliant blue dye for coloring food. Both dye compounds were filter-sterilized and a total volume of 100 uL of the selected compound was administered to mice while under isoflurane anesthesia. Mice were monitored for dye distribution/dissipation, behavior, body condition, and metabolic function. We found that the FD&C blue No 1 dye distributes instantly and develops a vibrant blue hue throughout the mouse. Additionally, the FD&C blue No 1 dye was visually undetectable approximately 6 h post-injection. This group was injected 2 additional times at 2-wk intervals. The group of mice injected with 2% Evan's blue developed a much lighter blue hue that after 6 wk of monitoring did not clear. We were unable to repeat 2% Evan's blue injections due to the visual lack of clearance. The control group was injected following the same techniques as the FD&C blue No 1 dye group. Upon completion of the study all mice were euthanized and intracardiac blood samples were taken for serology. Serologic analysis of both dye groups were consistent with the baseline values established with the control group. Throughout the study all mice exhibited normal activity noted by the presence of proper nesting behavior, adequate hydration, urine/fecal output, and body condition. From this pilot study, we were able to establish that the FD&C blue dye No 1 proves favorable as a survival validation technique for retroorbital injections in mice, thereby refining the validation tool and reducing the number of mice needed to train this procedure. We have implemented this validation tool into our current rodent training program for each mouse to be injected with FD&C blue dye No 1 up to 3 times with 2-wk intervals between training sessions.

P16 Technical Refinements in Assisted Reproductive Technologies for the 3Rs

P Qi*, OL Franco Mahecha, T Chavarria, E Umana, S Erdman, JG Fox

DCM, MIT, Cambridge, MA

Assisted reproductive techniques (ARTs) are used for all aspects of transgenesis, including rescue or rapid expansion of genetically engineered mouse (GEM) lines. Our centralized transgenic (Tg) facility has been working for 14 y on refinement of ARTs to fulfill the challenging needs of the academic research community, while also prioritizing the principles of the 3Rs. This retrospective study analyzed in vitro fertilization (IVF) procedures as one subset of ART used routinely to facilitate novel scientific activities. Our facility initially adopted conventional IVF procedures, and next implemented a novel laser-assisted IVF (LAIVF) technique. LAIVF was superior to conventional IVF because of the partial dissection of the zona pellucida (ZP). LAIVF improved oocyte fertilization rate to 36% compared to 0–5% with conventional IVF when using cryo-recovery of a variety of mutant C57BL/6 mouse sperm. As a

result, it was possible to reduce the use of animals and at the same time achieve better gift animal health status and other re-derivation outcomes. However, LAIVF had the disadvantage of yielding a decrease rate of live birth of pups per number of transferred embryos in comparison with conventional IVF (12% and 30%, for LAIVF and IVF, respectively). Starting in 2017, the IVF procedure was further improved by chemical modifications of the pre-incubation fertilization medium (human tubal fluid medium-HTF). HTF was supplemented with methyl-b-cyclodextrin (MBCD) and reduced glutathione (GSH), to improve the fertilization capacity. The supplemented HTF (sHTF) medium increased pup birth rates to 30% compared to 12% (LAIVF). With sHTF, the number of pups born per donor had a 4-fold increase (4 versus 0.9 pups for sHTF and LAIVF, respectively). More importantly, these model refinements allowed us to reduce the number of donor females by 50% for all IVF procedures, and to reduce the need for repeated experiments. We conclude from this study that attention to improvement of transgenic methodologies for ARTs is crucial to refine procedures and reduce number of animals used in a transgenic facility making biomedical research a more sustainable enterprise.

P17 An Innovative Method for Confirming Sexual Maturity in Male Macaques (*Macaca fascicularis*)

C Rosales*

Charles River Laboratories, Houston, TX

Cynomolgus macaques (*Macaca fascicularis*) are used widely in multiple aspects of research, including the development and testing of biological compounds for therapeutic uses. In order to evaluate safety thoroughly, these medications often require studies performed in sexually mature animals. At this time, there are few options available to confirm sexual maturity in male macaques: electroejaculation, collection of seminal fluid, and measurement of testicular volume. Electroejaculation and collection of seminal fluid are invasive and require either anesthesia or prolonged acclimation. Testicular volume is at best a surrogate and does not prove that the animal is mature and producing sperm. Based on a review of the literature, it was hypothesized that spermatozoa could be found in a urine sample post ejaculation, thereby verifying sexual maturity. The procedure is done in the early morning before husbandry staff washes the animal's cage. Animals are singly housed and urine visible on pan or that the animal voids when approached is collected with a syringe. One to two drops of urine are evaluated under a microscope and the presence of sperm confirms sexual maturity. Urine collection, as a result, became a successful method of sexual maturity confirmation, virtually eliminating animal handling and subsequent stress while reducing time and effort by technical staff. The number of animals being confirmed increased from 2 animals a week to up to 9 animals a week with a total of 15 animals being monitored each week. Collecting a urine sample (preferably fresh) in lieu of ejaculate via electroejaculation refines the process, provides confirmed animals at a faster pace, reduces contact time between veterinary technician and animal, positively impacts animal welfare by reducing potential stress, while ensuring that we provide a viable animal model for critical research studies.

P18 A Survey of Laboratory Animal Veterinarians Regarding Mouse Welfare in Biomedical Research

J Marx,[1,*] K Jacobsen,[4] N Petervary,[3] D Casebolt,[2]

[1]Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; [2]Department of Animal Resources and Department of Pathology, USC, Los Angeles, CA; [3]National Institutes of Health, Bethesda, MD; [4]Unaffiliated, Middletown, MD

There is an undeniable link between the quality of animal welfare and the quality of scientific results generated from the use of animals in research. Mice are the most commonly used mammalian species in biomedical research. However, there is very little information about the progress that has been made to improve mouse welfare or what factors should be considered to ensure future progress. The Animal Welfare Committee of the American Society of Laboratory Animal Practitioners (ASLAP) surveyed laboratory animal veterinarians to assess their opinions about the welfare of mice, addressing 5 factors affecting animal welfare in biomedical research: husbandry, clinical care, experimental use, regulatory oversight, and training. Two hundred and nineteen veterinarians responded to the survey. The results showed that while veterinarians were generally positive about the overall state of mouse welfare in biomedical research, there were several areas in which opinions indicated that further improvements should be considered. These areas were the following: 1) the training of researchers undertaking experimental procedures (57% reported training was occasionally/frequently inadequate to ensure animal welfare); 2) the frequency of monitoring mice likely to experience pain and distress on experimental protocols (49% reporting that monitoring was occasionally/frequently inadequate to ensure animal welfare); 3) the inclusion of the institutional veterinary staff in the monitoring of mice likely to experience pain and distress significantly improved the assessment of animal welfare ($P < 0.01$); 4) continued improvement in the environmental enrichment provided to mice (30% reporting environmental enrichment is currently inadequate to ensure animal welfare); 5) the ability of the IACUC to ensure that noncompliances are fully addressed in order to prevent reoccurrence both within laboratories and among other research groups at the institution (only 22% of veterinarians report the IACUC is effective at both of these; and 6) the assessment of animal welfare was significantly better ($P = 0.02$) when veterinarians perform examinations, diagnose disease and prescribe the treatment of sick or injured mice.

P19 Identification of Humane Endpoint Markers in Naturally B6 Aged Mice

N Ogiso,[1,*] K Tomita,[2] K Takano,[2] S Tamura,[2] H Kawasaki,[1] N Morikawa,[1] J Almunia,[1]

[1]Laboratory of Research Animal, National Center for Geriatrics and Gerontology, Obu-city, Aichi, Japan; [2]KAC Corporation, Kyoto-city, , Japan

Our facility has kept many naturally aged animals (mice and rats) used for gerontology and geriatric researches. The mortality rate and incidence of specific pathologies in naturally aged mice to use for researches vary due to various genetic and environmental factors. The age-related physiological changes have important practical implications for the clinical management of aged mice. We report about the setting of the humane endpoint of the naturally aged mice. Male and female C57BL/6 (C57BL/6NCrSlc(B6N), C57BL/6J(B6J)) mice (4-wk-old) were purchased from Japan SLC and Charles River Japan every 3 mo and kept over their lifetime. Physiological (measurement of body weight (n = 1,000), body temperature (n = 500) and survival rates (n = 1,530)), biochemical (CORT: urinary corticosterone (n = 80), morphological (necropsy (n= 300), autopsy (n = 96)) and hematological (blood count (n = 160) analyses were performed. Body weight showed rapid decrease at around 23-mo-old in B6N male mice, and 24-mo-old in B6J female mice. Body temperature were relatively higher in B6J mice. Survival rates of B6N mice started to decrease from 18-mo-old (85.3%), while in B6J mice decreased from 21-mo-old (93.4%). Hair loss and other skins disorders were observed after 24-mo-old in both B6 mice (B6N = 19.2%, B6J = 45.9%). A variety of tumors (lymphoma, adenom) were found at necropsy of both strains after 18-mo-old (36.5%). Blood test showed that the total WBC count started to decline in both strains at around 18-mo-old and the composition of WBC tended to change with aging. CORT levels were relatively higher in female mice (168.5-551.4 ng/mL) and tended to increase with age in both B6 mice. The product of body temperature and body weight of individuals with high CORT values fluctuated more than 10% during the last 2 wk before death in both B6 mice. It was suggested that this would be a suitable marker for the humane endpoint of naturally aged mice.

P20 Using Positive Reinforcement to Build Compliance in Sinclair Mini-Pigs

JN Perez,[2,*] RL Collins,[1] KR Lambert,[1] JW Vaughan,[2]

[1]Animal Resources Program, Wake Forest School of Medicine, Winston Salem, NC; [2]Wake Forest Institute of Regenerative Medicine, Wake Forest School of Medicine, Winston Salem, NC

Lameness and unwillingness to bear weight on a limb is an anticipated outcome following orthopedic joint injuries. Exercising the effected limb is essential to facilitate healing and is important for gauging a patient's recovery, as it offers an opportunity for assessing stiffness, range of motion, and weight-bearing capabilities. The stifle joint of Sinclair mini-pigs have a thick layer of articular cartilage that closely approximates that of the human knee, making them an ideal model for studying treatments for articular cartilage. Despite the benefits of exercise after treatment, motivating pigs to exercise can present a challenge to their recovery. Positive reinforcement training was used to motivate the pigs and maintain a rehabilitative routine. The goal was to engage the animals in walking for 15–20 min per session, multiple times a week. This effort was expected to promote recovery in the limb and to desensitize them to touch so that their legs could be palpated without additional stress. Primary challenges included transitioning the pigs from their raised floor housing to the ground level via ramp and identifying the right food motivators to induce consumption of the post-operative oral medications. Three intact male Sinclair mini-pigs were trained using the following training methods: preference testing, syringe training, target and station training, touch acclimation, and harness placement. Modifications to the environment included the use of a transport cart with a ramp to transition the pigs from their housing enclosure to the floor, using anti-slip shower decals on the ramp, and rubber mats placed on the floor to reduce re-injury caused by pad slippage on non-textured surfaces. Each pig received approximately 26 training sessions over the course of 6 wk , for an average of 8.67 total h of training time spent per pig. The training process had the added benefit of forging strong relationships with laboratory, veterinary, and husbandry staff. Daily routines, such as shifting pens for cleaning or holding at the station target during physical exams, became low maintenance and enjoyable tasks, creating a happy and healthy environment that continues to feed their motivation to exercise and provide further enrichment.

P21 Serial Survival Cerebrospinal Fluid Collection and Intrathecal Dose Administration in Intact Rats

S Wills, M Krueger,* J Relph, C Gocker, D Lunde, A Larson, V Walsh

Drug Metabolism and Pharmacokinetics, Covance Laboratories, Inc., Madison, WI

Central nervous system drug development programs often require cerebrospinal fluid collections and/or intrathecal dosing to assess pharmacokinetics. In rats, intrathecal dosing and cerebrospinal fluid collections have historically been performed via a surgically implanted cannula or as a terminal procedure, requiring potentially painful surgical procedures or an excess of animals to accommodate a sparse sampling profile. In this study, procedures for serial survival cerebrospinal fluid collections from the cisterna magna and intrathecal dose administration in intact male Sprague Dawley rats were developed. This technique allows serial cerebrospinal fluid sampling (reducing the number of animals on test) and/or allows intrathecal dosing in a nonsurgical model. Rats were anesthetized under sevoflurane gas to a surgical plane and were restrained on a purpose-built restraint device to appropriately position the head. After aseptic site preparation, the cisterna magna was accessed with a 28 to 31 gauge ½" needle using an insulin syringe, with a 30–45-degree entry angle below the occipital crest. Placement was confirmed by the presence of cerebrospinal fluid after negative pressure was applied. Intrathecal dosing (up to 30 µl) required removal of an equal volume of cerebrospinal fluid to alleviate potential spinal fluid pressure pain. Minimal needle redirection, up to 2 needle punctures per attempt, and a minimum of 3 h between anesthesia events (with up to 2 events per animal) were permitted. Both intrathecal dosing and cerebrospinal fluid collections were well tolerated. Rats were successfully dosed via intrathecal injection followed by up to 1 additional cerebrospinal fluid collection (n = 6 total with n = 3 per time point) or underwent up to 2 survival cerebrospinal fluid collections [n = 12 total with n = 3 per assessment (time point or volume collected)]. Animals recovered quickly (within 15 min) from the anesthesia and collection procedures. For cerebrospinal fluid, up to 50 µl was tolerated for a single survival collection; 30 µl was the recommended maximum volume for serial sampling and for single intrathecal dose administration. Overall, this technique provides an alternative method to terminal sample collections and surgical cannulation for intrathecal dosing or serial cerebrospinal fluid collections in rats.

P22 Species-specific Training Strategies for Vervets

C Scott,[1,*] KR Lambert,[3] B Frye,[1] D Bissinger,[1] C Copeland,[1] S Wise-Walden,[1] P Valure,[1] H Register,[2] C Shively,[1]

[1]Comparative Medicine, Wake Forest School of Medicine, Winston Salem, NC; [2]Internal Medicine, Gerontology, Wake Forest School of Medicine, Winston Salem, NC; [3]Animal Resources Program, Wake Forest School of Medicine, Winston Salem, NC

The use of African Green monkeys (*Chlorocebus aethiops sabaeus*) or vervets for biomedical research is increasing, but there is limited information available regarding the behavioral biology of this species. Our colony of vervets serves as a national biomedical research resource. The monkeys live in social groups of 6–25 individuals per pen with indoor and outdoor access. The indoor living space is equipped with a capture tunnel that can provide individual separations to allow for various activities such as closer examination of individuals or sedation for clinical or research procedures. A cohort of elderly and middle-aged female monkeys (n = 30) from various pens was selected to participate in a cognitive testing project for Alzheimer's research. Our goal was to train the animals to perform a cognitive task in the tunnel to minimize stress from being separated from their social groups for an extended period of time. The monkeys were presented with a puzzle feeder maze of various levels of difficulty. The vervets needed a different approach than those commonly used in macaques. First, we determined which food rewards would motivate the animals to work. Next, we implemented a systematic plan that included 3 phases of training, using positive reinforcement paired with a clicker to train the animals to interact with the puzzle. The first phase was acclimating the monkeys to the puzzle device being attached to the tunnel and the presence of the experimenters. Then we taught them to retrieve a carrot from the puzzle for a grape reward. The last phase introduced an element of the maze to ensure the monkeys had adequate dexterity to accomplish the task. Initially most animals showed signs of stress while in the tunnel and were reluctant to engage with the device. The training typically lasted from 3–5 d although a few animals required a longer training period of up to 2 wk. They were then tested in 30-min sessions for 5 d. After completion of the training 90% of the animals were tested successfully. This experience underscored the need for species-specific training practices that will enhance the welfare of those being utilized in research.

P23 Use of a Collaborative Housing Tracking System to Increase Rate of First-choice Housing for Large Animal

MA Burns,[1,*]C Wilbourn,[3] A Lange,[2] S Scott,[3] JL Lofgren,[1]

[1]Animal Welfare Compliance, Novartis Institutes for Biomedical Research, Cambridge, MA; [2]Laboratory Animal Sciences, Novartis Institutes for Biomedical Research, East Hanover, NJ; [3]Covance, Madison, WI

Housing space and configurations can facilitate species-specific

behaviors important to animal welfare. A pharmaceutical (PH) company and contract research organization (CRO) engaged in a collaboration to enhance animal housing choices for sponsored studies. While all CRO animal housing met or exceeded animal welfare regulations, some housing options were preferred to others by the PH company. The first step was to define first and second choice housing. While first choice housing provided enhanced opportunity for exercise and social interactions, second choice options added refinements to traditional US housing. For nonhuman primates (NHP), first choice housing was defined as EU-style pens while second choice was defined as stainless steel quad caging (*Guide* compliant) refined to facilitate social housing and vertical flight. For canines, first choice housing was defined as EU style runs, while second choice was defined as stainless steel rack caging (*Guide* compliant) refined to support social housing and additional opportunities for exercise. After alignment of housing type definitions, progress in the use of preferred housing was tracked. Fields were coded into the CRO scheduling system to capture housing selection. A weekly report was automatically generated from the system and placed onto a Sharepoint site for easy access. Through the use of this system, over a 9-mo period, studies run in first choice housing increased from 66 to 80% for NHPs, and from 40 to 74% for canines. This data allowed animal welfare teams from both stakeholder organizations to both track progress in the use of first choice housing and perform root cause analysis when second choice housing was utilized. This data facilitated capital investments at the CRO to improve first choice housing availability. These analyses also underscored the importance of advance notice regarding individual studies to the CRO site and highlighted study designs that should be prioritized for further refinement such that they can be performed in first choice housing. Finally, this collaborative effort demonstrated the value of communication and partnership between PH companies and CROs to set goals to enhance research animal housing and drive further program and research improvements.

P24 Characterization of a Novel Stereotypic Behavior in Laboratory Gerbils (*Meriones unguiculatus*) Housed in Individually Ventilated Caging

L Habenicht,[1,*] BM Clancy,[2] CA Manuel,[1] DL Fong,[1] A Klug,[3] JK Leszczynski,[1]

[1]OLAR, University of Colorado Denver Anschutz, Aurora, CO; [2]College of Veterinary Medicine, University of Illinois, Urbana, IL; [3]Department of Physiology and Biophysics, University of Colorado Denver | Anschutz Medical Campus, Aurora, CO

Though Mongolian gerbils (*Meriones unguiculatus*) have served as a common model for neurologic and auditory research for more than 50 years, only corner digging and bar gnawing have been reported as stereotypic behaviors. At our institution, gerbils exhibit repetitive corner jumping which has not previously been described. To better understand and ultimately reduce stereotypic behaviors in the gerbil colony, 17 breeding pairs were videorecorded in their home cages during the light cycle for 4 wk-long periods over 3 mo. For the first 5 d of observation, 30-min video segments during 3 time points of the light cycle were evaluated. These included 0630 at the beginning of the light cycle, 1230 mid-day, and 1930 at the end of the light cycle. Jumping counts, as well as frequency and duration of corner-digging were enumerated for each video segment using open-source behavioral observation software. The incidence of repetitive jumping was compared between different times of day to assess when the behavior was most common and whether it was temporally associated with stereotypic digging. The jump counts during the most active period were also evaluated over 4 wk to identify patterns in the study population over time. Total jumps were highest at the end of the light cycle compared to early morning and mid-day observation periods ($P < 0.0001$, Friedman test). Sixty-four percent of jumps at any time of day (1,480/2,307) were associated with a corner-digging event. All breeding pairs engaged in repetitive jumping, but 5 of the 17 pairs accounted for 63.4% of all evening jumps recorded over 4 wk of observation (4,813/7,589). Video monitoring facilitated objective evaluation of a repetitive behavior pattern in research gerbils. Understanding the prevalence of abnormal behaviors can provide a foundation for assessing the effect of interventions designed to promote optimal animal welfare.

P25 Successful Repairing of Adult Female Mice with Specialized Feeding Jars

V Forck*, K Vandeloecht, M Haney

Veterinary Services, Sinclair Research Center, Columbia, MO

Mice are social animals, yet may require individual housing for data collection, such as daily individual food consumption. In contrast, when animals are group housed, feeders should have enough access points to minimize competition for food. In particular, powdered feed requires feeders placed on the cage floor and may limit access to multiple mice. In the event that individual housing is no longer necessary in a long-term study, it is unclear if animals can return to social housing without conflict and undue stress. We investigated if individually housed mice could be paired in cages with specialized feeding jars without compromising study data or negatively affecting the behavior of the mice. Female Crl:CD1 mice were individually housed with standard chow and feeders for approximately 2 mo upon receipt to the facility. During a week acclimation, individually housed mice (n = 17; 4 mo of age) were introduced to glass jars with an approximately 3 cm diameter opening and filled with powdered feed. After acclimation, the mice were randomized and placed into 1 of 3 groups: single housed with 1 feeder (n = 5); pair housed with one feeder (n = 6); and pair housed with 2 feeders (n = 6). Body weights and clinical observations were collected at least weekly for 3 wk. At study termination, mice were euthanized, and blood samples were collected for clinical pathology on select parameters (glucose, calcium, cholesterol, BUN, TP, triglycerides, creatine, globulins, and albumin). During the study, there was no aggression or trauma regardless of the number of feeders or group and no differences in body weights. Chemistry samples revealed no statistical difference in any parameter. However, single housed animals displayed a trend for increased glucose (avg = 203 mg/dL) and lower cholesterol (150 mg/dL) compared to pair housed mice with 1 feeder (avg 165 mg/dL and 193 mg/dL, respectively) and pair housed mice with 2 feeders (avg 164 mg/dL and 169 mg/dL, respectively). Thus, repairing CD1 female mice likely has minimal effect on blood chemistry parameters. However, caution must be taken if changing housing status in a middle of a study, especially if glucose or cholesterol values are of interest. In conclusion, adult females may be pair housed after a period of single housing, improving the welfare of this social species in long term studies if individual housing is no longer necessary.

P26 SLAVT/BRAD Partnership: Accelerating Public Awareness and Acceptance of Animal Research

AL Murray,[2,*] LK France,[1] J Champion,[3] AA Bedwell,[2] PA Clifford,[4]

[1]Vanderbilt University Medical Center, Nashville, TN; [2]Indiana University School of Medicine, Idianapolis, IN; [3]BMS, Redwood City, CA; [4]Americans for Medical Progress, Washington,

Biomedical Research Awareness Day (BRAD) is an international outreach program devoted to honoring the role of animals in biomedical research and stimulating conversation about the necessary role of animals in medical advancements. Celebrated annually around the world, BRAD promotes peer education about animal research, offers a platform for those working with or supporting animals in research to discuss their work with animals, and highlights the devoted staff who care for these animals. The event also serves as a pipeline for future careers in biomedical research and laboratory animal care and medicine. We feature a partnership between the Society for Laboratory Animal Veterinary Technicians and BRAD, a program of Americans for Medical Progress. It will share how the partnership has accelerated the ability to engage specific audiences in meaningful conversations

about essential animal research while at the same time allowing the professional society to engage their current and potential members. In this case, the program empowers laboratory animal veterinary technicians to reach out to veterinary technician students about the role of animals in biomedical research and career opportunities in the field. Included are benefits of the partnership, responsibilities of each partner, a case study, and how to bring BRAD to your members and/or potential members and the public audiences within your area of influence.

P27 Developing a Human-derived iPSC Cerebral Organoid Model for Studies of Pediatric Anesthetic Neurotoxicity

C Krall,[1,2,*] M Schaefer,[2] H Hogberg,[3] R Johns,[2]

[1]Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine, Union Bridge, MD; [2]Anesthesiology and Critical Care Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; [3]Center for Alternatives to Animal Testing, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD

A concern exists over the potential consequences of early anesthesia on pediatric neurodevelopment. Indeed, preclinical research demonstrates that exposure during synaptogenesis leads to long-lasting cognitive dysfunction; however, data from clinical trials are both sparse and conflicting, thus necessitating the development of a human model in which to corroborate molecular investigations. Human-derived induced pluripotent stem cell (iPSC) cerebral organoids ("minibrains") emerge as a promising solution, although the inability to correlate their age to humans remains a limiting factor. We therefore aimed to pinpoint a key molecular event of synaptogenesis: the "developmental switch," in which the predominating subunit of NMDA receptors (and their associated scaffolding proteins [MAGUKs]) switches from NR2B-SAP102 to NR2A-PSD95, so to ensure refinement of brain circuitry. As we know when this developmental switch occurs in humans, its identification in minibrains could determine their relative age. Minibrains were generated by reprogramming human fibroblasts into iPSCs, which was performed using 2 different cell lines independently. These were next differentiated into neural progenitor cells, and cultured under gyratory shaking for 8 wk so to form spherical cerebral organoids, with collections occurring each week. At each time point, RNA was isolated for RT-PCR analysis in replicates for genes encoding NMDAR subunits (NR2A, NR2B) and MAGUKs (SAP102, PSD95), and markers of cell proliferation (Ki-67), mature neurons (NeuN), and myelination (MBG). We found that NR2A:NR2B peaked at weeks 2–3, with PSD95 rising above SAP102 at week 2. Cell proliferation decreased with time, while NeuN and MBG surpassed Ki-67 at week 6. Given that the NR2A:NR2B switch occurred prior to detection of mature cell markers, likely this corresponds to a prenatal event. Thus, these preliminary results suggest that minibrains cultured for 2–3 weeks may equate to prenatal synaptogenesis at approximately 20 post-conception weeks in humans. Future aims include assessing anesthetic toxicity in this age of minibrain, which may better recapitulate the clinical scenario, and therefore validate their potential as a human model to replace animal studies.

P28 When It Comes to Mouse Blood Collection for Inexperienced Users Chin Up, Don't Get Cheeky

DS Tu,[1,*] K Nolan,[2] AD Pavan,[2] J Stewart,[2] CL Freed,[2]

[1]College of Veterinary Medicine, Western University of Health Sciences, Bellflower, CA; [2]University Laboratory Animal Resources, The Ohio State Univeristy, Columbus, OH

The submental (SM) blood collection technique is considered an acceptable alternative to facial vein puncture (FV) in mice. Relative to the FV technique, SM collection provides visualization of the vasculature under the chin and skin puncture in an area lacking critical structures such as the facial nerve and auditory canal. To validate the implementation of SM collection into our training program, blood collection success rates and welfare parameters were assessed in mice that received either SM or FV collection from inexperienced trainees. We hypothesized that inexperienced trainees would have increased success in collecting large blood volumes with fewer clinical adverse effects using the SM method as compared to the FV technique. A total of 70 C57BL/6 (9–42 weeks old) were divided into groups of 5 and randomly assigned to FV or SM technique and trainees with no previous mouse phlebotomy experience (n = 7) were recruited. For each trainee, a pre-recorded video with technique demonstrations was administered at the start of the training session then trainees performed both FV and SM collection on a total of 5 mice per method. Body weight, number of punctures, and total quantity of blood was documented for each mouse. Mice were monitored for adverse effects for 60 min immediately post-collection and at 24 hr post-collection. A volume of 50µL was considered successful, with an allowable maximum of 100µL to be collected from each mouse. Success rates were 60% for the facial vein technique and 40% for the submental method. An average blood volume of 59 µL was collected from mice using the facial vein technique and 41 µL with the submental method. Results revealed no statistical differences between FV and SM methods for the success rate and the total blood volume collected, however 5 FV mice experienced adverse effects warranting euthanasia. We recommend new trainees use the submental method given there were no statistical differences between success rates and volume collected and fewer adverse events were observed compared to FV.

P29 Driving the 3Rs in the Outsourced Space: Innovation and Collaboration

MA Burns*, JL Lofgren

Animal Welfare Compliance, Novartis Institutes for Biomedical Research, Malden, MA

In recent years, outsourcing of some animal studies, including to contract research organizations, has become increasingly common. With this change comes the responsibility to ensure continued robust ethical oversight for external animal studies by the sponsor, as well as opportunities to create and promote collaborative 3Rs and animal welfare initiatives with external partner sites. To meet this evolving need, a unique role was developed to liaise between internal scientists and those conducting sponsored animal studies at external partner sites. The creation of an externally focused role allowed for identification of and engagement in new collaborative 3Rs advancement opportunities, development of direct relationships with veterinary and animal care staff at external sites for improved communication and collaboration, and alignment of expectations for animal care and use practices across the organization's external animal study portfolio. By centralizing the knowledge and experience related to the breadth of externally sponsored studies into one dedicated role, a unique vantage point is gained that allows for identification of trends and potential opportunities for refinement and collaboration in the external in vivo space. This has facilitated implementation of 3Rs initiatives related to key animal welfare topics, including improved housing, handling, and acclimation practices. It has also ensured a greater level of consistency in all aspects of ethical oversight, including external animal protocol review and conduct of in vivo facility audits. Finally, creation of a centralized point person for internal study team members has facilitated more timely and higher quality communication during study planning that has resulted in positive 3Rs implications such as enhanced analgesia and euthanasia practices. Abstract # 3446439 provides an objective example of the impact of this role on animal welfare. As the trend of outsourcing of animal studies increases, ensuring dedicated ethical oversight and 3Rs focus, such as through a new role like this, helps both the study sponsors and third party partners ensure animal welfare best practices are met and continual collaborative 3Rs innovations are achieved.

P30 Refining Transcranial Magnetic Stimulation in Rhesus

Macaques

M Klores*

New York University-Regeneron, New York, NY

Transcranial magnetic stimulation (TMS) is a noninvasive method for activating the nervous system using an external coil to induce a brief, high-intensity magnetic field, and is used to investigate neuroanatomical connectivity and behavior. Despite its significant clinical and experimental implications, TMS is not without potential adverse effects. Up to 40% of human subjects relate pain or discomfort during repetitive TMS, including headaches and muscle fasciculations, likely due to stimulation of the facial and trigeminal nerves, contractions of facial muscles, or activation of nociceptors in the scalp and bone beneath the coil. While nonhuman primates, most notably macaques, have been critical models to help better understand the physiology and application of transcranial magnetic stimulation, few studies have examined the associated welfare considerations. We sought to determine whether rhesus macaques experience muscle fasciculations related to the application of repetitive TMS and, if so, whether the use of local anesthetics would effectively ameliorate this. One male and 1 female rhesus macaque each underwent 2 sessions of anesthetized TMS (5 mg/kg ketamine, 0.015 mg/kg dexmedetomidine intramuscularly, 1–2% isoflurane in 2L/min oxygen, via facemask, 5 mL/kg/hr of intravenous Lactated Ringers Solution). Repetitive TMS (1-10Hz, 20–80% machine power) over the left dorsolateral prefrontal cortex led to localized muscle fasciculations in all anesthetized sessions. The severity of fasciculations increased with TMS intensity (% machine power), with severe unilateral fasciculations extending through the scapulae at 75% of the machine power. Localized tissue infiltration of anesthetics (2 mg/kg bupivacaine or 4 mg/kg lidocaine) within a session did not subjectively ameliorate fasciculations. Continued work is necessary to further characterize the welfare implications and potential refinements of TMS in macaques.

P31 Refinements to Intermittent Intravenous Infusion Procedures in C57BL/6 Mice to Achieve Study Endpoints and Improve Animal Welfare

TR Gleason,[1] A Evans,[1] M Stamen,[1] G Murphy,[1] V Karicheti,[2,*]

[1]Infusion Toxicology, Charles River, Ashland, OH; [2]Surgery, Charles River, Wilmington, MA

Intermittent intravenous (IV) infusion in mice is challenging. The traditional method for IV dosing is via the lateral tail vein. This requires animal restraint and the use of over-the-needle or through-the-needle catheters. Restraining mice can cause stress and potential injury. Catheters are technically challenging to place in the tail vein and may damage the intimal lining of the vessel. Alternatively, mice are tethered for dosing via surgically implanted intravenous catheters. Tethering is impractical for the typical dose regimen for an intermittent IV infusion study: once weekly for up to 30 min for 6 wk as mice remain attached to the tether system and individually housed for the duration of study. Five male and 5 female C-57BL/6N mice were surgically implanted with jugular vein catheters attached to a transcutaneous button and were transported to the study site. The mice were dosed with 0.9% sterile saline twice weekly at 2.5 mL/kg/minute for 6 min via an extension set attached to a button injector. Animals were removed from their cage, placed into a dosing cage, and were able to move freely. Various sizes of cages, with and without lids and enrichment, were tested. Ports were locked with 5 IU/mL heparinized saline following each dose. All mice were successfully dosed for 6 wk with no effects on body weights or clinical condition. Allowing mice to roam around a cage was an improvement to animal welfare and did not require restraint or tethering to deliver the dose successfully.

P32 Sales, Inventory, and Operations Planning Supports the 3Rs

TJ Receveur*, N Bartlett, J Lohmiller

Taconic Biosciences, Inc, Rensselaer, NY

Vivarium managers, principal investigators, and outside animal vendors are challenged with balancing the number of animals to be produced with the anticipated demand. Forecast too low and there are unhappy customers; forecast too high and there are unhappy institutional officials and veterinarians and animal waste. Institutions have the moral and ethical responsibility to follow the tenets of the 3Rs. One way animal model producers can mitigate the risk of overproduction is by implementing a robust Sales, Inventory, and Operations Planning (SIOP) process to align animal production with demand. SIOP is an integrated business management process, through which an institution continually achieves focus, alignment, and synchronization among all functions. SIOP can be applied to animal production colonies of any size and type. The goal of SIOP is to honor the value of researchers' lab animals through a disciplined approach to production. A properly implemented SIOP process for research animal production involves routinely reviewing customer demand (by animal model, sex, age, and zygosity) and production supply resources (e.g. required breeders, space for breeding and holding cages, and genotyping) and replanning quantitatively across an agreed-upon rolling time horizon. When SIOP is applied to animal production, the overall output of the process is a production forecast that is updated monthly leading to a use plan, animal production plan, holding and euthanasia plan, new animal model development plan, strategic initiative plan, and resulting financial plan. The development of several key performance indicators is vital to understanding and measuring SIOP impact. Measures of forecast and production accuracy, on-time to receipt (OTTR), overproduction, and product level profitability are all indicators of success. However, the true value of SIOP is exemplified by a reduction in the number of animals overproduced. A well-executed SIOP process enabled one animal provider to reduce overproduction by 38%, a phenomenal result that aligns with the 3Rs. The guiding principles of the 3Rs, in place for over 35 years, are intended to evolve and improve tools for responsible animal use. SIOP is such a tool, capable of helping ensure animals are produced responsibly.

P33 Adapting Positive Reinforcement Training to Novel Laboratory Species

A Tresler,* C Rasbach, TL Stevens, W Williams

Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK

Positive reinforcement training (PRT) as a component of a comprehensive species-appropriate enrichment program is a common tool used by animal handlers as a method to train specific behaviors and reduce stress on animals involved in research. Our facility has historically used PRT with nonhuman primates, so we adapted the PRT model and complex enrichment when we started a project with the goat, a novel species at our institution. Goats are very intelligent and highly food motivated which makes them good candidates for learning and clicker training. The goal for this project was to use a PRT program and clicker training to acclimate the goats to indoor housing, routine procedures including physical exams, leg and hoof manipulation, passive range-of-motion, walking on a lead, and medication administration. Human interaction and treat preference testing was used to optimize compliance with PRT, starting with short sessions of 5–10 min of slow introductions to select personnel. We gradually increased human interaction to 20 min. Once we identified the high-value preferred treats for each goat, we coupled it with clicker training to facilitate a comprehensive species-appropriate enrichment and training plan. Radio, mirrors, and social housing were used to satisfy sensory enrichment. Task-oriented feeding with hay, fruits, and vegetables satisfied the manipulative component. Lead walking, range-of-motion, and PRT satisfied the social component. Treats, hay bags, and fresh produce satisfied the gustatory component. Goats were successfully trained

to desired behaviors which included voluntary consumption of oral medication, calmly walking on a lead or halter to various locations within the facility, walking on and off a floor scale, hold, target, and others. In addition, the goats became well-adapted to various animal caretakers, technicians, and veterinarians without evidence of neophobia. Our goat-adapted program is a successful example of using preference testing, gentle handling, complex enrichment strategies, and positive reinforcement training to achieve a low stress environment and highly enriched animals to accomplish research goals.

P100 Using a 14-day Flash Glucose Monitoring System as a Clinical Management Tool for Cage-Housed, Diabetic Nonhuman Primates

E Jackson,* E Mitchell, K Black, V Collins, N McInnis

Wake Forest Baptist , Winston Salem, NC

Naturally occurring diabetes occurs in many primate species, especially within aged research colonies. Additionally, many studies require experimental induction of diabetes in a large number of animals. Whether naturally occurring or experimental, the nature of some laboratory goals and procedures may further complicate long term clinical management. Regular glucose monitoring can be difficult and time consuming, and may cause stress and inaccurate results if animals have not been trained for blood collection procedures. Based on our experience, a commercially available 14-day flash glucose monitoring system can be a useful veterinary management tool for cage-housed primates. Following external implantation of the device, serum glucose levels can be monitored consistently with the use of a scanner. Continuously recorded data allows for more accurate insulin titration in both newly diagnosed diabetics and for existing, difficult to manage cases without the need for repeated blood collection.

P101 A Novel Treatment for Rodent Rectal Prolapse

A Bland,[1] F Moore,[1] T Heather,[2] H Pisharath,[1] A Funk,[1,*]

[1]ARC, SJCRH, Memphis, TN; [2]Veterinary Pathology Core, St. Jude Children's Research Hospital, Memphis, TN

Rectal prolapse is a common clinical problem in mice that inevitably results in early euthanasia. Surgical placement of a purse-string suture is an option for severe cases, but it is time consuming and invasive. Nonsurgical palliative care involves the application of topical ointments for local analgesia to manage discomfort or osmotic agents to reduce the prolapsed tissue. As an attempt to identify effective alternatives to our standard of care of topical lidocaine jelly, we compared the efficacy of topical 2% phenylephrine, a potent vasoconstrictor, in combination with 50% witch Hhzel, a commercially available astringent. Initially, a proof of principle pilot study was performed in 10 mice. We randomized mice with rectal prolapses into 2 groups and assigned them to receive either the experimental treatment (phenylephrine) or standardized treatment (lidocaine jelly). Mice received up to 3 treatments within a 2-wk period and those that had severe prolapses were euthanized immediately. None of the 5 mice that received the standard lidocaine jelly had improvement in clinical signs or protection from progressively worsening disease. Three of the 5 mice receiving the experimental phenylephrine treatment had no rectal prolapses. At the conclusion of 2 wk, 9 mice were sent for diagnostic histopath. Given the favorable response from the pilot study and the historical lack of clinical improvement to our institution's standard of care using lidocaine jelly, we monitored efficacy of the new treatment on clinical cases where the researchers were offered the experimental treatment as an alternative to euthanasia. Over a 3-mo period, 8 of the 11 mice on this pilot clinical trial, treated once weekly with the combination vasoconstrictor and astringent, did not have a recurrence after an average of 6 wk of consecutive treatments. The remaining 3 mice continued to have rectal prolapses and were euthanized. Given the encouraging results, our plan is to conduct a larger facility wide randomized study to compare this novel approach to the standard of care. To our knowledge, this preliminary study is the first to report a noninvasive and practical symptomatic treatment for rectal prolapse in mice.

P102 Increased Incidence of Corneal Ulcers in 5xFAD Mice

D Lovell,[1] M Hendren,[2] S Rankin,[2] H Pisharath,[2] T Heather,[3] A Funk,[1,*]

[1]ARC, SJCRH, Memphis, TN; [2]Immunology, St. Jude Children's Research Hospital, Memphis, TN; [3]Veterinary Pathology Core, St. Jude Children's Research Hospital, Memphis, TN

Over a 7-mo period, 43 cases of corneal ulcers were found in a population of 230 5XFAD mice (18.7% incidence), which are used to model neurodegenerative diseases like Alzheimer's disease (AD). The incidence of eye problems in our facility during this same time span was 0.44%. Clinically, these mice presented with buphthalmoses, corneal masses, and some orbital tightening. Menace responses were abnormal, suggesting that there were possible optic tract lesions or other intracranial lesions affecting the lateral geniculate nucleus and/or rostral colliculus. Of those cases, 3 were submitted for diagnostic histopathology. Abnormalities found in the eyes included mild blepharoconjunctivitis, mild keratitis, unilateral or bilateral, healing corneal ulcers and/or, anterior uveitis. Additionally, all mice had multifocal amyloid plaques and foci of gliosis at all levels of the brain that were consistent with having a 5XFAD phenotype. The entire peripheral and central visual pathway must be intact for a normal menace response to occur. Abnormal menace responses with no visual deficits and normal facial nerve functions and in the presence of amyloid plaques throughout the brain suggest that lesions are either located in key anatomic locations that govern the blink reflex pathway like the rostral colliculus. Alternatively, cerebellar cortical degeneration affecting the functions of the corticopontocerebellar pathway that modulates blinking may also cause an abnormality. Therefore, the corneal ulcers were suspected to be associated with a delay in the blink response caused by a predisposition to develop neurodegenerative lesions and leading to keratitis sicca. It has been reported in human cases that altered neurological functions associated with having an AD phenotype may affect eye movements and blinking. The 5XFAD model may also have the same predispositions to developing corneal and other eye lesions like some human patients. To the authors' knowledge, this report is the first to characterize a predisposition for 5XFAD mice to develop corneal ulcers and other eye pathologies.

P103 Intrahepatic Cholangiocarcinoma in a Hamadryas Baboon (*Papio hamadryas*)

C Sella Paranzini,[1,*] MG Tamimi,[1] K Rivas-Wagner,[1] A Ellis,[2] PR Morales,[1]

[1]Veterinary, The Mannheimer Foundation, Hometead, FL; [2]Pathology, Antech Diagnostics, Fountain Valley, CA

During a routine biannual physical exam, a 21-y-old outdoor-housed, 13.70 kg, female, hamadryas baboon (*Papio hamadryas*), was noticed with generalized icterus and abrupt weight loss. The animal was transferred to the hospital for a comprehensive evaluation and medical care. Laboratory findings included anemia, hyperproteinemia, and severely elevated liver markers (ALT 352 U/L, ALP 10,493 U/L, GGT 341 U/L, bilirubin 12.6 mg/dl, cholesterol 453 mg/dl). Abdominal ultrasound revealed hepatomegaly with mildly distended biliary ducts and increased echogenicity of the gallbladder with thickening of the duct walls. Despite having a good appetite and normal stools, the animal was euthanized due to continued weight loss, abnormal liver values, and overall poor prognosis. At necropsy, the liver was grossly pale-yellowish and severely enlarged. A hard, off-white mass (2 cm in diameter) was extending from the gallbladder and biliary duct's wall. Mediastinal lymph nodes were enlarged. Sections of the liver,

spleen, and mediastinal lymph nodes were fixed in 10% formalin solution and sent for histopathology. The slides were prepared and stained with H & E. Under light microscopy, the liver contained a loosely demarcated unencapsulated, infiltrative mass that replaced the bile duct and hepatic parenchyma. The mass was composed of polygonal epithelial cells that formed branching, variably sized ducts bordered by a prominent scirrhous reaction. Biliary carcinomas tend to be aggressive with frequent and widespread metastasis; metastatic disease was not identified in this case, although the possibility of micrometastases in tissues not examined histologically cannot be completely excluded. Case reports of primary hepatobiliary neoplasms in nonhuman primates are scarce. To the author's knowledge, this is the first report of an intra-hepatic cholangiocarcinoma in a geriatric female baboon.

P104 Three Dog Night: Managing 28-Hour Survival Anesthesia in Canines

A Brown,[1,*] J McGrath,[1] C Mitchell,[2] GW Salyards,[3] M Niekrasz,[1,2]

[1]Animal Resources Center, University of Chicago, Chicago, IL; [2]Department of Surgery, University of Chicago, Chicago, IL; [3]Division of Veterinary Resources, National Institute of Health, Baltimore, MD

Prolonged survival anesthesia, while required for certain types of studies, presents unique challenges for the veterinary team to consider and manage to ensure animal welfare and comfort and data yield for the researchers. We recently provided support for survival anesthesia in 3, 25 kg mixed-breed female dogs previously implanted with microelectrode arrays in the visual cortex. These arrays needed to be stimulated constantly under anesthesia for 28 h, with measurements taken at set intervals. Due to the length of the procedure, both the investigative and veterinary teams worked in shifts to ensure the animals were properly monitored and to reduce the risk of errors secondary to fatigue. Anesthesia was induced with an intramuscular injection of buprenorphine (8.4 mcg/kg), ketamine (2.8 mg/kg), and dexmedetomidine (12.6 mcg/kg), followed by oro-tracheal intubation and maintained with isoflurane (1–2.5%). Anesthetic parameters including oxygen flow rate, isoflurane percentage, heart rate, body temperature, noninvasive blood pressure, end tidal CO2 (EtCO2), ECG tracing, ventilation rates, SpO2, and fluid totals were monitored continuously and recorded every 15 min. The canines were placed on a mechanical ventilator (tidal volume 10–15 mL/kg) and urinary catheters were placed to monitor urine output. Baseline hematocrit and blood glucose values were taken, and subsequent values were taken approximately every 3 h to adjust intravenous fluid rate and type as needed. At the same intervals, a technician would move the animals' limbs and adjust their positions to prevent decubital ulceration. At the end of the procedure, we assessed the success of these efforts and determined several modifications to be made in future procedures. We would not use a stereotactic frame, as we noted lesions on the inner lip of one dog corresponding to the placement of the mouth bar. We would also have extra anesthetic circuits readily available, as excessive moisture caused aberrant readings of increased EtCO2 that resolved when the circuits were replaced. Future staffing would also be split into 3 teams instead of 2, which would reduce or eliminate the overtime required from each staff member.

P105 Lipid Bound Extended-release Buprenorphine Effectively Attenuates Postoperative Hypersensitivity in an Incisional Pain Model in Mice (*Mus musculus*)

K Navarro,[1,*] K Jampachaisri,[2] M Huss,[1] C Pacharinsak,[1]

[1]Comparative Medicine, Stanford University, Sunnyvale, CA; [2]Mathematics, Naresuan University, Phitsanulok, Thailand

Sustained-release buprenorphine is used to manage postoperative analgesia in laboratory mice for up to 3 d. A new FDA-approved extended-release buprenorphine (XR) was recently released to the laboratory animal medicine market for use in rodents, but little is known about its use in mice. In this study, we examined the efficacy of XR. The aim was to investigate whether a high dose of XR effectively attenuates postoperative hypersensitivity better than a low dose of XR in a mouse model of incisional pain. Male C57BL6/J mice (n = 44) were randomly assigned to 1 of 4 treatment groups: 1) saline (1 mL/kg SQ, once); 2) sustained-release buprenorphine (Bup-SR, 1 mg/kg SQ, once); 3) low-dose extended-release buprenorphine (XR-lo, 3.25 mg/kg SQ, once); 4) high-dose extended-release buprenorphine (XR-hi, 6.5 mg/kg SQ, once). Mechanical and thermal hypersensitivity were evaluated daily on days 1, 0 (4 hrs), 1, 2, and 3. Both mechanical and thermal hypersensitivity were observed in the saline group on day 0 and day 1; values were comparable to baseline by day 2, indicating mice experienced pain on day 0 and day 1, but were comparable to baseline by day 2. Bup-SR, XR-lo, and XR-hi attenuated mechanical hypersensitivity on day 0 and 1 postoperatively. While XR-lo attenuated thermal hypersensitivity on day 0, neither XR-lo nor XR-hi attenuated thermal hypersensitivity on day 1, when we expected mice to be experiencing pain. There were no abnormal clinical observations or gross pathologic findings in any of the groups. Results indicate that a high dose of XR did not effectively attenuate postoperative hypersensitivity better than a low dose of XR in a mouse model of incisional pain. The data suggest that XR-lo (3.25 mg/kg) effectively attenuates both mechanical and thermal hypersensitivity postoperatively while XR-hi (6.5 mg/kg) attenuates mechanical but not thermal hypersensitivity. Our results also suggest XR-Lo and XR-Hi are comparable to Bup-SR in attenuating mechanical hypersensitivity on days 0 and 1, and suggest XR-Lo may be more effective in attenuating thermal hypersensitivity than Bup-SR.

P106 Peripheral Blood Vessel Diameter and Systolic Blood Velocity in the Yorkshire Swine Animal Model: A Report of Normals Data for Size and Age

S Gabriel,* WA Spinks, B Bellamy, K Szymczyk, CZ Cannon

CPDT, BD, Research Triangle Park, NC

Swine are a common animal model in the development, safety assessment, and refinement of medical devices due to their cardiovascular similarities to humans. There is limited published data to reference normal swine peripheral blood vessel diameter and systolic blood velocity. The purpose of this study was to measure maximum systolic blood velocity and vessel diameter in commonly accessed swine peripheral vessels for publication of swine normal data to apply to in vitro, in vivo, and/or in silico model development in consideration of the 3Rs. Six Yorkshire male and female swine, average body weight of 24.5 kg., were used in this IACUC-approved study. Swine were anesthetized and ultrasonography was used to visualize peripheral vasculature, rotating between 3 operators over the study time period. Sixteen vessels were visualized and measured per animal, including the left and right saphenous artery, cephalic artery, femoral artery, carotid artery, saphenous vein, cephalic vein, jugular vein, and femoral vein. Vessel diameter was measured on all 16 vessels. Systolic velocity was measured for 10 vessels using pulsed wave (PW) Doppler per individual swine. Vessel diameter and maximum systolic velocity were averaged across all animals and compared to average swine body weight. The current data set indicates that the carotid artery characteristics steadily increase with age and bodyweight; however, smaller diameter vasculature does not follow similar patterns. Data collection is ongoing in older, larger bodyweight swine to be reported at a later date. This data set provides references for swine peripheral vessel diameter and systolic velocity for use in development of translational models for procedure and medical device development. It supports the 3Rs with use of imaging for refinement of data collection, the reduction of future swine needed as it provides normal range reference data, and potential for replacement of swine by development of in silico models to assess early device designs.

P107 Mycobacteriosis in an Adult Zebra Finch (*Taeniopygia guttata*)

SJ Mangosing,[1,*] S Monette,[1,2] C Echeverri,[1,3] LL Diaz1,[3]

[1]Tri-Institutional Training Program in Laboratory Animal Medicine and Science, New York, NY; [2]Center of Comparative Medicine and Pathology, Memorial Sloan Kettering & Weill Cornell Medicine, New York, NY; [3]The Rockefeller University, New York, NY

A 20-mo-old, experimentally naïve, group-housed, female zebra finch presented with an approximately 0.6 cm diameter, right-sided, soft, periorbital mass. There were no clinical abnormalities observed in the cagemates. The animal was euthanized, and a necropsy performed. On gross examination, the mass was located in the inferior conjunctiva and extended over the cornea of the right eye. The surface of the solid mass was yellow, but it was pale tan on cut surface. Histologically, it was unencapsulated, moderately well-circumscribed, and composed of spindle cells arranged in interlacing streams with a scant fibrovascular stroma characteristic of a soft-tissue sarcoma of fibroblast, smooth muscle, skeletal muscle, or peripheral nerve sheath origin. Incidentally and most notably, moderate to marked lymphoplasmacytic and histocytic inflammation was also observed in multiple tissues, including the liver, heart, spinal cord, brain, intervertebral synovial joints, proventriculus, ventriculus, esophagus, kidneys, small intestine, pancreas, oviduct, thyroid, crop, skin, and skeletal muscles. Differential diagnoses included mycobacteriosis, systemic isosporosis (also known as atoxoplasmosis), and fungal infection. Ziehl-Neelsen staining of the inflammatory lesions revealed the presence of intracellular, acid-fast, non-filamentous beaded rods, consistent with *Mycobacterium* spp. Although the animal did not exhibit clinical signs attributed to the mycobacterial infection, the severity of the lesions indicate that the bird would not have been suitable as a research subject and could have served as an infectious source to cage mates and personnel. Importantly, avian mycobacteriosis is of zoonotic concerns for immunosuppressed individuals. It is most commonly caused by *Mycobacterium avium* and *M. genavense*, and is rarely reported in laboratory zebra finches. This case highlights unusual incidental findings in a zebra finch, which have not previously been detected in our facility, and the importance of pathogen screening and surveillance.

P108 Exercise and the Overweight Research Beagle: Will it Succeed when a Controlled-calorie Diet Has Failed?

S Tyhuis*

Merck & Co., West Point, PA

Canines are commonly used in the research environment, with the beagle being the most widely used breed. Obesity is nearly as common in research dogs as it is in household pets. Obesity is linked to a multitude of health issues leading to an overall unhealthy animal and increases the amount of compound required for study purposes. The ability to restrict calories is vital to achieving weight loss in these animals. Unfortunately, due to advanced age, genetics, and a lack of activity, some of these canines do not lose weight with a reduced diet alone. Implementing an exercise plan can be an important component of a weight loss program. This study evaluated exercise frequency as a variable, combined with a controlled-calorie diet, in achieving weight loss and improving body condition score. Overweight beagles were exercised outside of the pens in their housing room either not at all, 1, 3 or 5 days a week (groups 1, 2, 3 and 4, respectively) for 30 min per day and their body weights and body condition scores were collected and analyzed weekly during an 8-wk study period. Twenty animals were selected for study with 5 per exercise group. Study animals were over 3 y of age and had been on a restricted diet for at least 1 y with minimal weight loss. All animals but 2 lost weight, with groups 3 and 4 achieving statistically significant average weight loss (8.58 and 7.31%, respectively). Body scores also showed a decrease in most animals with groups 3 and 4 having the most significant changes (0.7, 0.6, 0.8, and 1.0 for groups 1–4, respectively). Another benefit was that the dogs undergoing exercise (and the dogs remaining in their pens) were engaged mentally and physically during the exercise times, thus providing enrichment for all the animals in the room, not just the study cohort. This may also account for the weight loss in the control group. Maintaining an appropriate weight is key to the general health and wellness of the canines, thereby making them better research subjects.

P109 Histiocytic Sarcoma with Bone Marrow Involvement in a Naïve CD-1 Mouse

G Mendoza,[2,*] S Kelly,[1] A Michel,[3] LL Diaz,[1]

[1]Comparative Bioscience Center, The Rockefeller University, New york, NY; [2]Tri-Institutional Training Program in Laboratory Animal Medicine and Science, Memorial Sloan-Kettering Cancer Center, Weill Cornell Medicine, and The Rockefeller University, New York, NY; [3]Center for Comparative Medicine and Pathology, Memorial Sloan-Kettering Cancer Center, Weill Cornell Medicine, New York, NY

A 16-mo-old female, experimentally naïve, CD-1 mouse presented with a distended abdomen. The mouse was housed in a large static isolator cage with 9 other clinically normal female mice. The animal was provided with irradiated corn cob bedding, hyper-chlorinated (~2 PPM) water and fed irradiated rodent chow ad libitum since arrival from the vendor. The mouse was used for restraint, venipuncture, and parenteral injection training. Physical exam revealed, in addition to the distended abdomen, moderate lethargy, a thin body condition (BCS 2.5/5), and perineal fecal staining. On palpation, the abdomen was soft and fluid filled. Differential diagnoses for distended abdomen in an aged CD-1 mouse included neoplasia (lymphoma, histiocytic sarcoma, plasmacytoma, mesothelioma, other), congestive heart failure, liver failure (hepatitis, hepatopathy, and cholangiohepatitis), protein-losing enteropathy or nephropathy, amyloidosis, acute necrotizing pancreatitis, peritonitis secondary to organ (GI or gall bladder) puncture and leakage following intraperitoneal injection, and less likely bladder rupture. The animal was euthanized due to age and submitted for post-mortem examination. Gross necropsy findings revealed a moderately enlarged liver, spleen, and kidneys. The cranial pole of the left kidney had a discoid, pale tan 0.8 x 0.8 x 0.2 cm mass. The left uterine horn was markedly expanded by a tan, multilobulated, semi-firm, partially encapsulated 2 x 2 x 1 cm mass. The right uterine horn had a circular, soft, tan, flat 1 x 10 x 2 cm mass. Histologic evaluation revealed changes within the heart, lungs, mediastinal lymph nodes, kidney, liver, mesentery, uterus, bladder, spleen, ovaries, adrenal capsule, perineurium of the mandible and bone marrow. The histologic and immunohistochemical findings were consistent with histiocytic sarcoma. This case highlights a relatively common tumor found in aged CD1 mice with atypical bone marrow involvement. The research significance, and clinical and cytologic features will be presented.

P110 Assessment of a Novel Procedure to Reverse Mouse Penile Prolapse

SM Setter,[1,*] IF Choi,[2] PW Setter,[1] HR Goold,[1] M Fink,[1] L Habenicht,[1] DL Fong,[1] LK Jori,[1] CA Manuel,[1]

[1]Office of Laboratory Animal Resources, University of Colorado Denver | Anschutz Medical Campus, Aurora, CO; [2]Department of Craniofacial Biology, University of Colorado Denver | Anschutz Medical Campus, Aurora, CO

Little is known about the pathogenesis of penile prolapses in male mice used in biomedical research. The current standard of care is to maintain tissue health and viability with daily lubrication in the hope of spontaneous resolution. Without resolution, unfavorable outcomes include chronic maintenance or euthanasia. In an attempt to reverse the condition, we developed a novel and noninvasive procedure that uses a lubricated stainless-steel probe to reinsert the penis into the prepuce. To determine the efficacy of this new procedure, as well

as the impact of a steroid-containing lubricant to aid reinsertion, we performed the following study. Following researcher consent, penile prolapse cases identified across the entirety of the campus mouse population were enrolled in the study. A physical exam was performed to record body condition, penis and genital health, presence of comorbidities, breeding status, and age to establish a severity-based scoring system. To determine if the lubricant used to reinsert the penis had an effect, mice were randomly assigned to 2 treatment groups. The first group underwent reinsertion using a sterile lubricant while the second group was given a lubricant containing dexamethasone. Twenty animals were enrolled in the study, with 10 animals per group. Our novel method of reinsertion reversed the condition in 85% (17/20) of cases with no reoccurrence after 2 wk. When inflammation was not present, 100% (10/10) of reinsertions were successful. In cases with penile inflammation, manual reinsertion was successful in 70% (7/10) of cases. No appreciable difference was observed in the success rate between lubricants with or without steroids ($P = 0.56$). We have identified 4 primary categories of comorbidities associated with penile prolapses: penis trauma or inflammation, foreign material or infection in the prepuce, secondary to a congenital issue like hydrocephalus, and idiopathic. Based on this study, manual reinsertion appears to be a successful and proven option for all cases except for those with congenital issues. It is clear that murine penile prolapse is a multifactorial condition requiring additional investigation into contributing factors and effective treatment options.

P111 Necrotizing Fasciitis of the External Genitalia in a Male Rhesus Macaque (***Macaca mulatta***)

RE Cooper*, SM Birch, MS Vermillion

Department of Comparative Medicine, Lovelace Biomedical and Environmental Research Institute, Albuquerque, NM

A 3-y-old individually housed male rhesus macaque (*Macaca mulatta*) presented for lethargy and inappetence of 2 d duratio and mild preputial erythema. Symptoms rapidly progressed despite treatment with broad-spectrum antibiotics and other supportive care. Within 1 d, erythema progressed to moderate scrotal and preputial swelling, and right-sided focal scrotal necrosis. Within 3 d, necrosis had expanded to affect the entirety of right scrotum, part of the prepuce, the left scrotum, and adjacent perineum. The animal was euthanized after 4 d of treatment due to rapid disease progression and poor prognosis. Necropsy and histopathology revealed severe necrotizing fasciitis with abscessation of the scrotum, prepuce, and perineum, originating from the right side and extending to the left; samples of the necrotic tissue yielded minimal growth of *Klebsiella pneumoniae*. Both testes were unaffected, and appeared normal by both gross and histopathologic examination. Although not previously reported in the rhesus macaque, the clinical symptoms, rapid progression timeline, and necropsy and histopathology findings were consistent with a diagnosis of Fournier's gangrene. This condition is rare but life-threatening in humans, in which it is associated with opportunistic polymicrobial infection; risk factors for Fournier's gangrene in humans comprise many causes of immunosuppression and microcirculatory compromise. In the current case, no underlying disease was identified by prior physical exam, bloodwork, or necropsy.

P112 Evaluation of Therapeutic Interventions for Rectal Prolapse in Mice Leads to Reduction in Early Euthanasia

CM Mitchell,[1,*] GW Salyards,[2] BR Theriault,[1] G Langan,[1] KR Luchins,[1]

[1]Animal Resource Center, University of Chicago, Chicago, IL; [2]Division of Veterinary Resources, National Institutes of Health, Bethesda, MD

Rectal prolapse (RP) in mice is a common clinical condition, and contributing factors include parturition, genetics, or infectious pathogens. There is no standard treatment, and options range from lubrication, surgical correction, to euthanasia. Due to the potential for pain, distress, and interference with research, euthanasia is commonly elected. The goal of this study was to assess potential treatment options that would maintain the RP tissue to reduce early study removal and euthanasia. This study utilized 120 mice with spontaneous RP, which were concurrently assigned to ongoing research protocols. Mice were randomly assigned to 1 of 3 treatment groups: petroleum jelly, lidocaine jelly, or no treatment (control). Treatment was applied 3 times per week for up to 3 mo or until reaching their protocol-driven research endpoint. At presentation, fecal samples were collected for pathogen testing, and all mice received an initial base score: RP length, gross mucosal health, pain and distress, and body condition. Mice then received weekly blinded scores, minus RP length. Euthanasia criteria was developed based on the individual scoring parameters. Upon euthanasia, RP tissue was collected for histopathology. Of the 120 mice identified, 47 (39%) were breeders, with 13 (28%) successfully producing 22 additional litters total. Of the 73 (60%) non-breeders, 67 (92%) were able to reach their intended research study endpoint or the 3-mo study endpoint. There was no statistical significance between the 3 groups based on gross mucosal health, pain and distress, or histopathology. In the lidocaine jelly group, there was a statistically significant decrease in the growth rate of the RP compared to the petroleum jelly and the control group. Statistical significance was also seen in the BCS of the lidocaine jelly group compared to the other 2 groups. Of the 120 mice, 96% tested positive for *Helicobacter* spp. In this study, no mice in any group were euthanized based on the aforementioned RP scoring criteria. These findings demonstrate that treatment is unnecessary to maintain the RP or animal welfare. In adherence to the 3Rs, this study supports animal number reduction and clinical refinement, allowing animals to contribute to their intended research study endpoints.

P113 Perioperative Care in an Adult Male Capybara (*Hydrochoerus hydrochaeris*)

KR Strait*, J Amilcar

GCMI, Atlanta, GA

Capybaras, the world's largest rodent, are infrequently found in biomedical research settings but have been proposed as an alternative animal model of ischemic stroke. Our lab acquired one 18-mo-old male capybara for a model feasibility study of ischemic stroke creation under anesthesia. Although rodent anesthesia methods are well described, there is little in the literature specific to capybaras. Here we describe perioperative care including anesthetic induction and maintenance in an adult male capybara undergoing cerebral vascular occlusion. The capybara was anesthetized in the home cage with 0.75 mg/kg tiletamine + zolazepam, 0.0075 mg/kg dexmedetomidine, and 0.075 mg/kg butorphanol IM. The initial dose of medication was insufficient to allow safe movement of the capybara and was supplemented with 5% isoflurane via mask. During surgical preparation, anesthesia was maintained at 2% isoflurane in 2L/min oxygen. An intravenous catheter was placed in the cephalic vein. Enrofloxacin 10 mg/kg SC and meloxicam 0.3 mg/kg SC were administered as a prophylactic antibiotic and preemptive analgesic, respectively. Endotracheal intubation proved to be challenging due to difficulty visualizing the airway. Ultimately, intubation was accomplished with the animal in sternal recumbency and positioned with the head hyperextended to align the oropharynx, larynx, and trachea. A bronchoscope facilitated airway visualization. In the operating room, the animal was maintained on 1–2 % isoflurane in 2L/min oxygen on a ventilator. Lactated Ringer's solution was delivered IV at a rate of 2–5 mL/kg/hr. Physiologic parameters were recorded every 15 m, including heart rate, temperature, blood pressure, blood oxygen saturation, and end tidal carbon dioxide. The animal remained stable throughout the procedure and no anesthetic complications were encountered. Euthanasia was elected in the immediate postoperative period due to complications from stroke. For future capybara procedures, the induction dose of anesthesia medication will require alteration to

achieve adequate sedation. Endotracheal intubation was the most challenging aspect of the procedure and can be approached similarly to methods described for rabbits.

P114 *Burkholderia gladioli* Infection in an Immunocompromised Rat Strain

BT Adedeji*, F Noto, S Moody, C Dunn, E Baldwin, D Scott, J Stanley, H Stills

Invivo, Hera Biolabs, Lexington, KY

Thirty of the about 190 rats in our barrier-maintained colony of immunocompromised rats, [a Sprague–Dawley *Rag2/Il2rg* double knockout (SRG OncoRat)] presented with varying degrees of lethargy, abdominal distension, and pustules on their limbs, muzzle, ears, and tails. About 50% of the infected rats during the initial outbreak became moribund within 5 d from the onset of clinical signs and were euthanized. On necropsy, milliary diffuse abscesses were found in the subcutis of 2 of the 30 reported cases. Other necropsy findings included hepatomegaly with multifocal pale areas on all lobes, splenomegaly, milliary abscessation of the testes and scrotal sacs, and enlarged discolored kidneys. Fresh fixed samples of the spleen, liver, kidney, and testes were collected and submitted for histopathology and swabs of the liver, kidney, spleen lungs, testes, and pustules were submitted for bacterial culture. *Burkholderia gladioli* was isolated from all the samples. Histopathology revealed a myriad of intrahistiocytic gram-negative rods in multiple samples. Severe disseminated, a histiocytic and suppurative disease diagnosed with *Burkholderia gladioli* as the etiologic agent. A literature search revealed no documented report of B. gladioli in any strain of immunocompromised rats and sparse reports in mice. Major presentation of the infection in mice include abscesses in the ear canal and cranium, seizures, torticollis, and rolling. Environmental testing was performed, and samples of the autoclave, water, and test articles were submitted for culture. The sanitation and microisolation procedures were reviewed and modified to improve effectiveness with retraining of all technicians. Although we were unable to directly identify the source of the bacterial contamination, we believe the likely source of exposure was through the water source or feed. The feed type was changed from a pelleted to an extruded diet, a less dense and heat-treated feed that improved sterilization via irradiation. After the initial outbreak, bacteria isolation and testing, all rats presenting with signs of infection were placed on a medicated diet containing 200 mg/kg Doxycycline Hyclate (Doxy diet) ad libitum. This treatment was effective at halting disease progression and mortality.

P115 Unilateral Renal Nephroblastoma with Ovarian Adhesion and Peritoneal Metastasis in an Adult Female Wister Rat

OL Franco Mahecha*, S Carrasco, HR Holcombe, JG Fox, S Muthupalani

Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA

An adult 5-mo-old naïve female Wistar rat presented for necropsy in poor body condition, with a right sided abdominal distension and a large palpable internal mass. Additionally, the animal exhibited hair loss over the right shoulder and porphyria. On gross necropsy, a large (6 x 5 x 4 cm), well-encapsulated, soft, reddish-brown mass filled with blood was found on the visceral aspect of ventral abdominal cavity in the region of the right kidney. There was adhesion of the right renal mass to the right ovary. The mesenteric lymph nodes were enlarged and red/brown. The urinary bladder was filled with red-dark urine. Histologically, the fairly well-demarcated and encapsulated mass replaced most of the normal renal parenchyma and was moderately cellular, pleomorphic with multifocal hemorrhage, edema, massive necrosis, and moderate amounts of fibro-vascular stroma. On a cellular level, the mass was composed of 3 distinct types of neoplastic cells representing epithelial, mesenchymal, and blastemal cell populations. Neoplastic blastemal and epithelial cells in nonnecrotic regions showed marked anisocytosis and anisokaryosis with hyperchromatic nuclei and prominent nucleoli. Mitotic figures were 5-6/40X HPF and few aberrant mitotic figures were also present. There was intracapsular multifocal extension and seeding of the poorly differentiated epithelial cords, tubules, mesenchymal and blastemal cells that extended to the extracapsular space, into the adjacent peritoneum and periovarian fibro-adipose tissue adhering to the mass. The mesenteric lymph nodes had medullary congestion/hemorrhage, and sinus histiocytosis with hemosiderin-laden macrophages. Immunohistochemistry revealed that neoplastic epithelial cords/tubules were positive for pancytokeratin. Mesenchymal and blastemal cells were positive for Wilm's tumor antigen-1 (WT-1). On the basis of the histomorphology and immunohistochemical features, the right renal mass was diagnosed as nephroblastoma with ovarian adhesions and extracapsular and periovarian metastasis. Spontaneous nephroblastoma is the only embryonal renal tumor reported in rats and has a very low incidence (≤0.1%). Notably, the pathology service at our institution has received 2 cases of nephroblastoma in rats during the last year.

P116 Hot Bead Sterilization of Rodent Surgical Instruments

J Holdridge,[1,*] M Wheaton,[1] W Dupont,[2] CP Jones,[1] K Shuster,[1]

[1]Department of Pathology, Biology, and Immunology, Vanderbilt University Medical Center, Nashville, TN; [2]Department of Biostatistics, Vanderbilt University Medical Center, Nashville, TN

Surgical aseptic technique is used to create and maintain a sterile working field, reducing the likelihood of microbial contamination and subsequent infection. One strategy employed for rodent surgeries is a "tips-only" aseptic technique, which restricts the surgeon to using only the sterile working ends of the surgical instruments to manipulate the surgical field and utilizes a hot bead sterilizer between consecutive rodent surgeries. Despite the common use of a "tips-only" technique, research is lacking on how many sequential surgeries the same set of bead-sterilized instruments can undergo without introducing bacterial contamination. The purpose of this study was to evaluate the effectiveness of hot bead sterilization in preventing aerobic bacterial growth on the tips of surgical forceps and needle-drivers. We hypothesized that hot bead sterilization (Germinator 500TM) utilizing the "tips-only" technique would effectively prevent aerobic bacterial growth on surgical instruments between five consecutive mouse splenectomies per surgical pack. Animals were aseptically prepped before surgery using three alternating applications of chlorhexidine scrub and 70% isopropyl alcohol. Between surgeries, instruments were manually cleaned of visible debris with sterile saline soaked gauze before being placed into the hot bead sterilizer according to manufacturer instructions. Instruments were then swabbed for aerobic bacterial culture and the presence of ATP as a marker for organic debris. In a preliminary experimental group for which a deliberate break in asepsis was introduced after completing each mouse splenectomy, using hot bead sterilization between surgeries maintained negative post-sterilization aerobic cultures through a series of 12 consecutive surgeries using the "tips-only" technique. On average, forceps had significantly higher ATP measurements than needle drivers, but there was not a significant difference in ATP levels over time for either instrument. These preliminary results support the use of hot bead sterilization between rodent surgeries when using the "tips only" technique. Future work will include establishing evidence-based guidance on the number of sequential aseptic rodent surgeries that can be performed with the same surgical pack.

P117 Rectal Prolapse in a Female Cotton Rat (*Sigmodon hispidus*)

TA Collins,[1,*] A Sparks,[1] D Coble,[2]

[1]University Laboratory Animal Resources, The Ohio State University, Columbus, OH; [2]Abigail Wexner Research Institute at Nationwide

Children's Hospital, Columbus, OH

A 2-mo-old female cotton rat (*Sigmodon hispidus*) presented with a hunched posture and ruffled hair coat. Physical examination revealed moderate dehydration and poor body condition. No dentition or oral cavity abnormalities were noted. Urogenital examination revealed a 1.5 cm opening at the level of the anus with impacted fecal, fur, and bedding material and a rectovaginal fistula was suspected. The animal was anesthetized with isoflurane for closer examination and all foreign material was removed. The skin of the affected region was erythemic and contained scabbed lesions. The animal was euthanized secondary to the clinical appearance and poor prognosis. Postmortem pathological examination ruled out the presence of a rectovaginal fistula. Sections of the reproductive and gastrointestinal tracts were submitted for histopathology. Microscopic examination of the rectum revealed a prolapsed and moderately proliferative rectum with moderate, localized edema. Histology confirmed that there was no fistula between the colon and reproductive tract. The definitive diagnosis was a severely prolapsed rectum with secondary self-mutilation. Rectal prolapses are rarely reported in cotton rats, however treatment options for mild cases can include monitoring and lubrication. As in other rodent species, clinical treatment is often unrewarding. Given the disruption of normal function and pain associated with this condition, the standard of care is euthanasia.

P118 Nasal Dermatitis in Small Ruminants

E Houston,* J Helbling, M Burton, AR Personett, LV Kendall, JD Ayers

Department of Laboratory Animal Resources, Colorado State University, Fort Collins, CO

Goats and sheep are common laboratory animals used in a wide range of research applications. Small ruminants can be sourced from a variety of vendors. Random-bred animals may have a multitude of diseases, some of which are zoonotic and pose occupational health risks to personnel. In our flock of 150 sheep and 30 goats, used for orthopedic research, concern of zoonosis was raised when multiple animals intermittently developed crusts and erosions around the nares and mouth. Precautions were taken to protect veterinary staff from contracting Orf virus during physical exam. Four goats and 1 sheep presented with raised crusts along the commissure or the lateral margins of the nares. These crusts were removed manually, revealing erythematous skin and mild erosions underneath. Removal resulted in proliferative scabs reoccurring within days. Upon presentation, animals were moved to isolation and additional safety measures communicated to staff. rtPCR from the lesion was negative for parapoxviruses, including contagious ecthyma virus or Orf. To rule out photosensitization related to ingestion of photodynamic plants, a chemistry panel was performed and revealed normal liver values, mild hypermagnesemia, and hyperglobulinemia. The dry lot was examined for such plants and affected animals were not on any medications. No cleaning agents that could contribute to photodynamic skin changes were used on the premises. All animals showed no evidence of depression, weakness, pruritus, or erythema associated with defective pigment synthesis. Physical exam revealed no signs of infectious or systemic disease causing liver pathology, and animals had not been recently vaccinated or exposed to biologics. No vesicles were observed and lesions were non-painful. Lesions were not present in the oral cavity and few animals were affected, making foot and mouth disease virus or vesicular stomatitis unlikely. No lesions erupted in the remainder of the herd following initial presentation. A full thickness punch biopsy was taken from the crusted lesions, showing nonspecific moderate chronic superficial inflammation with acanthosis and orthokeratotic hyperkeratosis. No bacteria were visualized on biopsy, nor were pustules, flakes, or nodules consistent with *Staphylococcus aureus* observed. Lesions were not present on the coronary band or teats, making co-infection with *Dermatophilus congolensis* unlikely. Two sections of sarcocysts were present in skeletal muscle and considered incidental. No *Oestrus ovis* or other parasites were observed on exam or biopsy, and no nasal discharge seen. Zoonotic poxviruses were ruled out, the diagnosis was consistent with idiopathic inflammatory nasal dermatitis. After approximately 1 mo, lesions resolved spontaneously in all animals. Nasal dermatitis that presents similarly to Orf may be due to idiopathic inflammatory conditions, and diagnostic testing should be pursued to formulate an appropriate treatment and handling plan.

P119 Unexplained Abdominal Distension in an Aged C3HeB/FeJ Mouse

J Walton,[1,*] J Eggenschwiler,[2] MA McCrackin,[1] SB Harvey,[1] U Blas-Machado,[3,4]

[1]Department of Animal Resources, University of Georgia, Athens, Georgia; [2]Department of Genetics, University of Georgia, Athens, GA; [3]Department of Pathology, University of Georgia, Athens, GA; [4]Athens Veterinary Diagnostic Laboratory, Athens, GA

A 22-mo-old, male C3HeB/FeJ mouse was submitted for necropsy after euthanasia for unexplained abdominal distension. The mouse was maintained as a breeder and had not been subject to any other treatments or formed part of any experiment. On gross findings, a 3 x 2 x 2 cm mass occupied most of the right cranial quadrant of the abdomen. Differential diagnoses included hydronephrosis, renal abscess, nephritis, and primary or metastatic neoplasia, including but not limited to renal lymphosarcoma, hemangioma/-sarcoma, renal adenocarcinoma, and nephroblastoma. On histopathology, an expansive, locally infiltrative anaplastic mesenchymal neoplasm, with multifocal hemorrhage and necrosis (in > 50% of the tissues examined) had displaced the right kidney and adrenal gland, infiltrated into the retroperitoneal space and epaxial muscles, and extended into the overlying subcutis. Other findings included mild peritonitis/mesenteritis; mild interstitial pneumonia, moderate myocardial degeneration, and necrosis; mild exocrine pancreatic atrophy; moderate lymphoid hyperplasia; moderate splenic extramedullary hematopoietic hyperplasia; moderate left adrenocortical degeneration; mild rhinitis; and bilateral retinal degeneration, with loss of photoreceptor cell layer. Based on the histopathology findings, the mass was characteristic of an anaplastic hemangiosarcoma of the renal capsule, which had displaced the right kidney and extended into the retroperitoneum. No evidence of neoplasia was detected in other organs and other histopathology findings were most likely abnormalities associated with malignancy. Primary capsular hemangiosarcomas of the kidney are uncommon in both human and animal species, and diagnosis of spontaneous renal neoplasms are underreported in this strain of mouse. This case represents a new group of tumors for investigation and consideration in mouse oncology.

P120 Transient Hyperlipidemia in a Closed Specific-Pathogen Free Cat Colony (*Felis catus*)

M Burton*, E Houston, AR Personett, J Helbling, JD Ayers

Colorado State University, Fort Collins, CO

Transient hyperlipidemia is a heritable condition that occurs in kittens ranging from 3 to 8 wk of age. Clinical signs include inappetence, poor body condition and weight loss, lethargy, pale mucous membranes, and neurological signs ranging from unsteady gait to paraplegia in advanced cases. Over the past 3 y, there has been 3 incidences of transient hyperlipidemia in our closed breeding colony of approximately 20 specific-pathogen free cats. In 2017, a 6.5-wk-old female kitten from a litter of 3, presented with depressed mentation, pale mucous membranes, tachypnea, and a grade III/VI heart murmur. Bloodwork showed anemia (8%), marked lipemia (1003 mg/dl), and hypercholesterolemia (332 mg/dl). The entire litter was weaned from the queen and provided a low fat and high protein canned diet. Within 3 d the kitten was bright with pink mucous membranes. The murmur resolved within a week. The kitten was slowly transitioned to a standard dry kitten diet over the course of 4 wk and remains in the colony. As an adult, this female gave birth to

a litter of 3 kittens in 2020. At 6 wk of age, 1 of the female kittens was noted to be smaller than her littermates with a depressed mentation, mild dehydration, pale mucous membranes, and neurological signs. Blood obtained from this kitten was grossly lipemic and bloodwork revealed anemia (12%), hypertriglyceridemia (563 mg/dl), and hyperbilirubinemia (0.4 mg/dl). This kitten was weaned, started on a low fat and high protein canned diet, and received a whole blood transfusion. This kitten's condition declined, and she was euthanized. A third kitten, a 4-wk-old male from a litter of 2 from an unrelated queen, was observed to be smaller than his littermate and had pale mucous membranes. Consistent with the previous cases, his blood was also grossly lipemic. Within 2 d of switching his diet, the kitten's condition improved, and he gained weight. Transient hyperlipidemia is a hereditary condition that may appear in breeding cat colonies. To facilitate early detection of these cases, animal care staff was trained to identify kittens with pale mucous membranes or with decreased activity levels compared to littermates. We found dietary modification was successful in managing these cases if the condition is identified early in the course of disease.

P121 Comedocarcinoma with Severe Skin Ulcerations: A Unique Presentation in a Retired Sled Dog

J Draper*

Cornell University, Ithaca, NY

Mammary tumors are the most common neoplasms in female dogs, with carcinomas being the most common malignant type. Here we report a case of comedocarcinoma, a rare and highly malignant mammary neoplasm in a 9-y-old female spayed retired sled dog used in an aging study. The dog presented for erythema on the ventral abdomen. Physical examination revealed multiple raised ulcerated erythemic masses in the right mammary chain (20 cm x 5 cm) with no other abnormalities noted. Differential diagnoses included neoplasia, bacterial, or fungal skin infection, and autoimmune skin disorder. Fine needle aspiration confirmed mammary neoplasia with inflammation and necrosis. Histopathology yielded a diagnosis of grade III out of III comedocarcinoma with evidence of lymphovascular invasion. Medical management was elected over surgery due to negative prognostic indictors including the size, ulceration status, and lymphocytic invasion. The mean survival time of comedocarcinoma in dogs is 14 mo, although, in this case, the dog was euthanized 3 mo later due to increased severity of ulcerations. At necropsy, distant metastasis to pulmonary tissue and the muscle and serosa of the urinary bladder were noted. To our knowledge, this is the first case report of comedocarcinoma in a dog with severe skin ulcerations.

P122 Metastatic Hemangiosarcoma in a Young Lewis Rat

Ä Sipos,[2,*] J Brazzell,[1] JA Scholz,[2]

[1]BioAnalytics, IDEXX, Columbia, MO; [2]Department of Comparative Medicine, Mayo Clinic, Rochester, MN

A naive 5-wk-old intact male Lewis rat presented with a firm, nonmobile mass adjacent to the dorsal lumbar spine. The rat was intended to be used for training under an IACUC-approved protocol, and the decision was made to monitor the mass and assess the quality of life. Euthanasia was elected at 11 wk of age due to the growth of the mass and lethargy of the rat. Gross examination revealed a multilobular mass closely associated with the lumbar spine and multifocal dark nodular discolorations throughout the lung. Histology of the lumbar mass and pulmonary nodules revealed an infiltrative, multifocally necrotic and hemorrhagic malignant spindle cell neoplasm that formed subtle vascular channels and occasionally wrapped collagen bundles. Histopathology and gross pathology findings were compatible with a diagnosis of subcuticular and pulmonary hemangiosarcoma. Evidence of neoplasia was not identified grossly or histologically in the heart, liver, kidney, spleen, or adrenal gland concurrently sampled at necropsy. The subcuticular malignancy was presumed to be the primary lesion with metastases to the lungs although a primary pulmonary hemangiosarcoma with subcutaneous metastasis cannot be excluded from consideration. Hemangiosarcoma has been reported in aged laboratory animals but has only rarely been reported in young animals. The rare cases of hemangiosarcoma in young animals were limited to the liver and spleen of Sprague–Dawley rats. To the authors' knowledge, this is the first case report of subcutaneous and pulmonary hemangiosarcoma in a young Lewis rat.

P123 A Well-differentiated Hepatocellular Carcinoma with Pale Cytoplasmic Bodies in a Common Marmoset (*Callithrix jacchus*)

M Stair*, JL Haupt, JG Fox, S Muthupalani

Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA

A 9-y-old female experimentally naive common marmoset (*Callithrix jacchus*) was diagnosed with a cranial abdominal mass on routine physical exam. Ultrasound identified a vascular, heterogenous 13mm length mass cranial to the left kidney of uncertain origin and other clinical and hematological work up was unremarkable. Follow up exams 6 and 12 mo later revealed no changes in mass size or abnormal bloodwork. However, 18 mo after initial examination, elevated liver enzymes (GGT- 27 IU/L) and mild anemia (33%) were noted and the mass was identified to be of hepatic origin on ultrasound with no palpable change in mass size. Between 2.5-3.5 y after initial presentation, the animal's condition worsened over time with significant increase in liver enzymes (GGT and AST), persistent anemia, and decreasing body condition leading to euthanasia. On necropsy, a well-demarcated, soft, red to tan, cystic mass measuring 4cm x 3.7cm x3cm was found within the right lateral liver lobe. Both kidneys were tan with irregular pitted cortices (consistent with chronic nephropathy, a common age-related condition) and the animal had minimal fat stores. On histological examination, the multinodular liver mass was consistent with a well-differentiated hepatocellular carcinoma (HCC) composed of dense compact sheets or trabeculae of large neoplastic hepatocytes with mild atypia, frequent large pale cytoplasmic ground glass-like inclusions and rare cytoplasmic hyaline bodies. Within the neoplasm, large areas of peliosis hepatis and necrosis were noted. On special stains, there was an abnormal reticulin staining pattern and the large cytoplasmic inclusions were PAS-ve and weakly Trichrome +ve. Electron microscopy revealed that the neoplastic hepatocytes contained 1 or more membrane-bound dilated endoplasmic reticulum filled with finely granular electron dense material typical of pale bodies of HCC. Pale bodies are an uncommon feature in human HCC. Hepatic neoplasms are uncommon in marmosets, with HCC only rarely reported. This case represents the first report of pale bodies in a hepatocellular neoplasm in a common marmoset.

P124 Branchiobdellidans Parasitization of a Laboratory Crayfish Colony

D Savson*, EK Daugherity

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

A single-tank colony of crayfish (*Procambarus clarkii*) used for neurophysiology teaching purposes was reported by husbandry staff for the presence of multiple thread-like organisms adhered to their exteriors. Initial examination revealed dozens of 3–4mm long, white organisms that were firmly attached to the crayfish's chitin exoskeletons. All crayfish were bright, alert, and responsive to stimuli with no visible lesions or clinical abnormalities at the individual or colony levels. Several of the organisms were collected for further identification via light microscopy, and photographs were taken. Consultation with parasitologists and the literature produced a likely diagnosis for this organism of the annelid branchiobdellidans, which are considered obligate ectosymbionts. Historically, literature reports

appear widely divergent, describing either a mutualistic relationship that is characterized by few significant deleterious host effects, or a detrimental parasitic relationship between branchiobdellidans and their crayfish hosts. The precise nature of the relationship has been shown to be dependent on several branchiobdellidan factors, including species, abundance, and ecological context. There are currently no published reports of these organisms exhibiting zoonotic or harmful potential for humans, so the risk to researchers that interact with the host crayfish is considered negligible. Given the lack of external lesions among this crayfish colony no treatment was elected. Necropsy of a colonized crayfish and sampling of hemolymph with comparison to naïve colonies as an indicator of stress are presented along with a discussion of the need to ensure the health of invertebrates when striving for best research and teaching models.

P125 Unexpected Tumor Regression in Multiple FVB/N Mice following Orthotopic Inoculation with HER2-positive Mammary Gland Tumors

JA Herrod,[1,*] K Mulka,[1] K Gabrielson,[1] P Korangath,[2] R Ivkov,[2] JS Villano,[1]

[1]Molecular and Comparative Pathobiology, Johns Hopkins University, Baltimore, MD; [2]Oncology, Johns Hopkins University, Baltimore, MD

A cohort of 50 8-wk-old, female FVB/N mice that had been inoculated orthotopically in the mammary fat pad with serially passaged HER2-positive mammary gland tumors presented for unexpected failure of tumor development and spontaneous tumor regression. Half of the cohort never developed tumors while the remaining half developed tumors that reached approximately 2 cm3 by week 8 post-inoculation, instead of the previous laboratory record of 4 wk. Tumors developed in both untreated control and nanoparticle treatment groups then spontaneously regressed in all mice by week 10 post-inoculation. Following tumor regression, 3 mice presented with abdominal distension, and a large, discrete, mass was palpable in the caudal abdomen of all animals. One of the 3 mice was found dead and the remaining 2 were euthanized and submitted for diagnostic necropsy. Additionally, several mice had alopecia along the dorsum, which prompted fecal and skin PCR testing for *Corynebacterium bovis*, which has been reported to decrease tumor growth in mice. History revealed that there had been no change in personnel, technique, or animal vendors and that the researcher had been performing this exact procedure without issue for many years. PCR for *C. bovis* was negative. Both mice submitted for necropsy had large, soft, tan to pink, multinodular masses in the area of the ovary. Histopathology revealed an unencapsulated, poorly demarcated, densely cellular, multiloculated neoplasm composed of tissue representing 3 primordial germ cell lines consistent with an ovarian teratoma. Ovarian teratomas are uncommon in mice but have been reported in up to 2% of aged FVB/N mice. Tumor expansion requires appropriate host conditions and metabolic competition by another tumor has the potential to inhibit growth. We postulate that the decreased growth and eventual fast regression of tumor burden in these three mice was secondary to competition with ovarian teratomas.

P126 Continuous Glucose Monitoring in the Diabetic Ossabaw Pig: Sensor Placement

M McIntyre*, GE Geist

Center for Comparative Medicine, Northwestern University, Chicago, IL

Type-I diabetic pigs are a robust translational model in the preclinical evaluation of novel diabetic therapies. Adequate glycemic control is essential for optimal study outcomes and animal health, however, repeated conscious blood sampling can be technically challenging and aversive. Recently, there has been increasing usage of skin-patch continuous glucose monitors (CGM) in the veterinary setting. These noninvasive devices are FDA approved for human use, and have the potential to replace standard cageside blood sampling. While emerging research indicates that CGMs can be effectively employed in diabetic swine, there are limited data regarding CGM sensor placement and maintenance. Here, we present a durable and reliable device placement method to preserve sensor longevity. In this dataset, one 37 kg alloxan-induced diabetic female Ossabaw pig was studied over 8 wk. The CGM brand was selected based on previous reports in swine, physical characteristics (low profile, water resistance), and 14-d sensor duration. Under sedation, the lateral neck caudal to the ear base was shaved, cleansed with betadine scrub and 70% alcohol, and allowed to dry. The single-use CGM sensor was applied in the prepared area of thin, minimally mobile skin. A small amount of tissue glue was applied around circumference of the sensor. A 4 x 4″ transparent adhesive film was placed over the sensor, and secured with a perimeter of 2″ elastic tape and skin staples. Elastic stockinette dressing was fitted over the neck and cranial thorax for additional stabilization/protection. The sensor was interrogated twice daily using a corresponding handheld reader, and demonstrated adequate comparison to time-matched ear-prick glucometer and/or lab chemistry samples (n = 6 samples, Bland Altman mean difference 6±23% vs. CGM). Sensors were replaced in the same location every 14 d (n = 6 sensors). No episodes of device dislodgement or malfunction were encountered, and no observations of animal discomfort or behavioral disturbance were noted. Taken together, the CGM is a viable and practical option for chronic glycemic monitoring in the diabetic pig. Optimization of device location and placement permits a dependable, low-stress refinement for colony management, and the CGM should be considered as an alternative to conscious blood sampling.

P127 Factors Affecting Hematologic and Serum Biochemical Parameters in Healthy Common Marmosets (*Callithrix jacchus*)

RM Kramer,[1,*] A Sheh,[1] SC Artim,[1,2] C Toolan,[1,3] MA Burns,[1] JG Fox,[1]

[1]Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA; [2]Merck Research Laboratories, Merck, South San Francisco, CA; [3]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA

Common marmosets (*Callithrix jacchus*) are an increasingly popular animal model for neuroscience, aging, toxicology, and other areas of research. However, published data on normal parameters in captive marmosets is limited. Our objective was to determine reference ranges for hematology and serum biochemistry parameters in healthy marmosets in our colony and investigate how intrinsic animal factors, including age, sex, animal source, and pregnancy status, affect those reference ranges. Understanding the influence of these factors on diagnostic test results can aid in interpretation of tests performed during routine health exams and diagnostic work-ups. We retrospectively examined marmoset medical records to correlate animal signalment and source with clinical pathology results from our in-house diagnostic laboratory obtained during routine health examinations. Samples were categorized by age (235 juvenile, 316 adult, 74 geriatric), sex (331 female, 294 male), pregnancy status (45 pregnant and 286 non-pregnant) and original source population (5 colonies with 22 to 352 samples per colony). Reference ranges for blood parameters in healthy animals were calculated after excluding potential outliers using the 1.5x interquartile range rule. Statistically significant differences occurred for at least some parameters (Kruskal–Wallis test with post-hoc pairwise Wilcoxon rank-sum test with Benjamini–Hochberg corrections), for all categories (sex, age, source colony, and pregnancy status), with variation more likely in serum biochemistry than hematology panels (n = 20 versus n = 19 laboratory tests per panel). No variation was observed in amylase, potassium, and mean corpuscular hemoglobin concentration across animal categories, while the parameters most likely to be statistically different were alkaline phosphatase, total calcium, and cholesterol. Of all factors, animal source had the potential to affect the greatest number of

parameters. Age and pregnancy status had the smallest effect on clinical pathology parameters, and animal sex had an intermediate effect. Overall, this analysis supports previous recommendations that institutions should determine their own in-house reference ranges for optimal interpretation of clinical pathology results in marmosets.

P128 Idiopathic Bone Disease in a Common Marmoset (*Callithrix jacchus*)

J Brockhurst,[1,*] C Peterson,[1] S Beck,[1] JM Izzi,[2]

[1]Molecular and Comparative Pathobiology, Johns Hopkins School of Medicine, Baltimore, MD; [2]Research Animal Resources, Johns Hopkins School of Medicine, Baltimore, MD

A 10-mo-old, group-housed, male common marmoset (*Callithrix jacchus*) presented with acute onset nonweight bearing lameness in the right arm. The animal was experimentally naive with no significant clinical history. Mild, diffuse muscle atrophy and generalized pain in the right arm was noted on physical exam. Radiographs revealed lytic lesions affecting the right radius, ulna, and humerus, with diffuse cortical thinning and expansion of the diameter of the radius and ulna compared to the left limb. A suspect pathologic, mid-diaphyseal humeral fracture was also noted. Bloodwork was unremarkable aside from elevations in AST and ALP. The marmoset remained painful with complete disuse of the arm despite multimodal analgesia, and euthanasia was ultimately elected due to poor prognosis. Postmortem imaging showed evidence of marked osteolysis and bony proliferation of the bones of the right arm. Histopathology revealed extensive myelofibrosis with cortical bone changes and periosteal bony proliferation. No parathyroid, renal, or gastrointestinal lesions were noted. Bone disease is well recognized in common marmosets, although disease presentation is diverse. Metabolic bone disease is frequently associated with "marmoset wasting syndrome" (MWS) which can lead to mineral and hormonal imbalances. Clinical signs and hematologic changes typically associated with MWS, such as significant weight loss and decreased serum albumin levels, were not present in this animal, suggesting a diagnosis of idiopathic bone disease. In cases of idiopathic bone disease, underlying etiology is not well understood; however, the age, focal distribution, and histopathologic findings in this case most closely resemble fibrous dysplasia. This disease presentation is rare, with only 1 similar case identified in our colony and few reports in the literature. Further work needs to be done to clarify bone disease in the common marmoset.

P129 Refinement of Standardized Care of Ocular Lesions in Mice

C Hunter,[1,*] A Skorupski,[1] J Lawrence,[2] PS Allen,[3]

[1]Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI; [2]Iowa State University, Ames, IA; [3]Boehringer Ingelheim, Ridgefield, CT

Spontaneous, non-experimentally induced, ocular lesions are a common clinical concern in research mice. Well-defined humane endpoints are essential in treatment considerations as ocular lesions may quickly progress and be a major source of pain. In order to streamline clinical care for common ocular cases, a revised standard of care (SOC) providing guidance for clinical care of mouse ocular cases was implemented. The revised SOC provides a clinical flowchart with clinical scores of 1 to 4 based on progression and severity of ocular lesions dictating if a case is suitable for continued monitoring by veterinary technicians, treatment options provided by veterinary consult, or is severe requiring euthanasia. Cases with scores of 3 or above are to be immediately transferred to veterinarians for treatment options. In a prospective study, we hypothesized that the revised SOC would lead to clinical resolution for appropriate cases and create a more efficient workflow for animal care staff. Strain, sex, diagnosis, treatment, and outcomes of spontaneous ocular cases were followed over a 4-mo period. Out of a total of 59 cases, conjunctivitis and corneal ulcers accounted for 70% of reported cases. Lesions occurred in females at 64%, in the right eye at 51%, and on mice with a C57BL/6 background in 95% of cases. For cases in which topical therapy was appropriate, combined topical treatments of antibiotics and analgesics led to resolution or maintenance to study endpoint in 60% of cases. Four of these cases required an enucleation, most commonly for perforated ulcers. No significant difference was found for topical treatments applied once versus twice a day. Twenty percent of cases required euthanasia after no improvement. Remaining cases were placed on continuous monitoring. Our findings suggest that topical treatment or enucleation is a viable option for ocular lesions deemed clinically appropriate by our revised SOC. The SOC provides technicians with a clinical score scale dictating escalation to veterinary consult and shows that ocular cases should be evaluated for possible treatment, providing a useful option for researchers using mice on C57BL/6 backgrounds known to commonly be effected.

P130 The Effects of Increased Therapeutic Doses (20 Mg/kg) Of Meloxciam and Carprofen on the Welfare of Laboratory Mice

AL Bailey, B Singh, BJ Smith, W McGee, LV Kendall*

Laboratory Animal Resources, Colorado State, Fort Collins, CO

Nonsteroidal antiinflammatory drugs are among the most commonly used drugs for analgesia in laboratory mice. However, little to no clinical data exists for those commonly used analgesics in mice and dose ranges to effectively treat their pain. The common doses currently used appear to be inadequate as an analgesic, and increased doses may be more appropriate. However, NSAIDs can potentially cause gastrointestinal and renal toxicity. The aim of this study was to evaluate the toxic effects of high-dose meloxicam and carprofen in female Crl:CD1(ICR) mice. Mice were assigned to 1 of 3 groups (n = 12 per group) and received 20 mg/kg of meloxicam (Melox), 20 mg/kg of carprofen (Carp), or an equivalent volume of saline, subcutaneously daily for 7 d. Mice were euthanized on day 8 and day 15 (n = 6 per group per day). Feces was collected for occult blood; liver, spleen, kidney, lung, heart, stomach, duodenum, and ileal-cecocolic junction were collected for histology; and blood was collected by cardiocentesis for serum chemistry. All mice appeared clinically normal for the 15-d duration of the study. At day 8, 5/6 of the mice treated with Melox were positive for fecal occult blood compared to the Carp and saline treated mice that had 0/6 and 1/6, respectively. Histologic evidence of gastritis was present in 4/6 mice treated with Melox, compared to Carp and saline treated mice that each had 1/6. At day 15, 1/6 mice treated with Melox and 1/6 mice treated with Carp were positive for fecal occult blood and none of the saline treated mice had fecal occult blood. Gastritis was present in 5/6 of Melox treated mice, compared to the Carp and saline treated mice that each had 1/6. There were no significant differences in the serum chemistry profiles among the groups, and there were no histologic lesions in any other tissues examined. The high-dose Melox caused a gastritis when used daily for 7 d with persistent gastritis for at least 1 wk, whereas high-dose carprofen has minimal side effects. These findings suggest that Carp given at 20 mg/kg may be safely administered in mice for analgesia; whereas high dose Melox causes toxicity.

P131 A Novel Presentation of Mouse Urologic Syndrome In Lpgat1 $^{+/-}$ Male Mice

M Klores,* S Rasmussen, S Peneyra

Division of Comparative Medicine, New York University Langone Health, New York, NY

Multiple adult male C57BL/6NJ-Lpgat1$^{em1(IMPC)J}$/Mmjax (LPGAT1 $^{+/-}$) mice, a novel transgenic strain developed to determine extramitochondrial cardiolipin's role in spermatogenesis, presented with lethargy and penile erythema, edema, and ulceration. Upon physical examination, palpable firm masses within the urogenital region were appreciated. Clinically symptomatic mice were all adult

male breeders and approximately 23% of sires within the colony were affected. Differential diagnoses included penile trauma, ulcerative dermatitis of the preputial area, preputial abscessation and cutaneous neoplasia. Due to a poor clinical prognosis mice were euthanized and were subsequently submitted for histopathology. Upon postmortem examination, the bladder was found to be severely distended, and there was a firm, yellow, friable mass encasing the distal aspect of the penis. On histopathology, the preputial lumen contained abundant colonies of Gram-positive cocci admixed with proteinaceous concretion and spermatozoa. Bilateral cystic dilation, adenitis of the preputial glands, and similar colonies of Gram-positive cocci were observed within the lateral excretory ducts. The accessory sex glands, testes, and urinary bladder had no significant pathology. Due to the clinical presentation, gross necropsy findings, and abundant proteinaceous debris noted within the urethral lumen, an obstructive uropathy was suspected. A diagnosis of ulcerative balanoposthitis was made, presumably secondary to a form of Mouse Urologic Syndrome (MUS). MUS is an obstructive uropathy that presents variably depending on the chronicity, but is often associated with ulcerative balanoposthitis, urinary bladder distension, urolithiasis, chronic suppurative cystitis, and hydronephrosis. Interestingly, a lack of *Lpgat1* and the subsequent alterations in cardiolipin synthesis may predispose male mice to the development of MUS due to alterations in spermatozoa development and lipid characteristics, and may represent a novel model for obstructive uropathy.

P132 Reducing Stress in Rabbits during Multiple Noninvasive Ocular Imaging Procedures

E Tolley,[1,*] M MacMillan,[1] M Ortega,[1,2] K Krueger,[2]

[1]Animal Care, Mass Eye and Ear, Boston, MA; [2]Schepens Eye Research, Boston, MA

Stress can alter physiologic and metabolic processes within the body, and refining procedures to minimize stress is one of the 3Rs adhered to in animal research. In our program, adult New Zealand white rabbits undergo weekly anesthetic events to obtain ocular images several times over 1 h using a slit lamp, which allows visualization of structures of the eye. Over time, we noticed the rabbits appeared stressed, by behavior changes (e.g. jumping/kicking) upon entering the procedure space prior to anesthetic injection. Rabbits are transported from their housing to the procedure room, where they are anesthetized with ketamine (35mg/kg), xylazine (5mg/kg), and acepromazine (1mg/kg) IM. Once anesthetized, they are placed on a portable stand and for each image their head is manipulated to align the eye and camera. Room lights are repeatedly turned off/on to acquire images and record data throughout the procedure. The rabbits frequently woke up prematurely, requiring additional ketamine. We believe this was caused by initial stress and external stimuli. Our multimodal approach began with administering the sedative acepromazine (1mg/kg) SC in their home cage ~15 min before transportation. The researchers were consulted, and agreed to minimize head movement and talking, and implemented placement of dim tap lights throughout the room in place of overhead lights to allow imaging to remain functional with minimal light interruptions. Additional ketamine usage was determined by exhibited signs of waking up (e.g. heart rate, blinking, head movement). Over 10 anesthetic events, acepromazine was given IM in 6 events and SC in 4 events. In the IM group, all rabbits required additional ketamine—averaging to 4/6 events in each rabbit. For the SC group, the average dropped to 1/4 events in each. Rabbits receiving acepromazine SC had a longer duration of anesthesia from time of induction compared to the IM group and were noticeably easier to handle with reduced excitation/kicking when induced. The frequency of supplemental ketamine was decreased, with no complications and normal recovery. The combination of the pre-emptive use of acepromazine, reducing noise, movement, and low light levels resulted in researchers reporting a serene atmosphere, calm and less fractious animals, and more efficient procedures.

P133 Isolation of *Jeotgalicoccus* spp. from the Tail Lesion of a Laboratory Mouse

TE Whiteside,[1,*] MD Johnston,[1] M Cesta,[2] DM Kurtz,[1]

[1]Comparative Medicine Branch, National Institute of Environmental Health Science, Research Triangle Park, NC; [2]Cellular and Molecular Pathology Branch, National Institute of Environmental Health Sciences, RTP, NC

Dermatitis in laboratory mice (*Mus musculus*) is an endemic issue in most animal research populations. Dermal lesions can range from mild alopecia and inflammation to severe ulceration requiring euthanasia. The cause of murine dermatitis is multifactorial and usually involves a combination of dermal trauma, primary or secondary bacterial infection, and genetics/immune response. Treatment is palliative and most often via the use of topical antimicrobials in combination with antiinflammatories with varying efficacy. The most common bacterial isolate in cultures of dermal lesions we perform are *Staphylococcus* spp., including *S. aureus*, *S. xylosis*, and *S. lentus*. Recently, we cultured a member of the genus, *Jeotgalicoccus* spp., from a dermal lesion on the tail of an adult, female, Swiss Webster mouse (Tac:SW). *Jeotgalicoccus* spp. are gram-positive cocci that tend to prefer high salinity environments and are members the family *Staphylococcacea*e first reported in 2003. To date, 10 species have been reported with the fist, *J. halotolerans*, isolated from a fermented Korean seafood called jeotgal. Since then, a few species have been isolated directly from animals (oral cavity of a southern sea lion) or animal housing areas (poultry and swine). The genus has also been reported as a commensal member of the mouse gut microbiome, and the gut flora is the most likely source of the isolate. However, there have been no reports of any members of this genus associated with disease. Here, we report the isolation, biochemical analysis, and sequence determination of *Jeotgalicoccus* spp. isolated from the tail lesion of a laboratory mouse and the histological evaluation of the lesions.

P134 Degenerative Myelopathy Secondary to Aberrant Engraftment of Intracranially Injected, Human-origin Induced Pluripotent Stem Cell (ipsc)-derived Microglia In Nsg-csf1 Mice

D Annamalai*, S Carrasco, OL Franco Mahecha, S Maddur Ganesan, A Aguilar, JG Fox, S Muthupalani

Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA

Hind limb paralysis and motor dysfunction are sporadic clinical conditions in mice. There are several causes for these conditions, including trauma, infection, neoplasia, and genetics which often require detailed evaluation. As part of an experimental study, a cohort of NSG-CSF1 mice were injected intracranially at postnatal d 1 or 3 with myeloid lineage cells differentiated from iPSC expressing GFP to generate human-mouse microglial chimeric mice to study neurodegenerative diseases. A subset of mice from this study showed clinical signs of either hind limb paralysis (2/4) or mild to moderate motor dysfunction (2/4) with poor body condition between 26 to 37 w post-injection. Due to poor prognosis, the affected mice were euthanized for detailed evaluation. On gross necropsy, 2 mice with paralysis showed marked kyphosis of the thoracic vertebral column and the other 2 mice showed no obvious gross abnormalities. Histopathologically, the cervical thoracolumbar spinal cord of all the mice (4/4) had mild to moderate multifocal degenerative myelopathy and radiculo/ganglionopathy of the spinal nerve roots characterized by myelin sheath dilation, axonal swelling, fragmentation, axonal loss with multifocal white and grey matter foci of abnormal poorly differentiated spindloid to vaguely polygonal ovoid cellular aggregates with occasional mitosis, reactive fibrosis, congestion, and mild gliosis. Additionally, these abnormal poorly differentiated cellular aggregates were also noted within the brainstem with none to minimal reactive or degenerative changes. On immunohistochemistry, these poorly differentiated cells in the brainstem and spinal cord segments showed positive GFP immunoreactivity. These findings indicate the aberrant translocation

of the injected myeloid cells from the brain to the spinal cord and secondary engraftment and proliferation with associated spinal cord degenerative lesions leading to hind limb paralysis and/or motor dysfunction. Further, there was no evidence of any associated pathology and/or GFP immunoreactivity in any visceral organs. Hence, it is imperative to perform thorough pathological analysis to differentiate natural causes from treatment related pathological lesions causing paralysis and motor dysfunction in laboratory animals.

P135 Atypical Clinical Presentation of Ferrets (*Mustela putorius furo*): Same, Same, but Different

V Mrotz*

Centers for Disease Control and Prevention, Atlanta, GA

Three atypical clinical presentations of ferrets demonstrate the need for animal care staff and researcher preparedness for such conditions. Ferret A, 7-y-old MC, was reported for hind limb lameness and on exam the left hind stifle was found to be swollen. Radiographs showed osteolytic lesions at the proximal tibia; however, no evidence of metastases were seen. An FNA on the left tibia found the bone soft and easily penetrated with a 18G needle; microscopic review confirmed lymphoma. Ferret B, <1-y-old MC, was found in his cage, unresponsive in lateral recumbency. On exam Ferret B became responsive and blood glucose was found to be normal; however, hind limp paresis remained apparent and was confirmed with a neurologic exam which also found absence of deep pain and anal tone. Due to poor prognosis and the assumed diagnosis of intervertebral disc disease, Ferret B was euthanized. Histology of lesions found on the meninges, kidney, and liver confirmed multicentric B-cell lymphoma. Ferret C, 1.5-y-old MC, was reported for a lump on his back. A soft subcutaneous approximately 1 in long and 0.5 in tall mass was found on the right dorsal abdomen. Radiographs showed a soft tissue opacity that extended anteriorly and posteriorly to the vertebra and fragments of mineral opacity suggesting involvement of the last right rib. Ultrasound confirmed no clear association to surrounding organs and an initial FNA was inconclusive. Within 2 wk, the mass doubled in size, the animal was euthanized, and histology diagnosed the mass as a non-differentiated sarcoma. Ferrets are the gold standard model of respiratory diseases in humans, such as influenza and more recently SARS-CoV-2. As the demand for this model grows, so must the ability of animal care teams to quickly and correctly identify and address atypical presentation of clinical conditions.

P136 Spontaneous Basal Cell Tumor in a Laboratory Rat (*Rattus norvegicus*)

C Si*

University of Michigan, Ann Arbor, MI

A 6-mo-old intact male Sprague Dawley rat (*Rattus norvegicus*) socially housed under specific-pathogen free conditions was examined for a skin lesion. This rat was used in behavioral studies involving sucrose rewards and lacking surgical intervention. Physical examination revealed a 1 cm diameter round, raised subcutaneous lesion on the right ventral thorax. The lesion had serosanguineous discharge and central cavitation and the rat was reactive to its manipulation. Non-neoplastic differential diagnoses included an iatrogenic lesion, self- or cagemate trauma with secondary inflammation, or bacterial infection. Neoplastic differential diagnoses included papilloma, keratoacanthoma, basal cell tumor, and squamous cell carcinoma. Analgesia and topical therapy were instituted and toenails were trimmed to prevent self-trauma but the lesion did not resolve. At experimental endpoint, 3 wk after initial presentation, the rat was euthanized. Gross necropsy did not reveal any organ abnormalities apart from the skin lesion, which had grown to be 1.5 x 1.2 x 0.5 cm with irregular, proliferative edges. Microscopically, the lesion consisted of a multilobular dermal mass of basaloid epithelium connected to an exophytic epidermal portion of well differentiated squamous epithelium. Basaloid cells within the dermal portion of the mass were monomorphic, had a low mitotic index, and formed nodules and cribriform structures with occasional cystic central degeneration. These findings were consistent with a basal cell tumor. Basal cell tumors arise from germ cells of the hair follicle and form slow-growing expansile dermal masses. They are not generally common skin tumors in rats but have been described, particularly in older Sprague–Dawley rats. As in this case, they have also been seen rarely in younger Sprague–Dawley rats. Local dermal expansion is common but since the tumors are slow growing and metastases are rare, surgical excision would most likely have been curative.

P137 Whonet Surveillance Facilitates the Identification of Multidrug Resistant Pathogens, Including Meropenem Nonsusceptible *Enterobacteriaceae* from Macaque Cephalic Implants

A Garcia*, EM Buckley-Jordan, JG Fox

Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA

Neurosurgical procedures performed in nonhuman primates for implantation of cephalic recording devices are critical in cognitive neuroscience research. However, despite the use of prophylactic antibiotics, cephalic implants can become colonized with bacteria exhibiting different antibiotic susceptibility profiles. Analyses of these profiles can provide empirical guidance for prevention and/or treatment of potential disease sequelae including intracranial infection. We employed WHONET, a free software from the World Health Organization (WHO) Collaborating Centre for Surveillance of Antimicrobial Resistance, to analyze the profiles of 166 cephalic implant and 8 brain abscess isolates collected during a period of ~4 years from macaques (N = 14; 6 females and 8 males) in our facility. The most prevalent organisms were *Staphylococcus aureus* and *Corynebacterium* spp., cultured from 86% and 79% of the macaques, respectively. Preliminary antibiograms using disk diffusion data showed that all *S. aureus* isolates were 100% and 92% susceptible to cefazolin and ceftriaxone, respectively, but 56% were nonsusceptible (NS) to enrofloxacin. Most *E. coli* isolates were multidrug resistant (MDR) exhibiting 100% nonsusceptibility to ceftriaxone and ampicillin (21 of 21 isolates), including 19 *E. coli* isolates that were 100% NS to both cefazolin and enrofloxacin. All *Enterococcus faecalis* isolates (6) were MDR with 33% exhibiting combined nonsusceptibility to linezolid, vancomycin, and meropenem. Potential extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* (ceftriaxone NS) (45 isolates) included 98% MDR organisms, 4 of which were brain abscess isolates. MDR Enterobacteriaceae included 4 meropenem NS isolates. Confirmation of nonsusceptibility by other microbiological and molecular methods is warranted. The intra- and inter-institutional creation of bacterial isolate databases using WHONET analyses may enable epidemiological studies of laboratory animal isolates with nosocomial, research, and public health implications, and the development of protocols to curtail their proliferation.

P138 Pregnancy-associated Thrombocytopenia and Bone Lesions in a Common Marmoset (*Callithrix jacchus*)

S Maddur Ganesan*, OL Franco Mahecha, S Muthupalani, JL Haupt, JG Fox

Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA

A 2.6-yr-old, primiparous pregnant female common marmoset (*Callithrix jacchus*) presented with left arm lameness during late gestation (130 d). Physical examination showed no palpable abnormalities of left arm, however diffuse petechial hemorrhages were noted on left arm and thorax. Ulcerations were present on the ventral surface of both tarsi from presumed decreased

activity. Bloodwork revealed marked thrombocytopenia (76k/ul), polycythemia (9.6x106/ul), hemoconcentration (Hct 65%), and hypocalcemia (1.0 mmol/L). Ultrasound revealed at least 2 feti with normal fetal heart rates. Differentials for thrombocytopenia in late-stage gestation included gestational/hereditary/immune thrombocytopenia and preeclampsia. The animal was treated daily with dexamethasone (1 mg/kg IM), supportive fluids, meloxicam, orbax, famotidine, oral calcium supplementation, and closely monitored. After 48 h of treatment, the animal delivered 4 stillborn feti (3 normal size, 1 mummified). On the following day, retained placenta was removed by gentle traction. However, petechial lesions progressed to purpura and ecchymosis with subcutaneous edema on ventral thorax, both forelimbs and hindlimbs. Repeat bloodwork revealed worsening thrombocytopenia (64k/ul). Radiographs indicated osteolytic lesions in radius, ulna, and humerus. Based on the deteriorated clinical condition, euthanasia was performed. Necropsy revealed extensive subcutaneous hemorrhages in left arm, thorax, right axilla, mammary gland, and softening of affected bones. On histopathology, the left humerus and scapula showed severe multifocal osteoclastic bone resorption, bone loss, hemorrhages, immature woven bone formation, and fibrous stromal proliferation consistent with fibrous osteodystrophy and reactive bone lesions. Fibrous osteodystrophic lesions were also noted in the skull. The link between thrombocytopenia and bone lesions if any, is unknown in this case. Various bone pathologies have been reported in young to old marmosets of both sexes including pregnant animals similar to the findings in this animal. This case highlights the need for close monitoring of marmosets in late gestation. To our knowledge, this is the first report of pregnancy associated thrombocytopenia in common marmosets.

P200 Post-transport, Maintenance, and Postoperative Care of Geriatric Care of Rats Using a Novel Commerically Available Feeding Device

H Maxwell*

Shimadzu Institute- ACF, University of Texas at Arlington, Arlington , TX

While the majority of research uses young, healthy animals, some studies require subjects to be aged or geriatric. Due to their aged status, they tend to be poorly conditioned and are more susceptible to morbidity and mortality during shipping and postoperatively than young, healthy adult animals. At our institution, an increased mortality rate of geriatric rats was observed post-shipping, despite standard supportive care during shipping by the vender and a high-caloric diet provided upon arrival at the facility. Additionally, there was a small, but increased mortality rate often during the standard acclimation period, as well as postoperatively. Diagnostic testing excluded infectious causes of death. To address this problem, moistened chow and sunflower seeds were provided immediately upon arrival post-shipping as a part of the daily standard of care, including postoperatively. The supportive care was provided via a novel commercially available administration device that allows for food to be provided in multiple ways (hung from wire bars, cage floor) rather than standard petri dishes. The novel device is composed of high-temp polysulfone that looks like an upside-down sombrero. To date, since implementing this simple change in husbandry using this device, there has been no mortality from shipping, daily maintenance, or postoperatively. The rats that receive that supportive care daily have maintained their hydration and body condition versus the geriatric animals that originally did not receive such supplementation. Before we began giving the geriatric rats supportive care daily, we were losing 20% of the animals from the vendor. Since starting the geriatric animals on supportive care, at the time of arrival, we are seeing 100% survival rate.

P201 Low-cost Alternatives for Group Housing Rabbits on the Floor

T Humbird*

CCM, Baylor College of Medicine, Rosharon, TX

When the *Guide for the Care and Use of Laboratory Animals* was updated in 2011, there was a change in height requirements for rabbit cages from 14″ to 16″. This rendered the existing caging systems at many facilities obsolete. Our facility took advantage of this opportunity to try group housing female rabbits on the floor. In order to transform an open room into a rabbit paradise, we purchased several sets of doggie play pens panels so that we could set up large introduction pens and still section off study groups. These panels were able to withstand normal cage wash temperatures up to 190 degrees, which made them a perfect, low-cost choice. A small, temporary play pen or full-sized shift space was used on full change out days. Daily spot cleaning was done with the animals in place as they enjoyed the human interaction. The floors were lined with cardboard-like sheets sized 48″ x 60″, which we covered with fluffy cellulose, paper bedding. On a weekly basis the paper and bedding could easily be rolled up and disposed of by technicians working in pairs. To provide privacy when needed, large 24″ PVC tunnels were placed in the runs as well as extra panels secured with carabineer clips in a lean-to area for rabbits to rest. Extra-large mouse cages (5″ h x 9″ w x 17″ l), lined with straw, were strategically placed in the corners of runs which the rabbits used as "potty boxes." These boxes were changed out at least 2 times per week and greatly helped to keep the living space drier and cleaner. A variety of floor and hanging enrichment toys are provided and changed out weekly. Other enrichment items included timothy hay and produce placed in empty, autoclaved glove boxes, to encourage foraging behaviors. In conclusion, open-topped pens enriched the rabbits by providing ample room for group sizes of 2 to 6 rabbits to periscope as well as demonstrate other species-specific behaviors such as digging, standing, and "binkying" (jumping and twisting in the air, which expresses happiness and comfort). The best suited studies for the floor housed rabbits are ones that allow for a lot of activity of the animals and interaction between animals and humans. Between 2017 and 2019, 96 female rabbits ranging in weight from 1.8 to 2.8 kilograms were successfully group housed in a low-stress environment.

P202 Effectiveness of Hybrid Hydrogen Peroxide in Decontamination of Isolators, Their Contents, and Their Filters

FM Grinstead,[1,*] D Holland,[3] L Watkins,[2]

[1]CURIS System, Oviedo, FL; [2]Animal Facility, UT MD Anderson Cancer Center, Houston, TX; [3]CBClean, Madison, WI

Risks associated with pathogen exposure from "germ-free" chambers and enclosures are well known and documented. Therefore, bio-decontamination devices are fundamental to maintaining the safety of these enclosures. Traditionally, enclosures have been decontaminated via service providers due to the inherent hazards in exposure to high concentrations of chemical sterilant in vapor or gaseous form. In addition, disassembling isolators, discarding filtration, and spraying with caustic chemical like peracetic acid and chlorine dioxide result in an average of 3 d for the chambers to dry out completely and become reusable. This laborious process delays delivery of food, bedding, and supplies to the chamber. Innovations that maintain efficacy but increase the safety, ease, and speed of gnotobiotic decontamination would greatly enhance research capabilities. Is safer, low-level 7% hybrid hydrogen peroxide (HHP) a viable method to achieve 99.9999% decontamination of a chamber, its contents, and its filters while decreasing overall treatment time and labor? Building on the proven success of a 7% pulsed hydrogen peroxide system in a biological safety cabinet and its plenums, researchers tested a modular, integrated 7% HHP system's ability to decontaminate a gnotobiotic chamber. An 1100-cubic foot isolator was precleaned using standard site protocol and 23 *Geobacillus stearothermophilus* biological indicators (BIs) were placed in critical locations throughout the chamber (including inside the gloves, transfer port, and filters, plus 1 control). The HHP system delivered 1 hour of primary/secondary injection, 2 hours of extraction, and

1 hour of neutralization. At the end of 4 hours, BIs were collected and incubated. All 23 BIs were successfully inactivated, proving it is possible to successfully decontaminate a chamber with safer, lower levels of H2O2 vapor via a modular, integrated 7% HHP system. No other known technologies decontaminate the whole chamber and its filters in one treatment. In contrast to current 3-d treatment times, a safer, easier, 6-log decontamination and turnaround of an isolator in only half a day is a revolutionary innovation for chamber decontamination.

P203 Edible Nail Polish as a Novel Enrichment Strategy for Singly Housed Nonhuman Primates

J Martin,[2] E Liebl,[2] W Williams,[1,*]

[1]Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK; [2]Oklahoma School of Science and Math, Oklahoma City, OK

Nonhuman primates in biomedical research require highly complex enrichment strategies to keep them engaged in species-appropriate behaviors and provide for psychological well-being. When nonhuman primates need to be singly housed for research purposes or veterinary care, it can be difficult to keep them optimally enriched which sometimes results in destructive behaviors such as picking at sutures and bothering wounds or incisions. In this project, we sought to determine if edible nail polish applied under sedation would stay on long enough to serve as a detractor for singly housed primates receiving sutures or wound repair. All primates that were sedated over a 6-mo period received edible nail polish made from a combination of ingredients including fruit extract, corn starch, flour, xanthan gum, and fresh blended fruit. The color (red or orange) and recipe type were recorded, along with individual animal ID, gender, age, and injury status. Recipe A stayed on significantly longer than recipe B at 1.28 days and 0.04 days, respectively ($P < 0.01$), and the polish stayed on significantly longer in animals over 15 y old compared to animals under 15 y old at 1.5 days and 0.23 d, respectively. ($P < 0.01$) Polish color, animal gender, and injury status did not have any effect on the length of time polish stayed on the nails. While no quantitative data was obtained to evaluate the effectiveness of nail polish as a detractor, all animals paid more attention to the nail polish than their incisions or wounds while the nail polish was still on the nails. These observations were confirmed in the daily notes for the evaluators. These results indicate that edible nail polish may be used as novel and safe enrichment provision or distractor in animals that are singly housed for veterinary care or research purposes. Specifically, older animals and recipes with xanthan gum as a thickening agent may have the most success when using edible nail polish in the enrichment plan for nonhuman primates.

P204 Where Do You Place Your Hydrogel?

J Locklear*

CMB/QAL, NIEHS, Research Triangle Park, NC

Rodents are shipped all over the world for biomedical research collaborations and there are guidelines and regulations that oversee the rodent shipping procedure. Typically, when exporting mice and rats from NIEHS, shipping crates are lined with hardwood chipped bedding and the rodents are given feed pellets, a water source (hydrogel), and enrichment to maintain their nutrient, water, and behavioral well-being while in transit. An issue we often encounter during the transit process is that bedding sticks to the hydrogel and in most incidents the hydrogel cups or pouches are buried under the bedding. We felt this mixture of bedding and hydrogel may affect the animal's ability to adequately hydrate especially during long transit times when the water source is significantly reduced or depleted due to absorption by the bedding. The objective of this report is to describe more efficient methods to provide a cleaner water source to our animals during transit. We identified 2 inexpensive methods: (1) hanging the hydrogel pack on the crate partition insert which divides the container into compartments and (2) if a partition insert is not available, the hydrogel packs can be secured to the wall of the shipping crate by melting a laboratory pipet tip to an air vent hole in the shipping crate. Both methods effectively secured the hydrogel packs to shipping crates while maintaining a clean water source for the animals and did not compromise the integrity of the crate. By using these simple novel approaches, we were able to consistently provide a clean water source to our animals while housed in shipping crates for transit.

P205 Husbandry and Management of Breeding Colonies of Jamaican Fruit Bats (*Artibeus jamaicensis*) for use in SARS-CoV-2 Research

E Houston,[1,*] C Lovelace,[1] T Schountz,[2] LV Kendall,[1] JD Ayers,[1]

[1]Department of Laboratory Animal Resources, Colorado State University, Fort Collins, CO; [2]Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO

During times of emerging disease outbreaks, bats are frequently tested as a possible source for viral mutation and spillover. Today, the role of bats in COVID-19 is under investigation. Healthy bats are needed in adequate numbers to complete studies on viral shedding, immunomodulation, and transmission. Captive bats require care to minimize nutritional deficiencies and toxicosis. Here we describe the husbandry and management of 2 captive breeding colonies of Jamaican fruit bats used in research of emerging viral diseases, with emphasis on optimizing the feeding program. Bats were maintained in 2 indoor flight rooms measuring 12′ x 19′ x 8′ at 75–75F, 40–50% RH, and on a 12:12 light cycle beginning at 10:00 p.m. Enrichment included social housing, hanging black cloths, metal frames, and baskets for roosting. Colony 1 contained approximately 200 bats maintained on 178 g of monkey biscuits and 17 kg assorted fruit prepared fresh daily. Colony 2 contained 400 bats fed 600 g pelleted feed formulated for iron-sensitive birds and 30 kg of fruit prepared daily. Fruit was processed into small chunks and distributed on flat feeders. Powdered milk was used to top dress pellets, but resulted in decreased palatability. In attempt to decrease food wastage and improve efficacy of food preparation, their diet was modified. Milk powder was discontinued, and fruit was decreased 10% in both colonies. Soft fruits were hung whole from rings, and melons halved or processed into small slices. No changes were made in concentrate feeds. After implementing dietary changes, increased feed consumption and decreased fruit waste was observed. Bat weights, checked weekly, demonstrated a mild decrease initially. Opportunistic necropsy of euthanized bats showed appropriate body conditioning and no evidence of musculoskeletal diseases, hepatic abnormalities, or wasting. Jamaican fruit bats can be maintained in a healthy and productive manner on a variety of fruits, plus monkey biscuits or iron-sensitive bird feed as additional protein and calorie sources. Fruit can be adjusted to the amount that will be eaten entirely between feedings and can be fed whole to decrease labor. These feeding practices allow entry of healthy, healthy animals into research investigating the emergence of novel diseases.

P206 Assessment of Opaque Tubing Enrichment to Reduce Stereotypic Behaviors and Promote Breeding Efficiency in Gerbils (*Meriones unguiculatus*)

L Habenicht,[1,*] S Bozan,[1] BM Clancy,[2] CA Manuel,[1] DL Fong,[1] JK Leszczynski,[1] A Klug,[3]

[1]OLAR, University of Colorado Denver Anschutz, Aurora, CO; [2]College of Veterinary Medicine, University of Illinois, Urbana, IL; [3]Department of Physiology and Biophysics, University of Colorado Denver | Anschutz Medical Campus, Aurora, CO

Mitigation of stereotypic corner digging behaviors in gerbils (*Meriones unguiculatus*) has been described using cage modification

to create an opaque, angled entrance to a simulated burrow. Rather than permanently modify gerbil caging, we sought to identify a more cost-effective strategy to reduce this nonproductive behavior using a straight or corner-simulating opaque tube fitted to the animals' preexisting opaque nesting box. Baseline incidence of corner digging behavior was video monitored for 17 gerbil breeding pairs over 6 d. Following opaque tube placement, cages were again monitored for several weeklong periods over 3 mo. An active period approximately 30 m before lights-out was used for data analysis. Videos were coded using open-source software to assess the frequency and duration of stereotypic behaviors and compared to baseline data. The effect of opaque tubing enrichment on breeding efficiency was also assessed. During the first week with opaque tubes, an increase in stereotypic behavior was seen across all cages in total digging frequency (147%), digging duration (178%), and repetitive jumping frequency (127%). Once the opaque tubes and nesting box combination had been in place for 4 wk, stereotypic behavior observations decreased below baseline by 26%, 9%, and 13%, respectively. Following 8 wk of exposure to the tubes, these metrics all rebounded to baseline levels. In addition, there were no trends that suggested a long-term effect of tube shape on incidence of stereotypic behaviors. Finally, the presence of opaque tubing in the cage for 3 mo was not associated with an increase or decrease in breeding efficiency (P = 0.78). Based on these initial findings, the addition of opaque tubing does not reduce stereotypic behavior or increase breeding efficiency in gerbil pairs long-term.

P207 The Genetic Diversity of Diversity Outbred Mice

H He,[2] V Philip,[2] KL Brackett,[1,*]

[1]JMCRS Finance and Operations, The Jackson Laboratory, Bar Harbor, ME; [2]Computational Sciences, The Jackson Laboratory, Bar Harbor, ME

The Diversity Outbred (J:DO) stock is a unique experimental resource that allows investigators the ability to perform high resolution QTL mapping, determine the genetic basis of disease, execute toxicology studies, or test pharmaceutical therapies in a genetically and phenotypically diverse mouse population. This colony was established by mating Collaborative Cross lines (consisting of 8 inbred founder lines), and has been maintained ever since by randomly breeding 175 lines 4 times per year. Theoretically, the population should have a 1/8 genomic contribution from each inbred founder strain and more closely represent human genetic diversity than other mouse strains in existence. In the fall of 2020, the J:DO will be producing its 40th generation. However, population genomics of the J:DO were last evaluated during Generation 21 (G21), due to the need to eliminate a genomic region under meiotic drive. Through MUGA data donated by users of the population, we have analyzed the population genomics for every generation since Generation 22 (G22). Here, we report heterozygosity, founder genomic contributions across each chromosome, average haplotype block size and rates of sex chromosome non-disjunction in cohorts from each generation, as well as values for litter size, sex ratio and non-productive breeding pairs. MUGA data from G22, and all generations thereafter, confirms that the meiotically driven region was successfully purged from the population in G21. While founder contribution can vary within and amongst chromosomes, contributions have remained stable since G21. Additionally, genome wide founder contributions do not significantly differ from the expected 12.5%. These data validate the breeding strategy as a means to preserve genetic diversity, and demonstrate the continued and unparalleled genetic and phenotypic variation offered by this remarkable population.

P208 Individual Tracking of LPS- or Chlorpromazine-induced Changes in Locomotion of Pair-housed Mice in Smart Cages

J Ramirez,[1,*] E Defensor,[2] C Liu,[1] LR Schaevitz,[2] JE Fenyk-Melody,[1]

[1]Global Science & Technology, Pfizer, Pearl River, NY; [2]Vium Inc., San Mateo, CA

Our institution collaborated with a commercial entity to perform a pilot study evaluating locomotion and body temperature metrics from socially housed mice in smart cages with 24/7 data capture and video recording. Data interpretation currently requires single housing in these cages. The study objectives were to determine whether locomotion metrics could distinguish between 2 mice in the same cage, and whether patterned ear tags and/or subcutaneously implanted microchips had utility for this. Fourteen female C57BL/6J mice were pair-housed in 7 cages surrounded with radio-frequency (RF) shielding installed in the rack. Mice were implanted with RFID microchips and received one identification tag in each ear. Baseline activity was monitored for 7 d during acclimation before the first treatment, in which an intraperitoneal injection (IP) of either 1 mg/kg lipopolysaccharide (LPS) or 10 mg/kg chlorpromazine (CPZ) was administered to 1 mouse in each cage, while the other received vehicle (saline). Activity was continuously monitored for 7 d. A second injection was then given of either LPS or CPZ to 1 mouse in each cage, with the cagemate receiving vehicle as in the first dose. Animals that received LPS in the first dosing received CPZ in the second, and vice versa. Activity was again monitored for 7 d before the study conclusion. Locomotion data retrieved from both the eartags and microchips was comparable. Significant differences were observed between mice in both LPS and CPZ treatment and vehicle groups, indicating that both eartags and microchips were effective in distinguishing data between 2 mice in a cage, and that both treatments were effective in decreasing measured activity levels. The maximum magnitude and duration of reduced activity was more profound in the mice receiving LPS (22.9% of baseline, $P < 0.0001$; 4 days) than CPZ (28.3% of baseline, $P < 0.0001$: 1 day). A significant drop in body temperature (6°C, $P = 0.0006$) during the first 24 h after treatment was also observed in the mice receiving CPZ. In conclusion, eartags and microchips are equally effective at differentiating between mice pair-housed in smart cages and both LPS and chlorpromazine detectably reduce activity levels.

P209 Introducing and Troubleshooting a Sealed Positive-pressure Rack System to House Germfree Mice

J Yamada, E Chua, K McEachin, R Garcia-Gonzalez*, C Sohn

gRED Animal Resources, Genentech, Inc., South San Francisco, CA

Gnotobiotic mice lack all the microorganisms that normal mice possess. They must be housed in strict, sterile conditions using isolators so as not to become contaminated. Working in isolators is labor intensive and poses some challenges. Using sealed positive-pressure caging, we performed a pilot study on a colony of C57BL/6 germfree mice to evaluate if we could keep them clean throughout weekly cage changes, routine handling, and manipulations. Cages were double-wrapped, autoclaved, and any supplies used were also sterile. Cages were changed out inside a Class II BSC with a 2-person system as close as possible to a sterile environment or operating room fashion. Full PPE (booties, hair bonnet, face mask, sterile gloves, and sterile surgical gown) was worn throughout. Cages were wiped down with 200 ppm MB10® disinfectant and allowed a rigid 15-m contact time. Mice were subject to routine bleeding and SQ dosing of sterile saline to mimic normal experimental handling conditions. PCR samples were collected weekly from the mice (oral/fur swabs, fecal pellets), biscuits, water, bedding, and enrichment items. We were able to maintain these mice germfree for 4 wk. One of the challenges we ran into is that, at some point, mice became contaminated, so we ended the study at 6 wk. We are currently working with cage wash operations and animal facilities to troubleshoot adequate sterilization of feed/bedding, our suspected sources of contamination, and analyzing how to improve methods to maintain the mice germfree for longer periods of time.

P210 Accessible Caging for Mice with Partial Paralysis

PB Hutchison*, NL Herndon

Division of Animal Resources, University of Illinois, Urbana, IL

Providing a suitable cage bedding substrate for mice with strain related or induced partial paralysis presents with multiple challenges pertaining to animal husbandry. Animal care staff noticed mice with partial hindlimb paralysis were unable to ambulate well in loose bedding substrate. Mice were unable to gain footing in corncob bedding and exhibited a paddling motion with front legs that did not promote ambulation throughout the cage. When using a paper bedding, circling behavior promoted furrow formation, which trapped mice, leaving them unable to free themselves. The substrate also made daily observations more difficult due to mice burrowing behavior. These reported activities led to secondary clinical concerns, such as dehydration, loss of body condition, and skin lesions. Researchers using paralysis models wanted to prolong overall survival rate while preserving animal wellbeing. Communications between research and veterinary staff could become strained due to competing interests of research and animal wellbeing. Due to these factors, our facilities began to explore a unique cage bedding substrate to improve animal welfare. From previous experience, we determined these mice required a cage liner that covered the bottom of the cage, provided a surface that enabled ambulation, and was absorbent. We tried commercially available compressed cotton pads marketed for neurologically debilitated rodents, as they are absorbent and can provide nesting material. This item offered many of the attributes we were looking for in a cage liner. We developed a novel cage setup for mice with hindlimb paralysis that was provided as soon as paralysis was reported. This cage set-up consisted of the compressed cotton cage liner, 6 grams of paper nesting material, and a petri dish of moist food provided on cage floor. The new cage setup did not prolong survival rate, but the cage liner offered great animal welfare advances by reducing the reports of secondary clinical concerns. This cage setup increased rodent mobility, which led to easier access to food and water and decreased reports of dehydration and skin lesions. This improved the quality of life for mice with hindlimb paralysis and improved researcher and animal care staff relations and communications.

P211 Is More Really Better? A Reevaluation of PPE Practices in a Barrier Rodent Facility

TA Collins*, CL Freed, E Brooks

University Laboratory Animal Resources, The Ohio State University, Columbus, OH

Personal protective equipment (PPE) minimizes occupational exposure to animal allergens and zoonotic agents, and protects restricted animal populations. PPE practices should be based on a risk assessment considering facility design, species, and research. Once established, the need to review PPE practices may be overlooked. PPE policies for a large rodent barrier facility were initially designed such that each suite, 11 total, would function as its own containment unit. A set of PPE (isolation gown, bonnet, facemask and gloves = $1.93) was donned at the suite level and removed before exit, regardless of health status of the next location. Study design required individuals to track changes for a period of 2 wk allowing us to quantify the impact of this policy on animal care staff, as multiple PPE changes were required throughout the day. Data analysis calculated average PPE changes based on job assignment (medicine versus husbandry) with a subsequent cost analysis performed. On average, each medicine tech (n = 5) used 5 sets/day of PPE while each husbandry tech (n = 8) used 6 sets/day. Proposed changes would allow donning 1 set of PPE and wearing that throughout the vivarium, including the corridor. All staff are trained to understand expectations for movement in the facility, working from most restricted to least restricted areas and continued compliance with preestablished room entry orders was key. Anticipated use on average would decrease to 3 sets/day across all staff in the facility (n = 31) resulting in a 2-wk cost savings of $2,377 for the entire group. Following thorough analysis, a change in PPE practices allowed us to reduce staff time donning/doffing PPE, minimize PPE expenses, and support green initiatives by reducing waste. In light of PPE shortages, this has also improved supply efficiency. Given a strict barrier, requiring the use of biosafety cabinets and clearly identified room health status, we do not anticipate this change will have any negative consequences on animal biosecurity or allergen exposure in the corridor. It is important that PPE practices are reevaluated periodically to ensure they are meeting facility and personnel needs as changing more often may not be necessary given the risk assessment at that time.

P212 Handling and Husbandry Technique Adaptations Involved in the Assessment, Blood Drawing, Euthanasia, and Dissection Of Juvenile Wild-caught Alligators

RK Banks*

Office of Research, UCF, Orlando, FL

When handling juvenile wild-caught alligators, extra precaution must be taken to ensure the safety of all researchers, staff, management, and veterinarians present. Safe handling is important in efficiently performing dimensional measurements, sexing, blood drawing, euthanasia, and dissections on this unique research species. Anatomy and physiology of the species should be considered when performing these techniques. Specialized equipment may also used to limit specimen mobility and increase safety of the procedure. If all necessary handling precautions are diligently practiced, then the scientific techniques involved with the alligators will be conducted efficiently. The scientific techniques used in this project included dimensional measurements (weight, snout to tail length, snout to cloaca length), sexing, blood drawing, euthanasia, and dissection. During measurements, a small rubber band or electrical tape was used to tightly secure the mouth of each alligator. If an alligator had become too rambunctious while on the scale, a holding technique was performed by raising the animal off the ground, perpendicular to the floor. This technique would allow the redistribution of blood in the body, ultimately relaxing the musculature of the limbs. When performing blood draws from the tail and neck, cradling and flattening techniques were used to isolate and stabilize blood drawing sites. Primary and secondary forms of euthanasia were performed. When transitioning to dissection, string ties were used to immobilize limbs on the dissection tables due to continued postmortem tail and body movement. In addition, one individual was responsible for holding the tail from flailing and thrashing the surgeon performing dissection. Incorporating physical safety precautions and knowledge of the species proved efficient as no injuries occurred, and all scientific data needed was collected. Overall, if all necessary handling precautions are diligently practiced, then the scientific techniques involved with the alligators will be conducted efficiently.

P213 Establishment of a Shared Database to Facilitate a Reduction of Animal Use at a Large Academic Institution

A Meyer*, DL Hickman

Laboratory Animal Resource Center, Indiana University, Indianapolis, IN

Breeding colonies of transgenic can result in the production of surplus animals of the incorrect genotype or sex. In the absence of easy communication, these animals are often euthanized, even though they may be able to be used by other scientists on campus. To facilitate the overall reduction of animals use on our campus, we worked with our breeding colony software programmers to establish an online shared database where scientists could view and request surplus animals. In addition to the technical assessment, we established a logistics flow to ensure that available animals were tracked and either moved to a new study or euthanized. While being viewed in the surplus animal database, the per diem support was provided by the animal care and use program. This support was facilitated by the administration in recognition of the advantage

of effectively implementing the 3Rs on our campus. Our program has been successful due to a high level of centralization of our breeding colony support programs and administrative support. In the past 6 mo, approximately 1,000 rats and mice that would have been euthanized have been redirected to other studies. Our shared database has become an effective way to match surplus animals with scientists who can use them, reducing the overall use of animals on campus and helping to address compassion fatigue with our animal facility staff.

P214 Operational excellence, risk management and compliance supports the integration of an insectary into an established research vivarium

C Mateo, L Magee*, J Keding-Casey, M Fishbaugher, C Osborne

Novartis Institutes for BioMedical Research, Inc., Oakland, CA

Malaria is a severe disease caused by parasites of the genus Plasmodium, transmitted to humans by a bite of an infected mosquito of the species Anopheles. It affects an estimated 219 million people worldwide causing 435,000 deaths annually. To better understand parasite lifecycle, disease biology, and develop novel therapeutics, the use of mosquitoes as a research tool in an in vivo setting has become increasingly important. Therefore, in order to support a research portfolio, it was necessary to design and assimilate an insectary into an already functional rodent vivarium. Operating within the current footprint, available space was repurposed to create a specially designed work environment. Rearing and maintaining a live stock of mosquitoes in a insectary requires strict parameters for environmental metrics, biosecurity, and biocontainment. With the insectary being contained in the rodent facility, already approved and established protocols for routine vivarium operations complemented the work in the insectary and ensured that the space was proactively managed and maintained at a high standard. To date, over 30,000 infected mosquitoes have been received from external suppliers, over 100,000 uninfected mosquitoes reared in the facility, and 5,000 in-house infections performed. Despite the high production volume, robust biocontainment and biosecurity protocols have yielded zero incidents of escape, infection, or bite, demonstrating the effectiveness of engineering controls and procedures. Some examples include multi-level containment, interlock doors, air showers, personal protective equipment (PPE), eliminating potential mosquito escape routes, and restricted security clearance. When working with infected mosquitoes, risks can be mitigated through thoughtful facility design, clearly established protocols, and a strong training program.

P215 Tox in a Box: Enhancement of a Closed Restraint Chair for Laboratory Macaques

CE Ferrecchia*, LF Matney

Comparative Medicine, Pfizer, Inc, Groton, CT

Working safely with unanesthetized nonhuman primates, especially mature male macaques, is a challenge shared across numerous research facilities. Recent literature shows that pharmaceutical companies and contract research organizations continue to use open chair restraint for nonhuman primates, though a physical barrier gained by a closed restrainer reduces the risk of injury and manual restraint of the animal. One of our goals was to develop a low- to no-contact method to administer compounds via oral gavage in a large-scale Good Laboratory Practice toxicology setting. Hence, we modified a colleague's closed restraint chair, which already included options for dosing and sample collection in the hind limb. Our most recent chair modification incorporates additional options for forelimb access, as well as optional oral access and restraint. In addition to enhanced safety and ergonomics, improvements to animal wellbeing over our previous processes include the opportunity for the animal to enter the chair voluntarily and present a limb for examination or blood collection. This also eliminates the need for collars for many animals. These refinements are applicable not only to a toxicological setting but may also be used across laboratory settings as well. Thus, technicians and researchers can perform tasks safely with minimal direct handling of the animal.

P216 Assessment of Existing and Novel Tissue Sample Collection Methods for Standard and Automated Rodent Genotyping

RC Dysko,[1,*] FC Hankenson,[2] F Benavides,[7] LA Colby,[3] PC Smith,[4] DK Taylor,[5] N Nowak,[6] DE Hoskins,[6]

[1]Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI; [2]Michigan State University, East Lansing, MI; [3]University of Washington, Seattle, WA; [4]Yale University, New Haven, CT; [5]Emory University, Atlanta, GA; [6]Transnetyx, Inc., Memphis, TN; [7]MD Anderson Cancer Center, Houston, TX

Numerous independent studies have been conducted examining the effectiveness of various genotype tissue sample collection techniques and their relative effects on animal well-being. However, those research activities have often relied on variable testing methodologies and data, bringing into question the broad applicability of the results. The Transnetyx Scientific Publications Committee, a multi-institutional panel of veterinary scientists, seeks to address issues of variability in genotyping and sample collection data by facilitating a parallel, multiinstitution research project to establish reliable, comparable, broadly applicable, and reproducible data. The pilot study herein was designed specifically to establish which novel (noninvasive) tissue collection techniques in rats and mice could generate sufficient biological material upon which to successfully conduct both standard and automated genotyping. For this study, the following 7 tissues and swabs were obtained from rats (n = 4) and mice (n = 10): ear punch (control), oral swab, rectal swab, skin swab, fur pluck, whisker pull (only rat), and fecal pellet. Tails tip samples, the most commonly used tissue source, were not obtained for the pilot study as it was decided beforehand that they would be assessed in the larger project. The rats were anesthetized and the mice were euthanized to facilitate the collection of multiple samples from each animal. DNA quantity and quality were determined using automated real-time PCR (performed at a commercial enterprise) or manual standard PCR genotyping (performed at an academic institution). For both species, the rectal swab provided the highest quality results (based on yielding the highest signal on internal control assays and the highest number of results that passed quality control and could be assigned a positive value), comparable with the ear punch control, followed in quality by the oral swab. The remaining tissues/swabs were inferior in quality. These results lead the authors to consider ear punch, rectal swab, and tail tip as the primary samples to be obtained in a multi-institutional study on tissue sampling and animal well-being.

P217 Evaluation and Implementation of Bagged, Crinkle-cut Paper Strips as Environmental Enrichment for Mice

JC Rodgers,[2,*] C Cheleuitte,[1] NS Lipman[2]

[1]Center for Comparative Medicine and Pathology, Memorial Sloan Kettering Cancer Center, New York, NY; [2]Center for Comparative Medicine and Pathology, Memorial Sloan Kettering Cancer Center, Weill Cornell Medicine, New York, NY

Nesting material is an important component of environmental enrichment for mice. At our institution with greater than 60,000 mice, two 1"x 1" compressed cotton squares have been provided to mice for >10 years. A commercially available nesting product, which incorporates ~7g of crinkled-cut paper strips within an easy-to-distribute, sealed filter paper bag was evaluated to assess: 1) nest quality and the longevity of high-quality nests; 2) changes in fecundity; 3) the ability to identify health issues and dead mice cage-side; 4) investigators impressions of the impact of the enrichment on their animal models via online survey; and, 5) its progressive implementation in a large animal care and use program. Nest quality

was evaluated using an established nest scoring system (0–5; poor–best) in cages provided either product A (N = 441) or product B (N = 448) with nest quality determined on day 7 and 14 by a single evaluator. On day 7, nests constructed from product B had a higher nest score (mean +/- SE = 4.26 +/- 0.12) than those made from product A (2.95 +/- 0.04) and by day 14 all product A nests were flat and dispersed in the cage, while product B scores remained high (4.19 +/- 0.17). In a longevity trial using product B (N = 266) in which the nests were transferred and evaluated weekly at cage change for 5 wk, the nest quality scores at the end of each week were 4.32 +/- 0.05, 4.41 +/- 0.04, 4.45 +/- 0.05, 4.08 +/- 0.05, and 3.35 +/- 0.08. Thus, product B maintains high-quality nests (≥ 4 of 5) for up to 4 wk. Fecundity (# pups weaned/breeding pair/month), was evaluated for 12 mo in 5 breeding rooms (N = 1600). A significant increase in the number of pups weaned was observed in cages with product B as compared to product A (7.5 vs. 5.6; $P < 0.01$). There was no difference in the number of dead mice identified (21.9 +/- 2.0 in product A vs. 21.2 +/- 2.1 in product B), but significantly fewer health issues were found in cages with product B (16.6 +/- 1.3 vs. 23.6 +/- 2.0; $P < 0.01$). Fifty-nine% of survey respondents (31/77) reported a positive impact of product B in their breeding cages, including increased pup survival. Product B was subsequently successfully implemented program-wide with a monthly replacement interval.

P218 Influence of Feeding Frequency and Brine Shrimp (*Artemia salina*) on the Growth on Zebrafish (*Danio rerio*)

R Giallella,[2] D Garrity,[2] P Robison,[1] J Owiny,[1,2,*]

[1]Laboratory Animal Resources, Colorado State University, Fort Collins, CO; [2]Biology, Colorado State University, Fort Collins, CO

Zebrafish in laboratory settings are often fed a diet that includes brine shrimp. Fish fed a commercial diet every other day have increased performance and fecundity compared to fish fed a diet consisting of only brine shrimp. However, to date no studies have investigated the effects of a diet consisting of a formulated diet plus brine shrimp compared to a diet consisting of only a commercially formulated food on the growth of zebrafish. Here, we aim to address the following questions. Does feeding frequency impact zebrafish growth? Does the exclusion of brine shrimp impact zebrafish growth? To answer these questions, we took assigned broods of zebrafish hatched on the same day to 1 of 4 groups: a diet consisting of brine shrimp fed once daily and formulated diet fed twice daily (n = 35), a diet consisting of formulated diet only fed either once (n = 33), twice (n = 24), or three (n = 20) times daily. Each group was fed the commercial diet at of 5% body weight, based on the average fish weight in each tank. Each group was weighed and length measured once weekly starting at age 30 d for a total of 12 wk. Data was analyzed using a linear regression in R. There was no difference between the weights and lengths of zebrafish fed three times daily with the commercial and those fed the same formulated diet plus brine shrimp ($P > 0.05$). Fish fed a commercial diet plus brine shrimp or commercial diet alone three times a day were heavier and longer than those fed a formulated feed only either once or twice a day ($P < 0.05$). Feeding fish a commercial diet alone 1 or 2 times a day may reduce performance characteristics. Feeding brine shrimp significantly increases labor costs and risks of introducing pathogens without necessarily providing additional benefits over commercial diets fed 3 times a day. Additional studies are planned to confirm effects the above dietary regiments on fecundity.

P219 Rearing and Vaccination of the Neonatal Muntjac Deer (*Muntiacus reevesi*)

J Helbling,[1,*] E Houston,[1] M Burton,[1] AR Personett,[1] E McNulty,[2] C Mathiason,[2] JD Ayers,[1] LV Kendall,[1]

[1]Lab Animal Resources, Colorado State University, Fort Collins, CO; [2]Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO

Chronic wasting disease (CWD) is a prion disease with an expanding prevalence among free-ranging cervid populations. Muntjac deer can serve as a useful alternative animal model for studying prion diseases to mitigate the challenges associated with housing larger CWD-susceptible cervid species, namely white-tailed deer and elk. We have established an indoor-housed breeding colony of Reeve's muntjac deer comprised of 2 adult males and 4 adult females at our institution. Muntjac deer are co-housed at a male to female ratio of 1:2 in secure rooms with concrete floors and walls. Wood shavings are used as a bedding substrate, with hiding huts, visual dividers, and litterboxes placed throughout the rooms. This breeding colony recently experienced diarrhea and neonatal losses, leading to husbandry and management changes. Following a 7-mo gestation period, pregnant does give birth to a single fawn. To estimate parturition dates, we performed sedated transabdominal ultrasounds on pregnant does. Upon parturition, does and fawns were moved into individual maternity suites with rubber mat flooring. Due to previous ingestion of wood shavings by fawns, wood substrate was removed from the suites and was replaced by reclaimed wood pulp. Extra chow and fine orchard grass hay were given to support the nutritional demands of the lactating does. Fawns were weighed at least once daily for a minimum of 2 wk. If weight loss occurred, fawns were syringe-fed with milk replacer and heat lamps were added to the enclosure. Healthy fawns were weaned at 8 wk of age. Two multivalent bacterin vaccines were administered subcutaneously at weaning while fawns were sedated for ear tattoo identification and again 2–4 wk after the initial dose. No adverse effects were observed in fawns. Adult deer began receiving annual vaccinations under a similar protocol. In addition to litterbox training, animal care staff and veterinarians used positive reinforcement training (PRT) to help decrease stress associated with human contact. Sedation is currently required for physical exams in adult deer, but with PRT we aim to conduct exams without sedation. The above husbandry and housing parameters have resulted in healthy captive neonatal Muntjac deer.

P220 Norovirus No More: The Power of Dry Heat Sterilization to Stop the Transmission of Murine Norovirus in Dirty Bedding

J Lee,* WA Bidot, EA Nunamaker

Animal Care Services, University of Florida, Gainesville, FL

Biosecurity is essential to an animal care program to protect the health of the animals and to control physiologic variables that could potentially affect research results. A critical component of the animal care biosecurity plan includes the sterilization of materials that come into direct contact with the animals, including caging and bedding. Dry heat sterilization is a method of sterilizing rodent caging and bedding that is gaining popularity in animal research facilities due to lower cost, less space usage, no water usage, and ability to sterilize water-sensitive materials. Currently, dry heat sterilization ovens are biologically tested and validated against *Bacillus atropheus* spore strips under the assumption that a lack of sporulation and growth is equivalent to successful sterilization of the sterilizer's contents. However, there are no published studies describing sterilization of rodent cages with relevant rodent pathogens using this method. To determine whether or not a dry heat sterilizer can adequately sterilize rodent cages and bedding against relevant rodent pathogens, we created Murine Norovirus (MNV) dirty bedding cages using mice with known infection and shedding of MNV. The MNV dirty bedding cages were either sterilized through a dry heat sterilizer oven with a validated cycle or not sterilized. Four-wk-old CD-1 mice were placed in the dry heat sterilized or non-dry heat sterilized cages for 2 wk, then subsequently placed into clean, autoclaved cages for the remainder of the study. Fresh fecal pellets were collected on weeks 0, 8, 12, and 16 and submitted for MNV PCR. Whole blood for MNV serology was collected on weeks 0, 12, and 16. At week 16, all animals in the non-dry heat sterilized cages were positive for MNV through both fecal PCR and serology. Comparatively, none of the mice in the dry heat sterilized cages were positive for MNV through fecal PCR or serology at any point. Our study demonstrated that dry heat sterilization is a viable sterilization method for rodent cages and

bedding.

P221 Implementation of an Electronic Abnormal Conditions Monitoring System

E Green,[1,*] J Brooks,[2] V Stanton,[2]

[1]Veterinary Science, Research and Support, Envigo, Indianapolis, IN; [2]Envigo, Indianapolis, IN

Preserving the genetic integrity of laboratory animals is a key component of colony maintenance. Unfortunately due to the high costs associated with genetic testing, many researchers are only able to facilitate testing a few times a year. This makes implementing an abnormal conditions monitoring system as a supplemental level of quality assurance advantageous. By tracking the abnormalities observed in each strain and developing a baseline for what are considered normal levels, one can easily identify surges of uncharacteristic phenotypes brought about by genetic drift, shift, or contamination. The objective is to detail the process of updating a manual abnormal conditions monitoring system to an electronic version and evaluate the benefits provided by this new database. This electronic model was established by first assigning representative codes to identify common abnormalities which would be used for all animals removed from the inventory tracking system. Next, a user-friendly training guide was created which contains pictures of the health conditions along with the respective codes. After distributing the guide and hosting a training session, a baseline level for the conditions was established averaging 6 wk of data. A graphical comparison of the actual number of reported conditions to the established baseline allows for easy visual detection of abnormal phenotypes. Along with the efficiency and the time savings that this new electronic model provides, it also promotes animal welfare by permitting early detection and management of abnormal phenotypes in accordance with the 3Rs.

P222 Ammonia Levels in Rat Double Decker Individually Ventilated Cage system

P Kowal*, m Defeo, M Mooney

Comparative Medicine, Pfizer, East Lyme, CT

Buildup of ammonia occurs as a normal biological consequence of animals excreting bodily waste following metabolism of their food and water. Research suggests that high ammonia levels in rodent cages can cause health effects, including lesions in the nasal cavity and olfactory mucosa. In rodents, 50 ppm is often used as a guideline for maximal ammonia exposure. We tested ammonia levels in double decker individually ventilated (IVC) rat cages with animals housed 2–3 per cage with bedding. Two different devices were used—a gas detection device and a colorimetric sensor. The results from both devices were comparable. PSARs are usually placed inside the animal's cage; however, after several hours, rodents chewed and damaged the sensors. We discovered a novel way to insert the PSARs below the IVC filter top, providing direct air exposure to the cage environment but prevented animals from accessing or chewing. This procedure allowed us to read ammonia levels daily, thus we were able to change cages prior to 14 d if the colorimetric sensor showed an ammonia reading of danger (blue) indicating an ammonia level of 50 ppm or greater. On average, results showed that approximately 20% of the cages of socially housed rats reached ammonia levels over 50 ppm before 14 d requiring early cage changes. The PSARs provided an accurate, immediate visual indicator when necessary to change the cage. We are studying double decker cages because they are much larger than standard rat ventilated cages (140 sq. in. floor space and 18.9 liters total volume vs. 280 sq. in. and 47 liters). We did not see a difference in cages that housed rats 2 per vs. 3 per. Where we did see a difference was in cages that were wet due to animals playing with the lixit. We had 40 cages in the study, males and females, 2–3 per cage, ranging in weight from 200g–400g. Most cages were changed within the 10–14 day timeframe. Smaller females tended to play with their lixits more often requiring more frequent changes and larger males tended to be less active, never playing with lixits and required less frequent cage changes.

P223 Is Your Disaster Plan Ready for Post-pandemic Normal?

J Brooks,[1,2,*] S Kirchain,[2,3] R Thompson,[2,3] G Cronin,[2,4] J Nichol,[2,5] P Holly,[2,4]

[1]Operations, Envigo, Indianapolis, IN; [2]Vivarium Operational Excellence Network, Boston, MA; [3]Brigham and Women's Hospital, Boston, MA; [4]Massachusetts General Hospital, Charlestown, MA; [5]McGill University, Montreal, Canada

As disaster plans failed miserably for world governments and industry when deployed against the COVID-19 pandemic, it appears that some industries adapted far better than others. Those that had plans that only revolved around natural disasters and utility outages needed to make swift adjustments. Amassing feed and supplies does not compute when decisions about cutting down rodent populations have to be made to follow stay-at-home guidelines or staffing absences due to COVID-19. We surveyed lab animal institutions across North America to determine effectiveness and impacts on disaster plans. Over 91% of respondents (n = 32) indicated their disaster plan would require modifications to address a pandemic situation. While our survey was focused on disaster plans, we were also interested in the human components that are not usually addressed in disaster plans. Survey topics included corrective actions, budgets, feed, supplies, vendor access, the working environment, plus staffing schedules, policies, and training. As our organization is geared towards sharing data for continuous improvement, the Vivarium Operational Excellence Network (VOEN) developed the survey specifically about this situation with the intent of sharing results of the survey for learning purposes. Briefly, we were able to identify trends which emerged from diverse organizations that implemented various disaster plan alterations and assessed human impacts that have sometimes been overlooked as an element of disaster planning. Staffing and staff-related issues were a reoccurring theme at most locations. The sharing of information allows others to improve their disaster plans and use the data to effectively navigate novel disaster situations in the future.

P224 Finding an Alternative Approach when Critical Medical Supplies Are in Short Supply during the COVID-19 Pandemic

RA Byrum,* MC St Claire

NIAID Integrated Research Facility, Frederick, MD

The COVID-19 pandemic has created shortages of various medical supplies, in addition to personal protective equipment that threaten to delay or prevent the timely initiation of important studies. At the height of the pandemic, researchers at the National Institute of Allergy and Infectious Diseases, Integrated Research Facility were working to develop a nonhuman primate (NHP) model for COVID-19. Computed tomography (CT) of the lungs is a critical component of these studies. This procedure involves placing intubated NHPs on a ventilator circuit to allow a breath hold to be performed on the animal during the chest CT. A pediatric-sized heat moisture exchanger (viral/bacterial) filter is placed on the endotracheal tube to prevent contamination of the ventilator circuit on the anesthesia machine. When planning and scheduling these studies, we learned the filters were not available or were back-ordered depending on the vendor. The filters are normally used once and discarded, but we quickly realized the supply would run out if we did not receive a new shipment. A plan was devised to reuse filters on noninfected animals or sterilize filters using ethylene oxide (EtO) gas after each use on virus-exposed animals before reusing the same filter on the same animal. After sterilization, a portable gas leak detector was used to check each filter for residual EtO. The first set of filters requiring gas sterilization still had low levels of residual EtO (2.6–2.9 ppm) after overnight aeration in the BSC. An

additional 24-h aeration period brought the EtO level to 0.0 ppm. After each gas sterilization, the integrity of the filter was evaluated with a handheld particle counter capable of counting particles as small as 0.3 µm. There were no particles detected which indicated the filtration integrity remained intact for particles ≥ 0.3 µm. We describe the procedures we employed to sterilize these filters and determine if they would be safe and effective for reuse. These procedural modifications allowed critically important research to find an animal model for COVID-19 to continue without compromising animal welfare.

P225 The Effect of Environmental Enrichment on Stress, Behavior, and Production in Gestating and Farrowing *Sus scrofa* Sows

C Peck,[1,*] J Kieffer,[2] S Moeller,[3]

[1]The Ohio State University College of Veterinary Medicine, Columbus, OH; [2]Office of the Attending Veterinarian, Department of Animal Sciences, The Ohio State University, Columbus, OH; [3]Department of Animal Sciences, The Ohio State University, Columbus, OH

The materials of confinement housing often limit periparturient behaviorial expression, which may increase stress. This has been shown to influence parturition, which affects production. Natural fiber cloth or rope may allow natural behaviors with little to no concern for ingestion. However, there is a lack of enrichment protocols for gestating and farrowing sows. This study aims to assess the impact of a natural fiber cloth on gestating and farrowing sow stress, behavior, and production. The purpose was also to evaluate appropriate provision criteria for future implementation of environmental enrichment in commercial settings. We hypothesized that enriched sows will display more natural behaviors, be less stressed, and have more live piglets and fewer stillborns. Sus scrofa sows were randomly divided into enriched and control groups (n = 6 each). A burlap cloth was hung with twine in the pens of enriched sows 6 d before the expected farrowing date and removed 24 h post-farrowing. Salivary cortisol was measured to evaluate stress level 6 d and 2 d before the expected farrowing date, 24 and 48 h after environmental enrichment, daily until farrowing, and at the start and end of farrowing. Video surveillance was used to monitor how often sows lied down, ate, drank, interacted with the burlap, bit crate bars, and bounced feeders. Thirty-minute timeframes were examined post-enrichment same day, 24 h post-enrichment, pre-farrowing, post-farrowing, and before enrichment removal (COVID-19 restrictions have delayed video analysis). Preliminary results demonstrate that, on average, enriched sows produce more live piglets (avg = 17.67) and fewer stillborns (avg = 3.17), than control sows (avg live = 17, avg SB = 5.17). No differences in salivary cortisol were observed (P = 0.78) between groups. As expected, salivary cortisol in all sows increased ($P < 0.05$) when farrowing began and remained elevated when the last piglet was farrowed ($P < 0.05$). 24 h post-farrowing, salivary cortisol levels decreased to a level similar to what they measured 24 h pre-farrowing ($P < 0.05$) in both groups. The current study results will facilitate the determination of an enrichment protocol and additional housing criteria to increase sow production and profit for swine farmers.

P226 The Cryodropper: A Novel Device for Mouse Embryo and Sperm Vitrification

B Stone*

ParaTechs Corporation, Lexington, KY

Cryopreservation of animal models as germplasm is ubiquitously favored by the biomedical research community for its ability to safeguard against catastrophic animal loss, provide effective vivarium management options, and as a convenient method to transport and share animal models. A novel tool for rodent embryo and sperm cryopreservation, the Cryodropper has a user-friendly design and employs simple but effective vitrification and thaw protocols. Embryos or sperm are vitrified in the device along with compatible thaw medium in liquid nitrogen vapor. Thawed germplasm is dispensed from the device by flushing with thaw medium allowing quick and complete recovery of material. By providing compatible thaw medium in the device, errors and confusion are mitigated during material retrieval. The system is compatible with standard storage and shippers, providing convenience for researchers and facilities commonly used for worldwide distribution. Cryopreserved CD1 mouse sperm recovered motility as well as sperm cryopreserved in conventional sperm straws. In vitro fertilization was used to determine sperm viability of samples vitrified in Cryodroppers and was not statistically different from IVF using fresh sperm samples. For embryo vitrification, development of thawed 2-cells and morula to blastocyst stage was assessed in tissue culture as a measure of embryo health. 85% of embryos developed to blastocyst stage. This data indicates that germplasm vitrified in the Cryodropper are viable for embryo culture and IVF with further applications in rodent assisted reproductive techniques.

P300 Serum Pharmacokinetics of a Highly Concentrated Buprenorphine Formulation in Female Sprague Dawley Rats (*Rattus norvegicus*)

K Galang,[1,2,*] H Knych,[3] R Oates,[1]

[1]Campus Veterinary Services, University of California, Davis, CA; [2]School of Veterinary Medicine, University of California, Davis, CA; [3]KL Maddy Equine Analytical Chemistry Laboratory, School of Veterinary Medicine, University of California, Davis, CA

Laboratory rats (*Rattus norvegicus*) are the second most common mammalian animal model used for biomedical, behavioral, psychological, and toxicological studies. Their long-established and varied research use has driven the progressive refinement of interventional and experimental techniques so that associated pain/distress can be ameliorated. One of these refinements is the use of opioids for pain management which has been shown in previous studies to provide effective analgesia for this species. Despite this, additional considerations such as handling stress and the growing opioid epidemic call for judicious use of this analgesic class. Buprenorphine, a partial mu opioid-agonist with high affinity for mu receptors, is a common opioid of choice in rodents as the longer duration of action reduces direct handling needs for administration. While conventional buprenorphine (CB) indicates BID-QID dosing as sufficient for pain control in many mammalian species, a novel, highly concentrated formulation of buprenorphine (HCB) is the first FDA-approved, veterinary-specific opioid labeled for q24h dosing in cats (*Felis catus*). We hypothesized that at the labeled feline dose of 0.24 mg/kg SC, HCB would achieve therapeutic concentrations ≥1 ng/mL in 6 adult female Sprague Dawley rats for at least 24 h. Mean peak serum concentrations occurred 0.5 h after administration at 13.79 ± 6.76 ng/mL. Twenty-four hours post-administration, the mean serum concentration was 1.02 ± 0.33 ng/mL with 4 of the rats maintaining a serum concentration of ≥0.99 ng/mL. With the exception of a minor, focal injection site reaction in 1 animal, none of the other known side effects of opioid administration in rats (severe pica, self-injurious behavior, sedation, bradycardia, respiratory depression, ileus) were observed. Our findings suggest that SC administration of HCB at the above-mentioned dose may provide therapeutic serum levels for 24 h in adult female rats, decreasing the frequency of administration and the need for multiple handling events.

P301 Development of a Noninvasive Transdermal Technique for Serial CSF Collection from Cisterna Magna In Rats

A Fiorenza,[1,*] P Lopez,[2] H Masakazu,[3]

[1]Institute for Neurodegenerative Diseases (IND)-Hunters Point Animal Facility, University of California San Francisco, San Francisco, CA; [2]Institute for Neurodegenerative Diseases (IND),

University of California San Francisco, San Francisco, CA; [3]DAIICHI SANKYO CO., LTD, Tokyo, Japan

Neurodegenerative diseases affect millions of people worldwide. Only considering Alzheimer's disease, it is estimated that there are approximately 44 million people affected but, although the high incidence, only around 1 in 4 people with the disease get diagnosed. Cerebrospinal fluid (CSF) is of particular importance in this kind of studies for its direct contact with brain and spinal cord. Unfortunately, in animal models, unlike other fluid like blood and urine, CSF collection is often a terminal procedure, requiring a large number of animals per experiment, or, if survival, the procedure is usually surgical, then producing tissue damage and distress that requires recovery and pre- and post-surgical care. We developed and tested an improved technique for nonsurgical, serial and survival CSF collection in Sprague Dawley rats. A total of 60, 8 wk-old rats were used, 30 females and 30 males. Although >100 uL of CSF could be collected from a single rat as a terminal collection with this procedure, we decided to set on 50 uL the maximum volume to be collected during serial collections. Animals were anesthetized using isoflurane and the dorsal head and cervical region was shaved and prepared. The experimenter gently handled the rat, flexing the cervical region to expose a depressible surface with the appearance of a rhomb between the occipital protuberance and the spine of the atlas becomes visible. Then the cisterna magna is accessed using a 29–31G needle connected to a draw syringe. The needle is inserted perpendicularly and centrally into the cisterna magna for CSF collection without making any incision at this region. Once the needle has pierced the skin, the operator generates small suction pressure in the syringe by slowly aspirating. The aspiration will make the CSF flow through the needle once the needle has penetrated the cisterna magna. We first tested that no brain damage results from this procedure using neurofilament light chain (Nfl) concentrations in CSF as a neurodegeneration marker. We then tested if serial collections were safe for the animals at different intervals (2, 4, and 8 wk) and compared these results with a single collection to define the safest and optimal time between collections. No deaths and/or clinical signs have been observed. Our CSF collection technique is, compared with the other existent techniques, not only safer but also faster (only few seconds needed for collection) and inexpensive since very common tools are needed. We developed and tested a simple, fast, and inexpensive technique for nonsurgical serial CSF collection in rats that allows to follow the progression of neurodegenerative diseases and/or the effect of potential treatments in the same rats, reducing the number of animals required for experiments and making results more precise due to the reduction in variability.

P302 Comparison of Two Isolation Techniques for *Macaca fascicularis* Peripheral Blood Mononuclear Cells: Cell Recovery, Cell Viability, and Purity

DR Rodriguez*

InVitro, Biomere, South Barre, MA

Peripheral blood mononuclear cells (PBMCs) are widely used in biomedical research to support pharmacokinetic/pharmacodynamic endpoints in drug discovery and development. However, PBMC isolation from nonhuman primates (NHP) can be problematic. The variation of mean corpuscular volume (MCV) in red blood cells, the time taken, and the skills of the technician to avoid contamination of red blood cells can all affect PBMCs isolation. We compared 2 different methods of PBMC isolation: 50 mL tubes with density gradient insert with 90% density gradient and standard 50 mL tubes with 90% density gradient, both using whole blood collected from 8 female *Macaca fascicularis*, between 8–11 and 14–21 y old. Using these 2 methods in a series of experiments, cell viability and cell recovery were measured using an automated cell counter and cell purity was measured using a hematology analyzer. A lipid profile was performed on all samples using a chemistry analyzer to investigate a possible relationship between the amount of lipid in samples and higher PBMC contamination. Statistical differences in parameter measured in PBMCs between methods were determined using a paired two-tailed Student test. Results showed that cell viability ($P = 0.02$) and cell recovery ($P = 0.01$) were remarkably better with 50 mL tubes with density gradient insert than with standard 50 mL tubes. Red blood cell contamination of the final PBMC preparation was significantly reduced; however, the removal of platelets and reticulocyte were similar between both methods. Moreover, blood samples that had above normal total protein showed higher PBMC contamination with red blood cells in both 50 mL tubes with density gradient insert and standard 50 mL tubes. Blood samples that had above normal total protein also showed lower PBMC contamination with reticulocytes using the standard 50 mL tube isolation method and higher contamination with reticulocyte using the 50 mL tubes with density gradient insert method. This needs further investigation. The results from these experiments indicate that the 50 mL tubes with density gradient insert method offered several advantages over the standard 50mL tube isolation method.

P303 Age-related Changes in GFAP-Immunoreactive Astrocytes in the Mouse Cerebellar Molecular Cell Layer

C Tyszkiewicz,[1,*] ID Pardo,[2] C Liu,[1] CJ Somps,[2]

[1]Comparative Medicine, Pfizer, Groton, CT; [2]Drug Safety Research and Development, Pfizer, Groton, CT

CBA/J mice are frequently used in studying autoimmune thyroiditis, atherosclerosis, kidney abnormalities, pancreatic defects, and enzyme deficiency. In addition, this strain develops hearing loss late in life, particularly at higher frequencies, and seizures related to handling and/or stimulation. However, age-related changes in the central nervous system (CNS) have not been reported in this strain. Using light microscopic and behavioral testing, we examined changes in the cerebellum of 8-, 10-, 16-, and 23-wk-old male CBA/J mice. Immunohistochemistry (IHC) assessments showed a significant increase in immunoreactivity for glial fibrillary acidic protein (GFAP, an astrocytic marker) in the molecular layer of the cerebellum near Purkinje cells in mice 23 wk of age (4/6, 67%) compared with 3 previous timepoints (0/18 for 3 time points, 0%). Increased GFAP immunoreactivity appeared in the form of clusters and distributed multifocally in this layer, but it was not associated with neuronal degeneration/necrosis or microgliosis. Three out of 12 animals at 16 and 23 wk of age exhibited clinical signs that were considered pre-convulsive in nature, typically upon handling, and which increased in frequency as the animals aged. Two of these 3 animals also showed increased GFAP immunoreactivity in the cerebellum. Rotarod behavioral assessment did not reveal any motor coordination changes associated with the increase in reactive glia in the cerebellum. Additionally, quantitative open field locomotor activity testing was perform, but also failed to result in any ambulation changes. These results suggest minimal to mild reactive astrocytosis and potential neuroinflammation in the CNS with age in this mouse strain. These neurological effects should be taken into consideration prior to using this mouse strain for studying neuroinflammation and aging.

P304 The Relationship of Body Temperature to Noise-induced Hearing Loss

C Yancey,[1,*] M West,[2] W Williams,[1]

[1]Comparative Medicine, University of Oklahoma, Oklahoma City, OK; [2]Hough Ear Institute, Oklahoma City, OK

In the animal model of noise-induced hearing loss, Hartley pigmented female guinea pigs are exposed to high decibel (dB) noise via a sound driver of approximately 121 dB for 3 h. This noise exposure results in damage to the hair cells of the cochlea, which produces hearing loss. To determine the efficacy of therapeutic drugs to regrow damaged hair cells, and potentially restore hearing loss, a specific amount of hearing loss is necessary for study inclusion.

Using Auditory Brainstem Response (ABR), we can evaluate hearing thresholds before and after noise exposure. Post-noise ABR readings began showing that fewer guinea pigs were meeting inclusion criteria for the experiment. After careful reflection of research records, an observation was made that the temperature setting of the circulating water blanket was reduced, resulting in lower body temperatures of the guinea pigs. To test if noise-induced hearing loss was related to core body temperature, historical data of 36 guinea pigs were analyzed. Study inclusion requires a specific amount of hearing loss with threshold shifts ranging between 70dB and 90dB in the 2k, 4k, 8k, and 16k frequencies. Of the 36 guinea pigs, 18 were assigned pass and 18 were assigned fail, dependent on if they meet study inclusion by analyzing their threshold shifts. Body temperatures for each guinea pig undergoing noise exposure are recorded every 15 min; these were entered into a data sheet and the mean and median body temperatures were calculated for each guinea pig. Using a 1-way paired t-test, the results showed that both the mean and median were significantly different between those guinea pigs that met the inclusion criteria, and those that failed (P = 0.02). Specifically, guinea pigs with body temperatures on average of 38.2°C or higher passed inclusion criteria more than guinea pigs that were not able to maintain an average temperature of 38.2°C. This finding suggests that guinea pigs that are maintained at a higher body temperature are more prone to hearing loss, determined by ABR threshold shifts, when exposed to high-decibel noise levels. This has important implications for future experiments that rely on reproducibility, and consistency with the 3Rs, specifically reduction in animal numbers and refinement of practices when utilizing the guinea pig model of noise-induced hearing loss.

P305 Longitudinal Characterization of Ladder-climbing Behavior in Young Duchenne Muscular Dystrophy MDX Mice: A Smart Cage Study

S Hwang,[1,*] C Liu,[1] J Pang,[1] M Montgomery,[1] B Manickam,[1] H Zhang,[1] LR Schaevitz,[2] R Goldstein,[1]

[1]Pfizer, Groton, CT; [2]Vium Inc., San Mateo, CA

In clinical trials for the treatment of muscle dystrophy, stair-climbing behavior is an important primary endpoint to evaluate muscle functions in humans. However, preclinically, there has been no report on climbing behavioral measurements in muscle dystrophy mouse models. Video image-based and cloud-based climbing behavioral analysis, especially with machine learning approach, could provide novel translatable outcome measures for preclinical efficacy studies in muscle dystrophy drug development. In this smart cage study 28 male mdx mice (no WT control) were housed in smart cages starting from 4 wk of age and their behavior was continuously captured with cameras starting from 14 to 27 wk of age. During this period, we measured general motion speed, 30 ladder-climbing counts, and daily climbing pattern in a 24-h period on a weekly or monthly basis. General cage-wide and ladder climbing behaviors were analyzed by the manual video-playback methods as well as python-based machine learning algorithm. The smart cage vendor provided algorithm analysis that showed a significant decrease in cagewide night time motion speed at 27 wk of age compared to the previous weeks of ages ($P < 0.001$). At night time (dark phase), mdx mice showed intermittent burst patterns in climbing with a peak in the first hour after lights-off in the evening and 1 h prior to lights-on in the morning ($P < 0.05$). Like the general motion speed, the average climbing count was significantly decreased in week 27 compared to week 14 and 19 ($P < 0.05$). In summary, in this first reported ladder-climbing mouse model, the mdx mice showed intermittent burst patterns in climbing. They significantly decreased general motion speed and climbing count at 27 wk of age. Climbing behavioral analysis may provide novel outcome measures for preclinical studies for muscle dystrophy biology and drug discovery and development.

P306 Hepatocellular RECK Overexpression Attenuates Nonalcoholic Steatohepatitis Susceptibility

R Dashek,[1,2,*] R Cunningham,[3,2] M Moore,[3,2] V Jepkemoi,[2] G Meers,[3,2] B Chandrasekar,[4,2] R Rector,[3,2]

[1]Department of Veterinary Pathobiology, University of Missouri, Columbia, MO; [2]Research Service, Harry S Truman Memorial Veterans Medical Center, Columbia, MO; [3]Nutrition and Exercise Physiology, University of Missouri, Columbia, MO; [4]Cardiovascular Medicine, University of Missouri, Columbia, MO

Nonalcoholic steatohepatitis (NASH) is characterized by hepatic steatosis, inflammation, and fibrosis. Reversion-inducing-cysteine-rich protein with Kazal motifs (RECK) has been shown to exert anti-fibrotic effects in the heart, though its role in liver inflammation and fibrosis is unknown. In this study, we assessed the effects of hepatic RECK overexpression specifically in cultured murine primary hepatocytes and immortalized AML-12 hepatocyte cell line in vitro, and transgenic mice that overexpress RECK in hepatocytes in vivo. Transgenic mice were generated by injecting hemizygous RECK-CAG mice (n = 4) with AAV8-TBG-Cre (1x1012) to overexpress RECK in hepatocytes. Hemizygous RECK-CAG transgenic mice injected with AAV8-TBG-Scr-GFP served as controls (n = 4). Mice were fed a western diet (WD; 45% fat, 17% sucrose, 1% cholesterol) for 8 wk, before being euthanized for tissue collection. In vitro, adenoviral-mediated RECK overexpression (Adv-RECK) in both cultured primary mouse hepatocytes and in AML-12 hepatocytes decreased pro-inflammatory TNFβ and IL-1β mRNA expression (n = 4, $P \leq 0.05$). Moreover, RECK overexpression in both cell types suppressed LPS-induced TLR4 mRNA expression ($P \leq 0.05$). In vivo, AAV8-TBG-Cre significantly increased hepatic RECK expression at both mRNA and protein levels ($P < 0.001$ vs AAV8-GFP control mice). Notably, hepatic RECK overexpression in mice significantly attenuated WD-induced NASH, including attenuated histological hepatic steatosis, inflammation, and ballooning, and tended to decrease hepatic fibrosis. These improvements corresponded with significant reductions in gene expression for markers of inflammation, fibrosis, and hepatic stellate cell activation (TNFα, αSMA, TGFβ1, ADAMs 10 and 17, MMPs 2 and 9, and TIMP1). These data represent the first observations that targeted RECK overexpression in hepatocytes in vivo and in vitro attenuates inflammation, NASH, and fibrosis. Future studies are needed to elucidate the precise mechanisms by which RECK elicits these hepato-protective effects.

P307 The Effects of a Decreased Light Adaptation Time on Electroretinogram (ERG) Results in Nonhuman Primates

KJ DePlancke,* R Boyd

Surgery, Charles River Laboratories, Paw Paw, MI

Electroretinograms (ERGs) are a diagnostic tool that utilizes a series of light stimuli to evaluate retinal function, which may be a sensitive indicator of toxicity from various drugs. The International Society for Clinical Electrophysiology of Vision (ISCEV) has established a standardized protocol that recommends 6 light flash intensities with scotopic and photopic phases being separated by a 10-min background light adaptation to suppress rod photoreceptor function. Our specific aim was to evaluate the effects of decreased light adaptation time on photopic light responses and the degree of variability between testing intervals. A reduced light adaptation time would increase the number of animals that could have ERGs collected in a given time period and it also significantly shortens the sedation period required to complete the ERG data collection, improving animal recovery time and reducing risk of anesthesia morbidity. To determine the effects of a decreased light adaptation time, we measured the a- and b-wave amplitudes for a scotopic single flash intensity of 10.0 cd*s/m2 and a photopic single flash intensity of 3.0 cd*s/m2, amplitudes for a cone 30 Hz flicker response, and the implicit time from a photopic single flash intensity of 3.0 cd*s/m2 with both 5- and 10-min light adaptations in six cynomolgus macaques (*Macaca fascicularis*) over 3 separate intervals. Anesthesia was maintained using an intramuscular injection of a ketamine/dexmedetomidine cocktail (10 mg/kg ketamine and

0.02 mg/kg dexmedetomidine). Following ERG data collection, an intramuscular injection of 0.2 mg/kg atipamezole was administered to reverse the dexmedetomidine. The photopic b-wave amplitude had an overall decrease of 9.2% when going from 10-min to 5-min light adaptation, the a-wave amplitude had an 8.9% decrease, and the flicker amplitude had an 8.1% decrease, all of which were statistically significant ($P < 0.05$). Additionally, the b-wave implicit time decreased by 0.24% and the a-wave implicit time increased by 3.4% when going from 10-min light adaptation to 5-min light adaptation. [BR1] Despite the reductions in waveform amplitude, variability between assessment intervals was not affected, suggesting the observed amplitude reductions are not likely to impact the assessment of retinal cone photoreceptor function.

P308 Chronic Systemic Injection of DREADDs Agonists Clozapine-n-oxide and Compound 21 Does not Change Behavior Relevant to Locomotion, Exploration, Anxiety, or Affect in Male Mice

SL Spears,[1,4,*] F Tran,[2] K Ahn,[2] AJ Eisch,[2,3] S Yun,[2,5]

[1]University Laboratory Animal Resources, University of Pennsylvania, Philadelphia, PA; [2]Children's Hospital of Philadelphia, Philadelphia, PA; [3]Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [4]Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA; [5]Department of Anesthesiology and Critical Care Medicine, University of Pennsylvania, Philadelphia, PA

Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) are a chemogenetic tool commonly used to manipulate rodent brain circuit activity. The most widely used synthetic ligand for DREADDs is Clozapine-N-oxide (CNO). However, CNO is back-metabolized to clozapine, which itself activates numerous endogenous receptors and therefore may influence rodent behavior. To eliminate potential off-target effects of CNO, a new DREADD agonist, Compound 21 (C21), has been proposed as an alternative as it lacks active metabolites. The literature is mixed on whether acute administration of CNO or C21 changes mouse behavior. In contrast, there is no substantial literature on whether chronic administration of CNO or C21 changes mouse behavior. Here we tested whether chronic injections of these 2 distinct DREADD agonists change key behaviors in non designer receptor-expressing mice relative to Vehicle (Veh)-injected control mice. Mice (CamKIIα-icre males) were injected i.p. with Veh (0.5% dimethyl sulfoxide in 0.9% saline, 5mL/kg), CNO (0.2mg/mL, 1mg/kg), or C21 (0.2mg/mL, 1mg/kg) 5 days a week for 16 wk. All 3 groups (8 mice per group) had similar weight gain over the 16-wk experiment, and showed similar measures in behaviors assessed during week 3 (beam breaks in a 30-min locomotion task, time in center of open field or open arms of elevated plus maze) and week 14–16 (ambulatory distance during 240-min activity monitoring, percent marbles buried, grooming time during the sucrose splash test). These data show chronic injections of CNO or C21 do not affect key behaviors as compared to chronic injections of Veh, and may be helpful for behavioral neuroscientists when study design requires repeated injection of these DREADD agonists.

P309 Isoflavone Intake Causes Adrenal Dysfunction Resulting in the Delayed Puberty In Prepubertal Male Rats

S Caceres,[1,*] A Alonso-Diez,[1] B Crespo,[1] G Silvan,[1] M Illera,[1] PC Carlos,[2] D Inmaculada,[2] JC Illera,[1]

[1]Animal Fisiology. Fac Veterinaria, University Complutense of Madrid, Madrid, Spain; [2]Departamento de Medicina, Cirugía y Anatomía Veterinaria, FAc. Veterinaria. Universidad de Leon, Leon, Spain

Puberty is considered a critical period of development that involves sexual maturation and morphological changes. Isoflavones have been described as endocrine disruptors in male rats. Recent studies suggested that isoflavones intake results in the maintenance of an oestrogenic environment in the testes, which blocks androgen production that delay the onset of puberty in male rats. Glucocorticoids also play an important role on he onset of puberty. Therefore, the aim of this study was to determine the effect of isoflavones on adrenal function and its consequence on the onset of puberty in male rats. One hundred and eight 30-d-old male prepubertal Wistar rats were randomly assigned to 3 groups: control, low doses of isoflavones, and high doses of isoflavones. Isoflavone doses consisted of 17 mg/kg/day genistein + 12 mg/kg/day daidzein for the low dose and 170 mg/kg/day genistein: +120 mg/kg/day daidzein for the high dose. Each group was gavaged daily for 5 wk (35 d) using a buttoned cannula. Every week, 6 animals of each group were sacrificed by cervical dislocation after collecting blood from the dorsal aorta. Adrenals were collected at necropsy. Serum and adrenal corticosterone (CT) and dihydroepiandrosterone (DHEA) were determined by a previously validated competitive enzyme immunoassay (EIA). The results revealed that in the control group, circulating and adrenal CT levels increase at the onset of puberty, suggesting that this increase is necessary to activate the hypothalamic-pituitary-gonad axis that activates the synthesis of androgens from adrenal precursors like DHEA by the sexual organs. On the other hand, the intake of low and high doses of isoflavones produces an increase in adrenal levels of CT and DHEA causing an adrenal dysfunction that alters the signals of the hypothalamic-pituitary-gonad axis and blocks the production of androgens necessary for the onset of puberty. In conclusion, isoflavone intake produces an increase of adrenal CT and DHEA levels responsable of blocking the hypothalamic-pituitary-gonad axis signals for the onset of puberty in male rats.

P310 Reduction of *Corynebacterium bovis* Viability following Freeze-thaw in Tumor Cryopreservation Media

G Mendoza,[1,*]RJ Ricart Arbona,[2] K Lertpiriyapong,[2]

[1]Tri-Institutional Training Program in Laboratory Animal Medicine and Science, Memorial Sloan-Kettering Cancer Center, Weill Cornell Medicine, and The Rockefeller University, New York, NY; [2]Center for Comparative Medicine and Pathology, Memorial Sloan Kettering Cancer Center, Weill Cornell Medicine, New York, NY

Corynebacterium bovis, the causative agent of *Corynebacterium*-associated hyperkeratosis, is an opportunistic pathogen known to confound research involving immunocompromised mice. Generally, *C. bovis* is spread via fomites, and recent studies have implicated *C. bovis*-contaminated, cryopreserved tumor tissue as a biosecurity risk. A study was conducted to determine the viability and recoverability of *C. bovis* following a freeze-thaw cycle in tumor cryopreservation media. Columbia CNA (CCNA) agar cultured *C. bovis* was suspended in sterile PBS to create stock solutions containing 1.62 x104 cfu/µl and 244 cfu/µl. Two aliquots containing 0, 1, 10, 25, 50, or 100 µl of each stock solution were added to tissue culture media (80% Roswell Park Memorial Institute media, 10% fetal bovine serum and 10% DMSO) generating a combined total of 1 mL that were frozen at -80°C. Each vial was thawed 24 h later and a 100 µl aliquot was removed and plated on CCNA agar to determine the concentration of the surviving bacterium. Aliquots were expected to contain 1.62x105, 4.05x104, 1.01x104, 2440, 1620, 610, 152, 24, 16, or 0 cfu/mL. The colonies/plate were enumerated with only aliquots containing > 610 cfu/mL yielding *C. bovis* growth. These results suggest that subjecting *C. bovis*-contaminated tumor stocks to a single freeze-thaw cycle could potentially, depending on the level of bacterial contamination, be used to derive *C. bovis*-free tumor for subsequent passage in mice.

P311 Impact of Thermal Support Systems on the Physiologic and Recovery Parameters of Anesthetized Common Marmosets (*Callithrix jacchus*)

AL Cates,[1,2] JL Haupt,[1,*] JG Fox,[1]

[1]Massachusetts Institute of Technology, Cambridge, MA; [2]Michigan State University, East Lansing, MI

Despite the increasing popularity of common marmosets (*Callithrix jacchus*) as research models, few publications exist regarding anesthesia and surgical support. General anesthesia is known to cause physiologic alterations in many body systems, as well as inhibit the regulatory control of thermoregulation. In rodents, the consequences of hypothermia during anesthesia yield a significant decrease in heart and respiratory rate. The purpose of this study was to evaluate the effectiveness of thermal support systems for the maintenance of body temperature in the sedated marmoset as well as the impact of hypothermia on the physiologic and recovery parameters following alfaxalone sedation. Marmosets were divided into 4 groups (n = 6): no thermal support; forced-air warming (variable setting); circulating warm-water blanket (42°C setting); or electric warming pad with conductive fabric (43°C setting). Following sedation with alfaxalone (10 mg/kg IM) vital parameters (rectal temperature, heart rate, respiratory rate, and oxygen saturation) were evaluated every 5 min until recovery. The duration of immobilization (loss of posture to first head lift) and recovery length (first head lift to climbing in capture box) was recorded. Our results indicated that without thermal support marmosets lost a significant amount of body temperature (mean loss 8oF) following sedation. The use of forced-air warming maintained a stable body temperature as compared to minor decreases with the warm-water (2oF) and electric warming systems (3oF). Hypothermia resulting from no thermal support caused a significant decrease in heart rate, with no significant differences in respiratory rate or oxygen saturation. Recovery from sedation was significantly prolonged in animals without thermal support with an average increase of 20 min in total recovery time. This study highlights that external thermal support is needed in sedated marmosets for maintenance of normal body temperature, stabilization of vital parameters, and expedient recovery from sedation and anesthesia. In addition, use of forced-air warming is recommended for the maintenance of normothermia during sedation and anesthesia in the common marmoset.

P312 Evaluation of Hot Bead Sterilizers Used for Rodent Surgery

JL Haupt*, H Nam, S Lackeyram-Owen, RM Kramer, JG Fox

Massachusetts Institute of Technology, Cambridge, MA

Aseptic technique, including sterilization of instruments, is required by the *Guide for the Care and Use of Laboratory Animals* for all survival surgeries. Rodent surgery offers unique challenges, as investigators may need to do multiple surgical procedures in a short time period. Hot bead sterilizers use small glass or metal beads and high temperatures for brief exposure times to inactivate microorganisms, hence their widespread use for rodent surgeries. These devices have been used for several years in the dental profession. However, the FDA has recently recommended their use be discontinued due to potential failure to sterilize dental instruments. This project evaluated the efficacy of hot bead sterilizers used for rodent surgery in terms of maximum temperature attained and time to reach a germicidal temperature (230°C/446°F). Units with a "ready" light indicator or exterior temperature gauge were evaluated for accuracy of the indicators relative to germicidal and core temperatures. Fifteen total units were tested, including 5 different models of sterilizers (variable number per model dependent on availability) were evaluated by serial core temperature measurement until high point temperature stabilization. For models with indicator lights or external temperature gauges, time points and temperatures were noted when ready status was indicated. Overall, the hot bead sterilizers took an average of 28 min to attain a germicidal temperature. However, this varied significantly by model, with some units only taking 16 min and other units never attaining germicidal temperature. The accuracy of indicator lights was poor with ready lights engaged on average at 340°F (23 min after turning on the unit), which was 110 degrees cooler than germicidal temperature. Exterior temperature gauges over-estimated core temperature 73% of the time. This study highlights that hot bead sterilizers used for rodent surgery vary significantly in their ability to attain germicidal temperature and in the accuracy of lights indicating units have attained germicidal temperature.

P313 Efficacy of a Commercial Primate Diet Containing Fenbendazole in Treating *Trichuris trichiura* Infection in a Captive Baboon Colony (*Papio* sp.)

JW Dutton,* LL Condel, JB Jimenez, SE Price, DB Elmore

Southwest National Primate Research Center, Texas Biomedical Research Institute, San Antonio, TX

We house a colony of approximately 1,000 baboons, including breeding animals and those on active research projects. This study examined the efficacy of a diet formulated with fenbendazole in a group of juvenile and adult baboons (*Papio* spp.) naturally infected with *Trichuris trichiura*. Although fenbendazole has been shown to be effective in elimination of *Trichuris trichiura* in singly housed baboons, this study evaluated treatment in an outdoor social housing environment where potential exposure to untreated animals increases the risk of reinfection. A commercially available diet containing 600 ppm fenbendazole was given to 25 baboons in 2 groups, aged 2.5 to 14.5 years, for 5 d. Following the initial treatment, animals were fed this same diet on week 9 for an additional 5 d. Animals were assessed by fecal egg count (FEC) prior to initiation of treatment, and again at weeks 4 and 14. The fecal egg count of *Trichuris ova* in each of these animals varied from 25–1675 eggs per gram of feces (EPG) prior to treatment (mean = 285). Two wk after completion of the first round of treatment, this was reduced to 0 EPG in all animals, for a 100% reduction. Animals remained negative by FEC through week 14, 1 mo following completion of the second round of treatment. On week 28, 3 animals were opportunistically sampled, and 2 were positive (0–75EPG, mean = 33). This information will be used to guide future parasite control measures in the baboon colony.

P314 Evaluation of Topical Treatment Regimens for Ulcerative Dermatitis in C57BL/6J Mice

BL Kick*, B Lyons, R Doty

Comparative Medicine and Quality, The Jackson Laboratory, Bar Harbor, ME

Ulcerative dermatitis is a common spontaneous skin disease in mice on a C57BL/6 background. An exact etiology has yet to be determined, with a wide variability in treatment effectiveness often resulting in euthanasia. This study evaluated the effectiveness of topically treating lesions with a 0.005% sodium hypochlorite (n = 18), type 1 collagen with silver oxide ointment (n = 20), and 2% chlorhexidine solution (n = 20) on a 3 times a week basis for a 2-wk period in combination with a toenail trim as needed. The control treatment consisted of toenail trimming as needed (n = 17). During the study, only 1 mouse needed to be euthanized due to lesion severity. Regardless of treatment, lesions that were limited to the dorsal neck exhibited a complete healing rate of 86%. In contrast, complete healing rates for lesions at multiple sites including the neck was 17%, and lesions located on the body excluding the neck was 9%. Complete healing based on treatment type occurred at a rate of 33% for 0.005% sodium hypochlorite, 30% for collagen ointment, 15% for 2% chlorhexidine, and 35% for nail trim only. Pearson's chi square test was used to determine whether there was a statistically significant difference between expected and observed frequencies when evaluating lesion location and treatment type. Results indicate that none of the treatment regimens evaluated were consistently successful in treating dermatitis lesions located on areas of the body outside of the dorsal neck region. The novelty portion of this study highlights distinction in the behavior of mice with ulcerative dermatitis based on lesion location. Regions that are more available for oral grooming are less likely to heal with topical treatments, since the behavior is not being prevented.

P315 Size of Cage Used for Euthanasia Can Affect the Welfare of

Rats During Euthanasia

DL Hickman*

Laboratory Animal Resource Center, Indiana University, Indianapolis, IN

When using low flow volume displacement per minute rates to euthanize with CO_2, the size and shape of the chamber can have an effect on the well-being of the rodents. Because CO_2 is heavier than air and tends to collect primarily at the bottom of the cage during the euthanasia process, this is especially of concern in species which regularly engage in a bipedal stance as part of their normal postures. This study was designed to assess how behavioral and physiological assessments of welfare were influenced by the volume and height of the cage used for euthanasia. The rats used on this study were all adult males of an outbred stock of alcohol-preferring rats (P rats) maintained at our institution. These rats were euthanized with 10% volume per minute displacement rate of 100% CO_2. The subject rats were individually housed and euthanized in 1 of 4 custom-made plexiglass euthanasia chambers: standard dimension rat cage, twice the height of standard cage, twice the floor space of standard cage, or twice the height and floor space of standard rat cage. The euthanasias were digitally recorded and the video was scored for time to estimated time to loss of consciousness (approximated by noting when the rat dropped its head and touched its nose to the floor of the chamber) and number of rears per minute were recorded for each rat. Additionally, terminal blood samples were taken and assessed for serum corticosterone. The rats that were euthanized in the standard caging took a significantly ($P = 0.0035$) longer time to loss of consciousness. The rats were euthanized in the cages with twice the floor space demonstrated a significant increase in the number of rears per minute ($P < 0.0001$) as compared to the other groups. There were no significant differences between the groups in the measurement of serum corticosterone ($P = 0.4714$). These findings suggest that when the rats were able to reach the top of the cage, they spent more time standing and exploring the dimensions of the cage as compared to those euthanized in cages that are twice the height. Additional assessment in other strains and species is warranted to shape future recommendations. As more institutions use cages with increased height to accommodate the normal postures of rats, this information is critical to understand how cage dimensions can affect welfare during CO_2 euthanasia in the home cage.

P316 Evaluation of Animal Welfare of Socially Housed Aged Male Sprague Dawley Rats

B Skiles, S Kulkarni, DL Hickman*

Laboratory Animal Resource Center, Indiana University, Indianapolis, IN

Rats are social animals and generally prefer to be housed in groups. When adult male rats grow to a size where only one can fit in a cage (according to the space requirements of the *Guide*), facilities must decide if the rats should be housed together, exceeding the space requirements of the *Guide*, or separately, removing the social interactions. To evaluate this welfare question, 56 paired male rats of at least 600g each were either left paired or separated into individual housing. Three days after the manipulation, a blood sample was taken from both rats and one was tested with the forced swim test to assess their initial response to the separation. One month later, the second rat was tested with the forced swim test followed by a terminal blood collection to assess their chronic response to the separation. The blood samples were assessed for corticosterone and the neutrophil:lymphocyte ratio. There were no significant differences between the paired or the separated rats in the corticosterone, the neutrophil:lymphocyte ratio, and latency to stop swimming when assessing the rats 3 d following manipulation. At 1 mo following manipulation, the rats that were separated into individual housing demonstrated a significant decrease in their corticosterone ($P = 0.0079$). There were no significant differences in the neutrophil:lymphocyte ratios between the groups ($P = 0.8059$) and the latency to stop swimming ($P = 00702$), though the rats that had been separated into individual housing showed a tendency to swim longer (71.75 +/- 8.17 sec) as compared to the paired rats (49.42 +/- 8.3 sec). These findings suggest that large rats may prefer to be housed separately as compared to being housed socially with reduced floor space available. Future studies should evaluate how this finding changes with the provision of increased floor space.

P317 Comparison of Gut Microbiota from 2 Different Colonies of Guinea pigs

D Chadalavada,[1,*] P Subramanian,[2] J Perez-Chaparro,[3]

[1]National Institute of Allergy and Infectious Diseases, National Institutes of Health (Charles River Laboratories Contractor), Bethesda, MD; [2]Bioinformatics and Computational Biosciences Branch, Office of Cyber Infrastructure and Computational Biology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD; [3]NIAID Microbiome Program, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Most microbiota studies are conducted on mouse models but exploring the microbiota of other species could complement mouse studies and generate new relevant knowledge for biomedical research in humans. This study aims to describe and determine differences in the gut microbiota bacterial and archaeal community profiles of 2 different colonies of wild type guinea pigs (*Cavia porcellus* Strain 13) from 2 distinct animal facilities, and to assess changes in the gut microbiota over time. To do this, 7 animals which had been housed from birth at a different federal facility (outside group) were brought to our facility. These animals along with 6 guinea pigs from our facility's colony (inhouse group) were housed in open top cages and maintained on water bottles in the same room for 12 wk. Fecal samples were collected from all animals at baseline and from 5 inhouse and 4 outside group guinea pigs at 12 wk. The sample microbiota were then profiled using V4 *16S rRNA* metagenomic analysis. Preliminary results show that alpha-diversity was considerably lower in the outside group at baseline but no at week 12. In addition, there was a trend for this group at baseline to cluster separately when beta-diversity, the difference in community composition across animals, was measured suggesting that observed differences may be due to the facility of origin. Differences across animals were driven by members of the bacterial *Ruminococcacea* and *Methanosphaera* (archaea) families at baseline in the inhouse group. At genus level, members of the archaea *Methanosphaera* genera and bacterial *Caproiciproducens* genera were significantly higher in the inhouse group, whereas members of *Bacteroides, Allestipes, Adlercreutzia, Bilophila and Lachnospiraceae_NK4B4_group* genera were enriched in the outside group. These significant differences did not persist at week 12. In conclusion, differences observed in bacterial and archaeal community profiles of the guinea pig gut microbiota in the studied groups appeared to be husbandry related from the colonies maintained in different micro and macro environments.

P318 The Impact of CO2 EuthanRsia on Sperm Quality, In Vitro Fertilization and Embryo Development Tates

S Gerb,* L Stevey, C Agca, Y Agca

Veterinary Pathology , University of Missouri, Columbia, NJ

The cryopreservation of sperm is an efficient and cost-effective method to preserve scientifically important rodent models of human diseases and safe guard them against genetic contamination, infectious disease, and natural disasters. Euthanasia via cervical dislocation (CD) is commonly used for collecting epididymal sperm for downstream applications such as cryopreservation or in vitro fertilization (IVF). While effective, it's aesthetically displeasing, requires technical proficiency, and scientific justification to perform. Alternative methods include CO_2 euthanasia. However, its effects on

sperm aren't well understood. In this study, 12-wk-old C57BL/6 male mice were euthanized either by low flow CO_2 (30% displacement/minute), high flow CO_2 (100% displacement/minute), or CD (n = 36). Flow rates were chosen by determining if the slowest acceptable or most rapid influx of CO_2 impacts sperm, allowing us to titrate the flow to determine an optimal rate. Time from onset of euthanasia to death was recorded, and sperm was collected immediately following euthanasia. Sperm motility analysis, including progressive motility, progressive velocity, average path velocity and track speed, was performed on fresh and frozen-thawed sperm samples for each mouse using the IVOS (Hamilton Thorne sperm motility analysis system). There were no significant differences in motility parameters across groups for either fresh or frozen sperm samples ($P > 0.5$). Frozen-thawed sperm from each group was used to perform IVF using fresh oocytes collected from 7-8-wk-old female C57BL/6 mice. Percentage of fertilized oocytes from each treatment group wasn't significantly different for low flow (52.09±27.28), high flow (51.01 ± 22.46), or CD (57.59 ± 25.64). Additionally, in vitro embryonic development rates of the resulting zygotes to morula and blastocyst stages were similar across the treatment groups ($P > 0.5$). IVF rates and subsequent embryo development rates are not affected when using sperm from animals euthanized via CO_2, suggesting CO_2 euthanasia is a viable replacement for CD when preserving sperm quality is essential.

P319 Functional Analysis of Novel Genetic Factor for Chronic Kidney Disease in Mice

Y Takahashi,[1,*] H Sasaki,[1] K Hiura,[1] M Watanabe,[1] K Nakano,[2] T okamura,[2] N Sasaki,[1]

[1]Kitasato University, Towada, Japan; [2]National Center for Global Health and Medicine, Tokyo, Japan

The ICGN mouse is a chronic kidney disease (CKD) model mouse that exhibits severe proteinuria at 8 wk and eventually progresses to CKD. Previously, we demonstrated that proteinuria in ICGN mice is caused by the deletion mutation in the *Tensin2* (*Tns2*) gene. Tns2 is a focal adhesion-localized multidomain protein expressed in various tissues, and its dysfunction leads to alterations in podocytes. To identify the modifier gene(s) to chronic kidney disease, we produced congenic strains carrying the Tns2-null mutation (*nph*) on the several genetic backgrounds and analyzed their severity. Interestingly, the C57BL/6J (B6) and 129/SvJcl mice congenic mice exhibited milder phenotypes than did ICGN, DBA/2, and FVB strains. Thus, we performed a genome-wide linkage analysis of backcrosses between 2 *Tns2*-deficient mouse strains, B6. ICGN-*Tns2*^{nph} (resistant) and FVB. ICGN-*Tns2*^{nph} (susceptible) and detected a novel major modifier locus on chromosome 10 and found the Nsas1 gene located on chromosome 10. In this study, we used a CRISPR / Cas9 system to introduce Nsas1 deficiency into FVB/NJ mice (FVB-^{nph}) with a deletion mutation in the Tns2 gene used in a chronic kidney disease model, and it was examined whether Nsas1 contributed to the progression of nephropathy. First, the prepared FVB-Nsas1KO mice did not show any remarkable clinical symptoms. FVB-*nph:Nsas1*^{KO} mice (7 males, 8 wk) were significantly lower ($P < 0.05$) urinary albumin levels than FVB-*nph* (5 males, 8 wk). To examine the localization of Nsas1 in the kidney, immunochemical staining was performed using an anti-NSAS1 antibody. Then there is no difference in the localization of Nsas1 between FVB-*nph* mice (male, 8 wk) and FVB-*nph:Nsas1*^{KO} mice (male, 8 wk). However, in 2 strains, expression was observed in glomeruli, particularly in proximal tubules. These results suggest that Nsas1 is localized in the glomeruli and proximal tubules of the kidney, and acts as an exacerbation factor for nephropathy. The endpoint is when the mice lose more than 20% of weight compared to the control group.

P320 Tensin 2 Suppresses Intestinal Tumor Progression via Inhibiting the Wnt/β-catenin Pathway

K Hiura1,*Y Takahashi,[1] M Watanabe,[1] K Nakano,[2] T okamura,[2] S Kakuta,[3] H Sasaki,[1] N Sasaki,[1]

[1]Kitasato University, Towada, Japan; [2]National Center for Global Health and Medicine, Shinjuku, Japan; [3]The University of Tokyo, Bunkyo, Japan

Tensin 2 (TNS2) is a focal adhesion localized multidomain protein expressed in various tissues and possesses protein tyrosine phosphatase (PTP) and Src homology 2 (SH2)-phosphotyrosine binding (PTB) domains. TNS2 expression is significantly decreased in many tumor cell lines and low TNS2 expression is associated with poorer relapse-free survival in some kind of cancers, suggesting that loss of TNS2 is associated with tumor progression. Meanwhile, deregulation of Wnt/β-catenin signaling is frequently found in colorectal cancer. Furthermore, it has been reported that integrin-linked kinase (ILK) activity induces the stabilization and nuclear import of β-catenin through inhibition of GSK3 activity. To investigate the role of TNS2 in intestinal tumorigenesis in vivo, we introduced the Tns2 mutation into *Apc*$^{Min/+}$ mouse (male, n = 7), a model for human familial adenomatous polyposis, compared to compound mutant mouse controls (male, n = 6). These mice were euthanized to collect samples. The compound mutant mice resulted in a significant increase in tumor number and size in the small intestine and colon compared with *Apc*$^{Min/+}$ mice. We found that TNS2 negatively regulated Wnt signaling by suppressing nuclear translocation of β-catenin via reduction of Integrin-linked kinase (ILK) activity in colon cancer cell lines. In conclusion, TNS2 may suppress the colorectal cancer growth and malignancy *in vitro* and *in vivo*, through dephosphorylation of ILK.

P321 A Unique Uncoupling of Cell Death and IL-1 Release in a Mouse Model of Meningitic *Escherichia coli* K1 Infection

C Chambers*, JA Skyberg

University of Missouri, Columbia, MO

Escherichia coli strains expressing the K1 capsule are one of the leading causes of sepsis and meningitis in newborn infants. Previously, we found IL-1 receptor knockout (IL-1R$^{-/-}$) mice were significantly more susceptible to intracranial infection with *E. coli* K1 than wildtype C57BL/6 mice. Furthermore, we found that IL-1 release from macrophages in vitro is dependent on the NLRP3 inflammasome. Here we dissected pathways of NLRP3 and IL-1 activation in response to *E. coli* K1 infection. NLRP3 is an inflammasome complex activated in response to K+ efflux during cell damage and leads to the downstream maturation and release of IL-1β. NLRP3 activation also leads to the cleavage of gasdermin-D, allowing it to form pores in the cell membrane, and initiating an inflammatory form of cell death called pyroptosis. Interestingly, while *E. coli* K1 induced significant cell death, as determined by LDH release, this death was independent of NLRP3. To confirm that cell death was not due to pyroptosis, we treated cells with the gasdermin-D inhibitor, necrosulfonamide and found no difference in LDH release. We also treated infected cells with glycine, a cell membrane stabilizer, and KCl which prevents K+ efflux. As expected, we found that glycine significantly decreased cell death, while KCl had no effect. We also found that IL-1 release was attenuated by necrosulfonamide and KCL, but not glycine. Finally, we treated macrophages with brilliant blue G (BBG), a purinergic receptor blocker, and found that BBG was able to block cell death in infected macrophages, without significantly altering IL-1 production. While more research is needed to understand the implications in vivo, these data reveal a unique uncoupling of cell death and IL-1 release during *E. coli* K1 infection which may lead to the development future therapeutics.

P322 Age- and Sex-related Changes in Hematologic and Biochemical Parameters of Dunkin Hartley Guinea Pigs

AR Personett*, MF Afzali, SB Bork, LH Burton, LB Radakovich, CA Seebart, KS Santangelo

Colorado State University, Fort Collins, CO

The Dunkin Hartley is the most common guinea pig strain used in biomedical research, particularly for studies of asthma, allergy, infectious disease, reproduction, and osteoarthritis. Minimally invasive blood tests, such as CBCs and serum biochemistry profiles, are often collected for diagnostics and laboratory analyses. However, reference intervals for these assays have not yet been well-established in this strain. The purpose of this study was to establish sex-specific reference intervals for hematologic and biochemical parameters of Dunkin Hartley guinea pigs and determine age- and sex-related differences. Hematologic and biochemical parameters of control animals from prior laboratory studies were retrospectively obtained from 91 male and 52 female guinea pigs between 2 and 15 mo of age. All blood parameters were analyzed by a veterinary clinical pathology laboratory. Reference intervals were established for male and female guinea pigs according to the American Society for Veterinary Clinical Pathology guidelines. Animals were categorized as juvenile (age, ≤ 3 months) or adult (age, > 3 months). Age- and sex-related differences were determined using unpaired t-tests or nonparametric Mann–Whitney tests. MCHC, lymphocyte %, phosphorus, albumin:globulin ratio, ALP, and anion gap decreased with age. MPV, heterophil %, eosinophil %, basophil %, glucose, BUN, creatinine, calcium, magnesium, total protein, albumin, globulin, ALT, AST, GGT, and HCO3 increased with age. Female guinea pigs had lower Hgb, Hct, RBC count, MCHC, total white blood cell count, heterophil %, ALT, sodium, and bicarbonate and higher MCV, platelet count, MPV, lymphocyte %, eosinophil %, basophil %, total protein, albumin, and cholesterol values compared to males. Establishing age and sex differences in hematologic and biochemical parameters of Dunkin Hartley guinea pigs provides valuable insight into their physiology to better evaluate diagnostics and experimental results.

P323 Effect of Social Housing on Changes in Monocyte Expression in Pigtail Macaques (*Macaca nemestrina*) during Acute Simian Immunodeficiency Virus Infection

N Castell,* S Guerrero-Martin, E Shirk, J Brockhurst, S Queen, M Li, B Bullock, B Carlson, R Adams, L Gama, D Graham, C Zink, J Clements, J Mankowski, KA Metcalf Pate

Johns Hopkins School of Medicine, Baltimore, MD

Simian immunodeficiency virus (SIV) infection in pigtail macaques (*Macaca nemestrina*) is a widely used model for AIDS pathogenesis. The course of infection in this model physiologically resembles HIV infections in humans, and disease progression is similarly affected by both internal and external factors. Because the deleterious impact of chronic stress on the immune response is well established, we hypothesized that singly housed macaques would have a less robust innate immune response post-inoculation than those housed in stable conspecific pairs. Monocytes are important effecters of the innate immune response, and progress from a CD14+ to a CD16+ phenotype when activated. Flow cytometry (FACS) was used to characterize the CD14 and CD16 expression profiles of peripheral blood monocytes in male juvenile pigtail macaques (n = 76) before infection with SIV, and at 7, 10, and 14 d post infection. At baseline there were no significant differences between monocyte numbers or profiles between socially housed and singly house animals. Post infection, monocyte counts significantly increase by days 10 and 14, driven by an increase in classical monocytes (CD14+) in all animals. Both intermediate (CD14+CD16+) and non-classical (CD16+) monocyte numbers remain static. Animals that were socially housed showed a significantly greater rate of classical monocyte proliferation at all post-infection time points than singly housed animals. These findings demonstrate that the immunomodulatory effects of chronic social stress significantly alter the innate immune response to acute retroviral infection. This variable should be considered in study design both to reduce inter-animal variability and for its translational significance for people with HIV.

P324 Effects of Oxygen Supplementation on Ketamine/Xylazine Anesthesia in C57BL/6 Mice

CE Blevins,[1,2,*] NA Celeste,[3] J Marx,[1,2]

[1]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; [2]University Laboratory Animal Resources, University of Pennsylvania, Philadelphia, PA; [3]College of Veterinary Medicine, The Ohio State University, Columbus, OH

Oxygen supplementation is rarely administered when anesthetizing laboratory mice, despite reports that mice become profoundly hypoxic under anesthesia. We investigated the physiologic effects of oxygen supplementation on C57BL/6 mice anesthetized with common injectable drug protocols, and its effects on anesthetic depth. Mice were anesthetized with 1 of 3 protocols: low-dose ketamine/xylazine (KX), medium dose ketamine/xylazine/acepromazine (m-KXA), or high-dose ketamine/xylazine/acepromazine (h-KXA). KX (100/8 mg/kg) was chosen as a dose to provide immobilization of mice, suitable for imaging procedures. M-KXA (100/10/1 mg/kg) was chosen as a dose commonly recommended for surgical procedures. H-KXA (150/12/3 mg/kg) was chosen following a pilot study to determine a dose at which all mice achieved a deep plane of anesthesia, suitable for long surgical procedures. In a cross-over design, mice were anesthetized twice at each dose, once with supplemental oxygen (100% oxygen at 0.6 L/min) and once breathing room air. Heart rate, respiratory rate, SpO_2, rectal temperature, and hindlimb paw withdrawal reflex were recorded every 5 min following loss of righting reflex. With KX and m-KXA, mice receiving supplemental oxygen had SpO_2 values above 92% and mice not receiving supplemental oxygen had SpO_2 values ranging from 55–80%. Oxygen supplementation did not affect heart rate or respiratory rate. In the KX and m-KXA doses, supplemental oxygen significantly decreased the time mice were at a surgical plane of anesthesia (KX with oxygen: 21.7 ± 14.45 min, KX without supplementation: 29.2 ± 17.81 min, m-KXA with oxygen: 43.3 ± 17.82 min, m-KXA without supplementation: 61.3 ± 27.1 min). In the h-KXA group, all mice not receiving oxygen supplementation died, while all mice receiving oxygen supplementation survived. This suggests that oxygen supplementation provides survival benefits at high doses of injectable anesthesia. Overall, these results indicate that mice anesthetized with ketamine/xylazine are routinely hypoxic, which increases duration of anesthesia and may be alleviated with oxygen supplementation.

P325 Use of Entyce in New Zealand White Rabbits (*Oryctolagus cuniculus*)

J Draper*, EK Daugherity

Cornell University, Ithaca, NY

Inappetence is a common clinical signs in laboratory and privately owned animals. It is a welfare concern, especially in rabbits, *Oryctolagus cuniculus*, as inappetence can lead to gastrointestinal stasis and death. There are numerous causes of inappetence in rabbits, including stress, pain, systemic disease, poor diet, and the use of opioids to treat or control pain. In rabbits the recommended treatment for inappetence and gastrointestinal stasis includes fluid therapy, enteral nutrition, gastrointestinal motility stimulants, and appetite stimulants. The use of appetite stimulants in rabbits is anecdotal as there are no published studies evaluating efficacy. We evaluated the appetite stimulant Entyce, capromorelin, a ghrelin receptor agonist effect on appetite in healthy New Zealand White (NZW) rabbits. Nine male NZW rabbits received 3 oral treatments: sterile water (control), capromorelin 4 mg/kg, and capromorelin 8 mg/kg, once a day for 3 d with a 4-d washout period in a randomized crossover study. Feed intake and fecal output were monitored daily with body weight monitored biweekly. Baseline data was collected prior to the start of treatments. The statistical analysis

using the linear mixed effects model showed that capromorelin at 8 mg/kg caused a significant increase in food consumption (P = 0.018) and fecal output (*P* = 0.0196) in healthy NZW rabbits when compared to the control. Furthermore, capromorelin at 4 mg/kg approached significance for food consumption (*P* = 0.08) and fecal output (P = 0.081) when compared to the control. There was no significant difference in body weight between the groups at any timepoint. Therefore, capromorelin at 8 mg/kg caused a significant increase in feed intake and fecal output in healthy NZW rabbits over a three day time period. To our knowledge, this is the first study evaluating the effects of capromorelin in NZW rabbits.

P326 Comparing the Effects of Irradiation and Mammary Fat Pad Implantation on Patient-derived Xenograft Model Characteristics

T Chan*, D VanBuskirk, R Banzon, M Cheng, K Draheim

Department of Innovation & Product Development, The Jackson Laboratory, Sacramento, CA

Due to its ability to preserve the heterogeneity and tumor microenvironment of the original patient tumor, patient-derived xenograft (PDX) tumor models are commonly used in preclinical oncology studies for evaluating drug efficacy and developing therapeutic strategies. However, PDX models can often have a long latency period before the tumor is usable. Additionally, the engraftment rates and the time to reach the target tumor size within the same cohort can vary widely. This variability and latency considerably limit the ability for researchers to apply PDX models to real-time treatment schedules. We developed a method that will decrease the time between engraftment and palpable tumor and reduce growth variability, while not substantially altering the genetic, histological, and drug response properties of the PDX model. Our lab has previously established a nonsurgical method to engraft PDX tumors into a non-cleared mammary fat pad (MFP). Using the same technique, we compared the effects of irradiation and MFP implantation, or both combined, on the growth kinetics of several pre-established PDX models in NOD. Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice (n=6-8 mice per group for each PDX model). We observed minimal effects from irradiation but improved latency and decreased cohort variability for the MFP-implanted tumors. We then analyzed the genomic and histological properties of the MFP-implanted tumors to confirm there were no significant changes. MFP-implanted PDX tumors also demonstrated responsiveness to standard of care drug treatment comparable to that of tumors engrafted subcutaneously. These findings show that MFP implantation can improve the engraftment and growth, as well as reduce the latency period of PDX tumors while preserving the fundamental characteristics of the PDX models. The results from this study has important implications for increasing the usability of PDX tumor models in future preclinical studies.

P327 Characterizing the Pathogenesis of Murine Chapparvovirus in CD-1, C57BL/6, and NSG Mice

ML Kain,[1,*] S Monette,[2] RJ Ricart Arbona,[3] NS Lipman,[3]

[1]Tri-Institutional Training Program MSK/WCMC/RU, New York, NY; [2]Laboratory of Comparative Pathology, MSK, WCM, The Rockefeller University, New York, NY; [3]Center of Comparative Medicine and Pathology, MSK & WCM, New York, NY

Murine Chapparvovirus (MuCPV), the etiological agent responsible for a long-recognized inclusion body nephropathy syndrome in mice, has been identified in laboratory mice in several countries with an estimated prevalence of 9%. This virus can cause significant renal pathology, morbidity, and mortality in immunodeficient strains, and subclinical infection in immunocompetent mice. Tissue and cell tropism was investigated using RNA in situ hybridization (ISH) in CD-1, C57BL/6 (B6), and NOD *scid* gamma (NSG) mice following oronasal infection of 40 mice per strain with 5.8 x 107 viral copies. ISH was supplemented with histological examination of all major organs and clinical pathology data (complete blood count, serum chemistry, and urinalysis) collected at numerous time points between 3 d and 4–6 mo post-inoculation (14 time points for the B6 and CD-1 mice, and 20 time points for the NSG mice). MuCPV replicates in both the gastrointestinal tract (stomach, large intestine, and small intestine) and kidneys within 1 wk of inoculation, much earlier than the onset of shedding in the urine and the feces (4–7 wk post-inoculation). MuCPV does not appear to replicate in other examined tissues. Replication in the gastrointestinal tract was patchy and temporally inconsistent, although CD-1 mice showed increasing viral replication in the kidney and gastrointestinal tract over time. Renal viral replication was associated with tubular injury and inflammation, whereas there were no associated gastrointestinal lesions. Renal lesions in immunocompetent CD-1 and B6 mice were mild and did not appear to affect renal function or general health, as no clinical signs or significant changes in complete blood count, serum chemistry, or body weight were observed. These results shed light on the pathogenesis of this novel virus and the effects it may have on animal health and biomedical research.

P328 Efficacy of Beta-defensin Derived Cationic Antimicrobial Peptides against Biofilm-producing Bacteria.

E Knebel*, Z Shen, S Zhang

Veterinary Pathobiology, University of Missouri, Columbia, MO

Host antimicrobial peptides, such as avian beta-defensins (AvBD), are cationic antimicrobial peptides (CAMPs) that are part of the innate immune system of many species. These peptides are promising alternative therapeutic strategies notably against mature biofilms, which are intrinsically resistant to conventional antimicrobials. Two short, linear CAMPs (A and B) ranging from 11–25 amino acid residues were designed based on AvBDs and demonstrated antimicrobial properties and stability against multiple bacterial species including multidrug-resistant isolates. In this study, CAMP A and B peptides were compared to ciprofloxacin in their efficacy against mature ATCC 9027 *Pseudomonas aeruginosa* biofilms using a crystal violet biofilm mass assay. Each peptide's minimum inhibitory concentrations in planktonic culture reflected previous findings of 16 µg/mL (CAMP A) and 32 µg/mL (CAMP B). Each compound was incubated with a mature 20–24 hr-old *P. aeruginosa* biofilm for periods of 30 min, 2 h, 8 h, and 24 h at a concentration ranging from 16–256 µg/mL in 2-fold incremental dilutions. Compared to untreated biofilms, the biomass decreased significantly in the 2h incubation period for all 3 compounds, but not in longer incubation periods. Ciprofloxacin significantly reduced the mass at all concentrations tested ($P < 0.05$). CAMP B significantly reduced mass at higher concentrations of 128 µg/mL and 256 µg/mL ($P < 0.05$). Biomass reduction was only observed with CAMP A at 256 µg/mL. None of the biofilms were fully eradicated, demonstrating biofilm persistence and regrowth. The results suggest that these CAMPs do not have the same antimicrobial effects on mature biofilm as seen in planktonic culture. Further evaluation with these and other peptide analogues against other bacterial species is indicated.

P329 Evaluation of the Aversive Nature of Carbon Dioxide as Compared to Isoflurane

S Kulkarni, DL Hickman*

Laboratory Animal Resource Center, Indiana University, Indianapolis, IN

The appropriate use of inhalant anesthetics, such as carbon dioxide and isoflurane, for euthanasia in rodents is an ongoing topic of debate due to concerns regarding the aversive nature of these gases. Specifically, welfare concerns have been associated with repeat exposure to these gases, though the gases are generally evaluated separately. The aim of this study was to expand upon a previously published study evaluating the aversiveness of carbon dioxide by introducing an isoflurane treatment group in parallel. Aversion was

tested using a forced exposure setup. Twelve naive female Sprague-Dawley rats were exposed during 4 consecutive d, once to each of 4 treatments: isoflurane (3% isoflurane in 3.5L O2/min), fox urine (5ul on a tissue in a tea ball), oxygen (3.5L O2/min), and carbon dioxide (18.8% volume per minute displacement rate of 100% CO_2). Order of exposure was randomized using 3 4x4 Latin squares. Following a 5-min acclimation period, rats were exposed to the treatment for 2 min. The exposure period was recorded and a baseline period (60 s before exposure) and active period (60 s after exposure) were scored for behaviors such as rearing, lid-pushing, bedding manipulation, horizontal locomotor activity, and immobility time. Consistent with the previously published work, we hypothesized there would be statistically significant increases in aversive behaviors for the rats exposed to carbon dioxide and isoflurane, but not oxygen or fox urine. We also hypothesized there would be no significant differences between the carbon dioxide and isoflurane treatments. Compared to the previous study, there were several inconsistencies in behavioral trends on consecutive days of exposure. For example, the previous study observed a decrease in line crosses between day 1 and day 2 of exposure for all treatments, while we observed an increase in line crosses for all treatments except fox urine. There were no significant differences between carbon dioxide and isoflurane treatments except in line crosses. Overall, rats were more active in the isoflurane and carbon dioxide treatments compared to the control groups. This suggests that isoflurane and carbon dioxide are similarly aversive.

P330 Location of Gas Inlet Can Negatively Affect the Well-being of Rats Euthanized with CO_2

DL Hickman*

Laboratory Animal Resource Center, Indiana University, Indianapolis, IN

When using CO_2, the gas is delivered in a variety of ways ranging from direct delivery to the cage to placement of the cage in a secondary chamber. Especially with the use of a secondary chamber, the location of the gas inlet can be variable between institutions, leading to potential welfare concerns during euthanasia. To evaluate the effect of location, this study used behavioral and physiologic parameters to assess male and female CD rats that were euthanized with low flow (30% volume displacement per minute [VDR/min]) and moderate flow (50% VDR/min] of 100% CO_2 that was introduced through a port located 3 in from the top of the approximately 85L secondary chamber as compared to a port located 3 in from the bottom of the approximately 85L secondary chamber. The rats were in a standard rat cage (approximately 14L) which was placed in the secondary chamber. Behavioral tests included the proportion of rears and line crosses (times the rat crossed the center of the cage, moving from one side to the other). Blood was sampled when the rat achieved a surgical plane of anesthesia, defined by lateral recumbency and slow, even respiratory rate. Physiologic parameters included noradrenaline and corticosterone. For both VDR/min of CO2, the rats demonstrated a significantly prolonged time to loss of consciousness when the gas inlet was located near the bottom of the secondary chamber (391.21 +/- 17.99 sec for 30% VDR/min; 108.69 +/- 5.15 for 50% VDR/min) as compared to the location near the top of the secondary chamber (79.94 +/- 16.82 sec for 30% VDR/min, 55.38 +/- 5.15 for 50% VDR/min, $P < 0.0001$). There was a significant increase the concentration of noradrenaline when the gas inlet was located near the bottom of the secondary chamber and the VDR/min was 30% ($P = 0.0155$), though there was a significant increase in crosses when the gas inlet was located near the top of the secondary chamber for both VDR/min grups ($P = 0.0004$ for 30% VDR/min and $P = 0.0134$ for 50% VDR/min). No other significant differences were noted between the groups. These findings suggest that, when using a secondary container for euthanasia, the gas inlet should consistently be placed in a location that allows gas to freely flow into the cage to minimize the potential distress associated with the euthanasia process.

P331 Biocompatibility Testing of a Novel Cranial Implant in a Rhesus Macaque

J Schoenberger*, D Mailhiot, N Pach, KR Luchins, M Niekrasz

University of Chicago, Chicago, IL

Cranial implants have proven to be indispensable to neurophysiology research in nonhuman primates. Since their inception centuries ago, cranial implants have continuously improved with one recent innovation being 3-D printing. This technique uses computed tomography and magnetic resonance scans to allow for individual customization, enhancing function and fit. Using 3-D printed devices evades the tedious task of reshaping titanium metal in an anesthetized NHP subjected to a craniotomy, hence lowering anesthesia time and risk of infection. The custom fit also reduces space between implant and skull, decreasing later possible complications such as foreign body reactions and implant failure. In addition, material modifications using polyamides, a synthetic polymer with proven human tissue biocompatibility, has improved flexibility, and decreased weight and cost of implants. Polyamide 12 (PA12) has been used to develop implants for bone, hip, and knee replacements in humans. Given this information, construction of PA12, 3-D printed, cranial implants for rhesus macaques was explored. As part of biocompatibility testing, we tested tissue reactivity to PA12 by surgically placing an approximately 5x6 mm2 sample into the subcutaneous tissue between the scapulae of a rhesus macaque. During the 6 wk it was in place, palpation and visual examination of the implant site occurred at least once per week, and no tissue reaction was ever noted. After explantation, no gross or histopathological foreign body reaction was seen in the tissue surrounding the PA12 implant. PA12 was then used to create a 3-D printed floating microelectrode array connector, and was successfully surgically placed onto the skull of this same rhesus macaque. To our knowledge, this is the first 3-D printed floating microelectrode array connector produced from PA12.

P332 Investigation of the Role of Il-22 In *Helicobacter pylori*-induced Chronic Gastritis in Murine Models

M Stair,[1,*] E Bryant,[2] HR Holcombe,[1] S Muthupalani,[1] Z Ge,[1] Z Shen,[1] D Puglisi,[3] A Aguilar,[1] JG Fox,[1]

[1]Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA; [2]Merck, Boston, MA; [3]Tuoro College of Dental Medicine, Hawthorne, NY

Helicobacter pylori infects approximately half the world's population, causing chronic gastritis that can progress to gastric cancer. This progression is well characterized histologically but the contributing immunological features are still being explored. IL-22 is a cytokine produced by immune cells that targets epithelial cells and contributes to mucosal immunity. It has been implicated in *H. pylori*- induced gastritis and cancer, but its mechanistic role is unclear. Here, we evaluate the role of IL-22 in *H. pylori*-induced pathology in 2 mouse models. We have generated a colony of mice deficient in IL-22 receptor (IL2ra1$^{-/-}$ "KO") on a C57BL/6 background, a strain susceptible to *H. pylori* mediated gastritis. The second model is the INS-GAS mouse (FVB/N. Tg(Ins1-GAS)1Sbr) which overexpresses human gastrin in the stomach. This results in spontaneous chronic gastritis and gastric carcinoma, with more severe disease in males. The process is accelerated by *H. pylori* infection. Male and female KO or B6 mice or male INS-GAS mice (n≥5) were infected with *H. pylori* SS1 via oral gavage at 6-8 wk of age. Animals were euthanized at 2, 3, 4, or 6 mo post infection (MPI) with half the INS-GAS receiving neutralizing IL-22 antibody in the 2 wk preceding necropsy. Gastric histopathological parameters, bacterial colonization levels, stomach proinflammatory cytokine expression, and stomach antimicrobial peptide gene expression were evaluated. Infection with *H. pylori* upregulated IL-22 by 4-fold and 50-fold at 3 and 6 MPI respectively, but not at 2 MPI in INS-GAS. Antimicrobial peptides RegIIIβ and RegIIIγ were also upregulated with *H. pylori* infection; however, expression was not inhibited by IL-22 Ab treatment in INS-GAS. As

expected, pathological changes were more advanced in INS-GAS mice compared to B6 or KO mice, but we found no impact of IL-22 on pathology or bacterial colonization in either model. Neutralizing IL-22 antibody or absence of the IL-22 receptor had no significant effect in *H. pylori* induced increases of IFNγ, TNFα, or IL-17a. These data suggest that IL-22 does not have a significant independent role in the development of gastritis and gastric cancer, though the impact of its interplay with other inflammatory mediators requires further study.

P333 Coagulation Parameter Reference Intervals for Inbred Strain 13/n Guinea Pigs (*Cavia Porcellus*) and Validation of Low Volume PT and aPTT Sample Analysis

JA Condrey,[1,*] T Flietstra,[2] K Nestor,[3] E Schlosser,[3] J Coleman-McCray,[2] S Genzer,[1] S Welch,[2] J Spengler,[2]

[1]CMB, CDC, Conyers, Georgia; [2]VSPB, CDC, Atlanta, GA; [3]CRL, CDC, Atlanta, GA

Inbred strain 13/N guinea pigs are used as small animal models for the study of hemorrhagic fever viruses (VHFs). Comprehensive evaluation of coagulation function is critical in model development, and studies of viral pathogenesis and therapeutic efficacy. Here, we develop reference intervals in both juvenile and adult strain 13/N guinea pigs for 3 coagulation parameters: prothrombin time (PT), partial thromboplastin time (PTT), and fibrinogen. In addition, to address research situations with limited availability of blood for clinical analysis, we investigated the validity of low-volume blood sample analysis of PT and aPTT. We hypothesized that a novel blood collection method performed in accordance with recommended anticoagulant-to-sample ratio will have consistent PT and aPTT values regardless of sample volume. Both male and female strain 13/N guinea pigs were used. Age groups were defined as 0–150 d old for juveniles and 151–900 d old for adults. 0.6 mL of blood was collected from the cranial vena cava under isoflurane anesthesia. A 0.1mL premeasured micropipette was used to transfer blood directly from the syringe to a 0.1 mL sodium citrate tube. The remaining 0.5 mL of blood was transferred into a 0.5 mL sodium citrate tube. All samples were then run per manufacturer recommendations. Coagulation parameter reference ranges were not significantly different by sex, but age did significantly affect aPTT values. Analysis of the smaller sample volume was found to only be statistically different for PT samples from juveniles. However, in this cohort, the average difference between the 0.1 mL and 0.5 mL samples was only 1.0 sec, which is well within 1 SD of our established reference range for PT, suggesting that this difference is not clinically relevant. Here we define reference intervals for coagulation parameters in strain 13/N guinea pigs, to aid in the study of coagulation abnormalities and possible therapeutic efficacy for hemorrhagic fevers. We also determined that the low-sample volume can be used in strain 13/N guinea pigs for evaluation of coagulation parameters.

P334 Cannabidiol and Cannabidiolic Acid-rich Hemp Oil for the Treatment of Diarrheal Disease in Cynomolgus Macaques (*Macaca fascicularis*)

TN Johns,[1,*] AI Cameron,[2] WM Burnside,[3]

[1]Animal Care Services, University of Florida, Gainesville, FL; [2]School of Pure and Applied Sciences, Florida SouthWestern State College, LaBelle, FL; [3]Haman Ranch, The Mannheimer Foundation, Inc, LaBelle, FL

Recurrent diarrheal disease is a leading cause of morbidity in captive macaque breeding colonies, with significant economic impact due to diagnostic costs, frequent medical treatment, and the inability to utilize these animals for research or breeding. Many of these animals become refractory to currently available treatments, resulting in euthanasia. Hemp-derived phytocannabinoids are a safe and novel option for treatment of diarrhea in macaques. Previous studies have shown their beneficial effects on the gastrointestinal tract and stress due to inflammatory, immunomodulatory, and anxiolytic properties. A pharmacokinetic study was performed in 2 male and 2 female 3.5-y-old cynomolgus macaques to determine the oral bioavailability of cannabidiol and cannabidiolic acid-rich (1:1) hemp oil at 2 mg/kg. Serum cannabidiol and cannabidiolic acid concentrations, measured at 0, 0.5, 1, 2, 4, 8, 12, and 24 h post-administration, were detected after 30 min. The peak mean concentrations of cannabidiol (8.61 ± 2.35 ng/mL) and cannabidiolic acid (32.62 ± 11.41 ng/mL) were 4 h post-administration, with half-lives of 7.9 and 7.4 hours, respectively. A clinical study was also performed in macaques less than 4 y old (n = 9) presenting with a history of 2 or more diarrheal events; a twice daily 2 mg/kg oral cannabidiol and cannabidiolic acid-rich (1:1) hemp oil was administered along with a standard treatment of antibiotics, bismuth subsalicylate, and probiotics. A complete blood count, serum biochemistry, and C-reactive protein level, and fecal floatation, direct smear, and calprotectin level were collected at initial presentation and 3 d after diarrheal resolution. Although not statistically significant ($P = 0.1016$) based on a Wilcoxon matched-pairs signed rank test, preliminary clinical results revealed that this hemp oil reduced hospitalization time by an average of 32.8% compared to standard treatment alone. In summary, cannabinoid enhanced hemp oil shows promise as a novel adjunctive therapy for nonhuman primate diarrheal disease. Our pharmacokinetic results are similar to those in cats and human adolescents. Based on these findings, our current studies are evaluating higher doses of this hemp oil to increase serum concentrations and clinical efficacy.

P335 Inhibition of Fibroblast Activation Rescues Cardiac Dysfunction Associated with Inherited Dilated Cardiomyopathy

KA Zabrecky,[1,*] K Kooiker,[2] D Bugg,[3] A Martinson,[3] J Davis,[3,4]

[1]Department of Comparative Medicine, University of Washington, Seattle, WA; [2]Department of Medicine, Division of Cardiology, University of Washington, Seattle, WA; [3]Department of Pathology, University of Washington, Seattle, WA; [4]Department of Bioengineering, University of Washington, Seattle, WA

Inherited cardiomyopathies affect 1:500 people in the general population. One of the primary clinical phenotypes is dilated cardiomyopathy (DCM) which is characterized by myocyte eccentric hypertrophy, left ventricular chamber enlargement, and systolic dysfunction. A hallmark of DCM is the accumulation of extracellular fibrosis further accelerating the clinical disease course. Little is known regarding the mechanical homeostatic mechanisms between cardiac myocytes and extracellular matrix (ECM) on regulating cardiac structure and disease progression. To determine the role of fibroblast activation in DCM disease progression, we crossed 2 existing transgenic mouse models: a DCM mouse, FVB-Tg(Myh6/tetO-Tnnc1*I61Q)1Jmol/J (I61Q TTA), and a tamoxifen conditional myofibroblast knock-out mouse, B6.Cg-Mapk14$^{tm1.1Otsu}$Tcf21$^{tm(cre/Esr1*)Mdt}$ (P38F/F TCF21+/-). It was hypothesized that knocking out one of the pathways of fibroblast activation, p38, in I61Q TTA mice would improve the DCM phenotype and reduce maladaptive cardiac remodeling. Tamoxifen induction was started at 1 mo of age by once daily intraperitoneal injections (40 mg/kg) for 5 d then maintained on Tamoxifen chow (40–48 mg/kg/day). Mice were studied by echocardiography, pressure-volume loops (PV loop), histology, and imaging at 2 and 4 mo of age. The outbred I61Q TTA mice exhibited features of DCM including systolic and diastolic dysfunction and left ventricular dilation. Loss of p38 function in cardiac fibroblasts rescued systolic function at both time points shifting the I61Q TTA P38F/F TCF21+/- PV loop back towards non-transgenic controls. To determine if these organ-level changes are due to altered cardiac myocyte function, strips of intact cardiac muscle were collected at 4 mo of age and subject to 1Hz twitches. When normalized to non-transgenic controls, these experiments demonstrated that loss of p38 function in the fibroblast also rescued the I61Q TTA phenotype at the myocyte level. Collectively, these findings demonstrate significance of extracellular mechanics on dilated cardiomyopathy disease progression.

P336 The Superfund Chemical, N-nitrosodimethylamine, Induces

an Early Onset Pattern of Mutations

AL Armijo,[1,2,*] S Chawanthayatham,[2] BI Fedeles,[2] RG Croy,[2] JG Fox,[1,2] JM Essigmann,[2]

[1]Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA; [2]Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA

Superfund sites are chemically contaminated areas that have been identified by the federal government as being eligible for cleanup and decontamination to protect human and wildlife populations from further exposure. These sites disproportionately affect economically challenged populations and underrepresented minorities due to proximity and exposure variables. A probable human carcinogen, *N*-nitrosodimethylamine (NDMA), is a key contaminant of the Superfund site in Wilmington, MA. For many years it contaminated the drinking water of people who drew water from several municipal wells. We hypothesize that high-resolution mutational spectra (HRMS) of DNA exposed to environmental toxicants, such as NDMA, can reveal early onset genetic signatures of environmental toxicant-driven human diseases that occur later in life. Twenty gpt Delta C57Bl/6J mice (5 treated, 5 controls of each sex) that are transgenic for the *gpt* Delta reporter gene that is used for the phenotypic detection of gene mutations, were dosed with NDMA using a carcinogenic regimen. Briefly, NDMA was administered via intraperitoneal injection at 8 d of age (3.5 mg/kg) and at 15 d of age (7 mg/kg). Ten weeks post-exposure, tissue samples were collected and liver DNA were analyzed by a new high-fidelity DNA sequencing technique (duplex consensus sequencing) to produce HRMS of the *gpt* Delta reporter gene. HRMS allowed the monitoring of the acquisition of toxicant-induced mutational patterns over time. Our results indicate that as early as 10 wk post-exposure to NDMA, a mutational spectrum is present in treated mice that is predominantly characterized by GC to AT mutations that appear in a distinctive pattern. The relationship of this pattern to that seen in human liver cancer will be discussed. Identifying these mutational processes can inform strategies for Superfund site remediation as well as clinical genetic disease early-detection, intervention, and prevention.

P337 Prevention of Corneal Opacities during Ocular Imaging in Rats

HB Culp,[1,*] M Ault,[1] X Ping,[1] L Yang,[2] C Nunes,[1] J Destefano,[1] L Gichuru,[1] L Chen,[1] I Petrescu,[1] C Johnson,[1] L Handt,[1] S Bellum,[1] N Li,[2] S Motzel,[1] X Shen,[1]

[1]SALAR, Merck, West Point, PA; [2]Cardiometabolic Disease, Merck, West Point, PA

Imaging of the posterior segment of the eye, requires transparency of the ocular media. Corneal-dystrophies in rats during ophthalmic imaging techniques like Optical Coherence Tomography (OCT) and Fluorescein Angiography (FA) can significantly affect accuracy and results of measurements. It has been reported that multiple factors such as corneal dehydration, injectable anesthetics, body temperature, oxygen supply, pH stress, and spontaneous occurrences can factor into the development of corneal abnormalities. Among them, maintaining eye hydration and body temperature are considered the most important factors that prevent the formation of corneal opacity. To minimize corneal opacity incidences and to obtain reproducible high-quality images in anaesthetized rats, multiple improved techniques were applied to provide adequate hydration of the eye beginning from anesthetic administration through imaging procedures and animal recovery with enhanced body temperature control. Flushing the eye with sterile saline prior to imaging and hydrating with lubricating drops (every 3-5 min) prevented dehydration of the cornea due to lack of blink reflex from anesthetic and exposure to a light source during imaging. The period of greatest risk for corneal dehydration was found to be during the recovery phase. Placement of a thin plastic film to act as a physical hydrating barrier on the eye in addition to a petroleum based ophthalmic ointment had the greatest impact on the reduction of corneal opacities. Animals were additionally placed on warming pads during all imaging procedures to maintain body temperature, especially during the critical phase of recovery. With adequate hydration treatment, the incidence of corneal opacity and lesion was reduced by 80% compared to those animals that did not receive proper hydration treatment (n = 24 per group). Therefore, the detailed methods described here minimize the corneal changes associated with ocular surface abnormalities. When these methods were used consistently, high-quality images of the posterior segment were obtained even upon repeated imaging of the same animal in our lab.

P338 Impact of Social Housing on Long-term Patency of Jugular Catheters in Rats (*Rattus norvegicus*)

L Krueger,[1,*] SE Chang,[2] M Motoc,[3] M Chojecki,[2] ZT Freeman,[1] SB Flagel,[2,4]

[1]Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI; [2]Michigan Neuroscience Institute, University of Michigan, Ann Arbor, MI; [3]Undergraduate Biology, Health, and Society Program, University of Michigan, Ann Arbor, MI; [4]Department of Psychiatry, University of Michigan, Ann Arbor, MI

Chronic vascular access devices are widely used in a variety of species for repeated sampling or infusion. Rats with jugular catheters are commonly used for studying addiction-related behaviors and have historically been single-housed for the duration of the study to prevent cagemate-related damage. The 2 goals of this study were to determine 1) the effects of social housing on catheter patency (experiment 1) and 2) the effects of social housing on catheter patency of rats participating in an opioid self-administration and cue-induced reinstatement of opioid-seeking behavior study (experiment 2). A refinement opportunity, the temporary use of a 'buddy barrier' with pair-housed rats, was assessed for its utility in the facilitation of social housing. Male Heterogeneous Stock (HS; n = 24) and Sprague Dawley (SD; n = 121) rats were allocated to either a single- or pair-housed condition immediately following catheterization surgery. To assess the effect of social housing on catheter patency, rats (HS, n = 24; SD n = 36) were monitored in their assigned housing condition for 1 mo, with patency and structural evaluations every 7–10 d. To examine the effect social housing on catheter patency during an opioid self-administration and reinstatement study, rats (SD, n = 85) were placed and monitored in their assigned housing condition for 1 mo with patency evaluations every 10–15 d. Catheter patency rates between single- and pair-housed rats were not statistically different, and this was also true for those rats participating in the opioid self-administration study. Additionally, we report that postoperative use of a 'buddy barrier' with our pair-housed rats allowed for continued social contact with no observed adverse effects. These results suggest that, when research allows, pair housing is a viable option for rats with chronic vascular implants and may improve their well-being by allowing for the display of species-typical social behaviors.

P339 Effects of Isoflurane on Engraftment Rates and Cytokine Release In Humanized Mice

M Harris,* M Chen, J Yang, G Yang

Product Development, The Jackson Laboratory, Sacramento, CA

Retroorbital (RO) bleeding is a widely used technique for blood collection of mice in the laboratory, which allows the technician to be able to get a precise amount of blood from the mouse while also allowing for the mouse to heal at a relatively faster rate than other methods. Although, RO does have a few limitations which include the fatigue and stress on the technicians' nondominant hand due to restraining a large number of mice for mass RO and an increase in possible eye injury if there is any difficulty to manipulate the eye opening. We investigated the effects of using isoflurane on engraftment rates and cytokine release in humanized mice to see if there is a difference from the results of the traditional RO method

with physical restraint. If there are no effects on the data then it would allow for technicians to interchange these methods which will allow for less fatigue, finer control of the mouse eye, decrease the possibility of eye injuries, and allow for newer technologists to learn and gain confidence with their RO technique more easily. Female NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ (NSG) mice were used to generate 2 different humanized mice. To humanize NSG mice, 3-wk-old mice were IV injected with CD34 cord blood cells (CD34-NSG) and 7-wk-old mice were irradiated and IV injected with peripheral blood mononuclear cells (PBMC-NSG). The mice were used 30 wk after CD34 humanization and 6 d after PBMC humanization. To check engraftment, 50 uL of blood was collected with or without Isoflurane via RO, and the cells were stained with hCD19, hCD56, hCD3, hCD14, hCD45, and mCD45, then analyzed with a flow cytometer. To assess the effect of Isoflurane on cytokine release, PBMC-NSG mice were IV injected with either PBS or OKT3 (0.25 mg/kg), and 75 ul of blood were collected 6 h after the injection and processed to serum. Tetracaine was applied to all mice among nonisoflurane groups, and BD™ Cytometric Bead Array (CBA) kit was used to measure cytokine levels. The results of these experiments illustrate that the effect of Isoflurane on engraftment rate was donor dependent and increased cytokine release. With this finding, we can conclude that the 2 methods are not interchangeable.

P340 Fecal Microbiome Monitoring in Colony and Sentinel Mice

PC Smith,[1,2] J Gu,[3] C Zhou,[3] CJ Zeiss,[1] S Compton,[1] PA Cirillo,[2] H Wallace,[2] J Macy,[1,2,*]

[1]Comparative Medicine , Yale University, New Haven, CT; [2]Yale Animal Resources Center, Yale University, New Haven, CT; [3]Biostatistics, Yale University, New Haven, CT

To determine if monitoring the fecal microbiome (FM) of soiled bedding sentinels informs changes of FM of colony mice, a study using 6 breeding pairs of in-house reared mice B6.129P2(C)-Tg(Nr5a1-cre)7Lowl/J (SF1) and 6 breeding pairs B6.129P2(C)-Cx3cr1$^{tm2.1(cre/ERT2)Jung}$/J (CXCR), 8 cages of 16 female C57BL/6NCrL (B6) mice, and 8 cages of 16 female Crl:CD1 (CD) commercially acquired mice was performed. Shallow shotgun metagenomic sequencing analysis of individual feces from all mice (56) was performed at 0, 2, 4, 14 and 28 d. At days 49, 63, 77, 91, 105, 119, and 133 soiled bedding combined from 6 cages of SF1 or CXCR were transferred into 2 sentinel cages of B6 or CD and soiled bedding combined from 12 cages of SF1 and CXCR were transferred into 2 B6 or CD sentinel cages. Two control cages of B6 and CD sentinels received no soiled bedding. On days 49, 77, 105 and 133 feces from all mice (56) and pools of feces from SF1 (12 feces) and CXCR (12 feces) mice were collected. Sequence analysis from all samples revealed that sentinels (B6, CD) had higher species richness and diversity than colony mice (SF1, CXCR) and there was no change in alpha diversity over time. For each genotype, beta diversity (inter-individual variation) of FM changed during the first 4 d, stabilized after 14 d, and increased in CXCR from days 28–91. Exposure to soiled bedding from colony mice did not impact alpha diversity but did increase beta diversity of the sentinel mice FM. Genotype had the largest variability with differences between groups segregating by mouse source (in-house vs. vendor), followed by the specific housing cage.

P341 Optimization of Superovulation Hormone Injection Intervals

JA Cayton,[1,*] C Bethune,[1] C Piotter,[1] J Walls,[1] D Davis,[2] H Men,[1] CL Franklin,[1]

[1]Veterinary Pathobiology, University of Missouri, Columbia, MO; [2]RSCH Core Facilities, University of Missouri, Columbia, MO

Superovulation is routinely used for many murine assisted reproduction procedures. It involves the use of exogenous gonadotropins to induce increased production of mature oocytes. Pregnant mare's serum gonadotropin (PMSG) mimics the oocyte maturation effect produced by endogenous follicle-stimulating hormone. Human chorionic gonadotropin (hCG) mimics the ovulation inducing luteinizing hormone. Current literature suggests use of a 46–48 h interval between PMSG and hCG injections to induce superovulation in mice. In this study, C57BL/6 female mice (n = 214) were utilized. We evaluated the number of zygotes and unfertilized oocytes produced using 46- and 48-h time intervals between PMSG (5 IU) and hCG (5 IU) intraperitoneal injections. During this study, our vivarium also removed dark cycle red lighting, which allowed for the evaluation of the impact of this variable on superovulation. There were no significant differences observed in superovulation outcomes between the 46- and 48-h intervals. However, a trend (P = 0.169) was observed indicating that a 48-h time interval may yield more zygotes and unfertilized oocytes. Mice in a 12-12 hour light cycle with no dark cycle red light exposure produced significantly (P = 0.031) greater zygote and unfertilized oocyte counts at collection. These findings highlight the need for further studies on the many factors that can be exploited to optimize superovulation protocols in rodent research models.

P342 Postsurgical Recovery Assessment of Mice Treated with Extended- or Sustained-Release Buprenorphine

M Saenz,[1,2,*] E Bloom-Saldana,[2] PT Fueger,[2] R Ermel,[1] J Finlay,[1]

[1]Center for Comparative Medicine, City of Hope, Duarte, CA; [2]Department of Molecular and Cellular Endocrinology, Beckman Research Institute at the City of Hope, Duarte, CA

The principles of *The Guide for the Care and Use of Laboratory Animals* emphasize that pain should be minimized using appropriate analgesia. The extra-label use of sustained-release buprenorphine (SRB) is commonly used in rodents to mitigate mild-to-moderate pain. A new FDA-indexed formulation of a sustained-released buprenorphine, known as extended-release buprenorphine (XRB), has recently become available. Being FDA-indexed, XRB is approved for use in minor animal species because its safety and efficacy has been affirmed by the FDA. However, no studies have directly assessed differences in the recovery of XRB- and SRB-treated mice after a surgical procedure. Thus, the purpose of this study was to quantify post-surgical recovery indices in mice treated with XRB compared to SRB. We hypothesized that post-surgical recovery of mice treated with XRB would be similar to mice treated with SRB. Male and female 13-to-15-wk-old C57Bl/6J mice were anesthetized, treated with either SRB (1 mg/kg, SC, once) or XRB (3.25 mg/kg, SC, once), and underwent a surgical carotid artery catheter implantation (6–8 animals per group). Body weight, body condition, facial pain recognition scale of eye closure, and visual pain-index assessment scores of coat condition, coordination/ posture, and overall condition were recorded daily for 3 d after surgery. As expected, all animals lost weight post-operatively. Post-operative weight loss was similar between groups; body weight declined by 11.7 ± 1.6 and 12.3 ± 0.7% in males and by 7.6 ± 2.2 and 8.1 ± 1.1% in females treated with SRB or XRB, respectively. Total pain-index visual assessment scores indicated both SRB- and XRB-treated animals had mild pain, appearing slightly hunched but able to move about, up to 3 d postoperatively, suggesting these analgesics are effective. There were no significant differences in body condition or individual pain-index visual assessment scores between SRB- or XRB-treated animals. Thus, in the post-surgical recovery assessment of mice, the newly available FDA-indexed XRB is comparable to SRB. Therefore, the availability of XRB increases the options for safe and effective analgesia in mice.

P343 Reducing Inter-operator Variability Using a Novel 3D and Thermal Measurement System when Measuring Subcutaneous Tumors in Mice

A Smith, K Turley, J Steed*

Marketing , Fuel3D, Oxford, , United Kingdom

Measurement (length and width) variability between technicians and scientists using calipers to measure subcutaneous tumors implanted

in mice is a common issue that can limit the ability to conduct studies. The lack of measurement consistency between operators can cause studies to be delayed or reduce facility capacity. When a single operator must take all measurements during a study to prevent data errors, this may ultimately impact scientific reproducibility. We explore the use of a new tool that uses thermal, 3D, and RGB photographic images to take automated measurements of the tumor length, width, and height to determine a tumor volume and its impact of inter-operator variance. In 3 separate preclinical studies (2 using Balb/C mice, the first with 64 animals in 8 groups and the second with 32 animals in 4 groups and 1 study used C57BL/6 mice with 40 animals across 8 groups), 3 technicians were tasked with capturing measurements of subcutaneous tumors on the flanks of female mice with both callipers and a thermal/3D measurement system on the same day, for the duration of each study protocol. The primary goal was to compare the inter-operator variability of the 2 techniques to see if the thermal/3D system outperformed callipers in the repeatability of measurements between operators. The results were analyised using an interclass correlation (ICC) statistical model and demonstrated that the thermal/ 3D measurement system showed statistically significant reduction in the variability of measurements between operators compared to callipers, when measuring subcutaneous tumours implanted in mice in these 3 experiments. The thermal /3D measurement system achieved an ICC score of 0.96 (out of 1) excellent level of agreement between operators while the callipers achieved an ICC score of 0.86 good to moderate levels of agreement. It is possible that improving measurement consistency between operators can reduce delays in studies and increase the facility's measurement capacity as a single operator does not have to conduct all the study's measurements. The ability to repeat the statistical results across 3 separate experiments which used 2 different mouse strains further supports this conclusion.

P344 Inactivation of Amplicons Using Gaseous Chlorine Dioxide

K Lorcheim*

ClorDiSys Solutions, Branchburg, NJ

Within the life science and medical industries, polymerase chain reactions (PCR) are used to diagnose and further research conditions by determining the presence or absence of a gene or to generate forensic DNA profiles from small DNA samples. However, there are scenarios when results can become inaccurate, namely because of amplicon contamination. An amplicon is a segment of DNA or RNA that is the source and/or product of natural or artificial amplification, which is a massive replication of a gene or DNA sequence. Amplicons can be formed by various methods, including PCR. With the increasing use of analyzation of DNA sequencing, the use of PCR equipment is expanding immensely. Digital PCR readers are useful in applications like mutation detection, copy number variation, rare sequence detection, gene expression analysis, MicroRNA analysis and next generation sequencing sample quantification. However, a challenge is the integrity of the results, as amplicons can accidentally contaminate parts of the PCR reader causing improper analysis of subsequent samples. If amplicons spread to other areas of the PCR reader, all subsequent readings would read inaccurately. To allay this issue, amplicons need to be eliminated completely from the PCR reader, including all cracks and crevices that they can get into within the machine or any other space. A successful method to accomplish this is with the use of chlorine dioxide gas. The chlorine dioxide gas decontamination process was independently validated by 2 major PCR equipment manufacturers. Validation consisted of inoculating amplicons on various surfaces within both systems. Subsequent to the validation treatment with CD gas, swabbing of the internal surfaces was performed and analyzed via the same type of PCR Reader. Controls were used to ensure the recovery of the swabbing process. Verification that the equipment was not impaired during the treatment process was also confirmed by running the equipment through a calibration cycle. This treatment process also does not leave any residuals on surfaces, helping to ensure that the quality of the unit is maintained. The application of chlorine dioxide gas to inactivate amplicons is a key attainment in ensuring dependable and consistently accurate results from PCR readers or any other device or area in question. Quality readings are vital to further medical and laboratory research and testing, and the method of gaseous chlorine dioxide decontamination is a way to ensure further quality progression in this arena.

P345 Zebrafish (*Danio rerio*) Embryo Surface Disinfection: Refining the Bleaching Protocol

A Winn*, K Prestia, S Peneyra

Division of Comparative Medicine, NYU Langone Health, New York, NY

Minimizing opportunistic pathogen transmission into zebrafish facilities is often combated through vigilant biosecurity practices. Many embryo surface disinfection protocols use chlorine bleach, as sodium hypochlorite has been proven effective against numerous pathogens. However, chlorine can cause significant mortality and morbidity in zebrafish embryos when not used at the appropriate pH, concentration, or exposure times. Reagent-grade sodium hypochlorite is more commonly used for surface disinfection, since commercial-grade sodium hypochlorite includes additional ingredients which may have deleterious effects on the embryo. Additionally, potassium peroxymonosulfate + sodium chloride and chlorine dioxide are effective equipment disinfectants; however, the impact of these 2 chemical agents on zebrafish embryos during surface disinfection remains unknown. This study aimed to determine the survival, hatch rate, and morphological defect rate of 5D zebrafish embryos exposed to 4 different chlorine-containing agents during the surface disinfection process: reagent-grade sodium hypochlorite, commercial-grade sodium hypochlorite, potassium peroxymonosulfate + sodium chloride and chlorine dioxide. Embryos 6 and 24 h post fertilization were exposed to 1 of 4 chlorine-containing agents in triplicate (n = 30) at either 50 or 100 ppm for 5 or 10 min. The experimental group that showed the highest survival and hatch rate with the lowest morphological defect rate were the 24 h post fertilization embryos exposed to 50 ppm potassium peroxymonosulfate + sodium chloride for 5 min. The embryo survival, hatch rate, and defect rate did not significantly differ from age-matched controls; however, the hatch rate (100%) was significantly higher ($P < 0.0001$) than the hatch rate of embryos exposed to the bleach gold standard – 50 ppm reagent-grade sodium hypochlorite for 5 min (23.33%). Although exposure to sodium hypochlorite generally led to high survival and low morphological defect rates in 5D embryos, potassium peroxymonosulfate + sodium chloride may be an alternative embryo surface disinfectant with improved hatch rates; however, in vivo efficacy against pathogens should be further investigated.

P346 Lessons Learned during Murine Microbiome Analysis on a Decentralized Campus

S Hashway,[1,*] J Reuter,[2]

[1]Office of Animal Resources, University of Colorado Boulder, Boulder, CO; [2]Research and Innovation Office, University of Colorado Boulder, Boulder, CO

The gut microbiome is a well-recognized factor in the physiology of health and disease in humans and corresponding animal models. The murine microbiome can be influenced by many external factors, including barrier status, husbandry practices, diet, and physical location. Thus, it can be difficult to draw broad conclusions about the composition of the microbiome and its relevance to model phenotypes within an institution. We conducted a pilot evaluation of the murine microbiome on our decentralized campus to assess the influence of extrinsic variables. Fresh feces were collected from outbred soiled bedding sentinel and inbred colony strains of mice at multiple facilities. Colony mice represented 8 inbred strains, both

sexes, ranging 2 to 9 mo of age. Detailed metadata was gathered for 41 independent samples that were sent for DNA extraction/quantification. Bioinformatics analysis was performed using the One Codex database. Shannon alpha diversity metrices were analyzed via t-test, and Bray-Curtis beta diversity dissimilarities were analyzed via PERMANOVA. Consistent with previous published studies, samples were dominated by the phylum Bacteroidetes. Secondary phyla included Firmicutes, Verrucomicrobia, and Proteobacteria. No significant differences were found in alpha or beta diversity between sentinel mice at different facilities, or between sentinel and the corresponding colony mice on the same rack. Differences in both alpha and beta diversity were found between inbred colony mice at different facilities and between mice fed standard versus breeder chow. Significant differences were also observed between newly arrived, naive sentinels and those that had been receiving soiled bedding for 3 mo. However, distinct variation in microbial composition among newly arrived sentinels confounded meaningful interpretation. This pilot provided confirmation that differences in location and diet can alter the microbiome, but power analysis was limited by a small sample size. Observed intrinsic variability in outbred stocks makes them a questionable model for microbiome analysis. This study underscores the importance of robust study design, detailed metadata collection, and large sample sizes when analyzing the murine microbiome at a decentralized institution.

P347 Assessment of Indicators of Well-being in Syrian Hamsters Euthanized with Ethanol

DL Hickman*

Laboratory Animal Resource Center, Indiana University, Indianapolis, IN

Intraperitoneal injection of ethanol has been proposed as an alternative method of euthanasia for small rodents, particularly in disaster situations when other compounds may not be readily available. In mice, intraperitoneal injection of ethanol is comparable to pentobarbital, but it did not result in loss of consciousness in rats, suggesting it may not be appropriate for all small rodents. To assess the reliability of this method in Syrian hamsters, ethanol (95%, max volume 1 mL), or pentobarbital (approximately 500 mg/kg, max volume 1 mL) injected intraperitoneal were used to euthanize 24 female retired breeder Syrian hamsters (12 per treatment group). Each euthanasia was digitally recorded from the administration of the euthanasia agent to the exhibition of ataxia to determine the approximate time to loss of consciousness. An observer blinded to treatment scored each video for rearing, freezing, jumping, and grooming behaviors. After achieving a surgical plane of anesthesia (defined as when the animal was in lateral recumbency with slow, even respirations), a terminal blood collection was performed. Serum noradrenaline, corticosterone, and glucose were compared between treatment groups. All hamsters were successfully euthanized with the injection of either ethanol or pentobarbital, and there were no significant differences between the times to loss of consciousness between the groups. Although there was a significant increase in the proportion of rearing behaviors expressed by the hamsters euthanized with ethanol, the serum corticosterone levels were significantly increased in the hamsters euthanized with pentobarbital. These findings suggest that ethanol may be a potential alternative to the use of pentobarbital for hamsters. Additional assessment of the effect of this compound on research outcomes is desirable.

P348 Assessment of Indicators of Well-being In Laboratory Rats and Mice Euthanized with Carbon Monoxide

DL Hickman*

Laboratory Animal Resource Center, Indiana University, Indianapolis, IN

Although carbon monoxide has been used to euthanize a variety of species, its use in laboratory rodents has been limited. In this study, the welfare of laboratory rats (Sprague Dawley and Brown Norway) and mice (ICR and SJL) euthanized with carbon monoxide was assessed as a potential alternative to carbon dioxide and isoflurane. Six males and 6 females of each strain were used for this study. All were offspring from a breeding study that were naïve. Volume displacement rates per minute of 30%, 50%, or 70% carbon monoxide (8% in room air) or an intraperitoneal injection of pentobarbital (approximately 500 mg/kg) were used to euthanize these rodents. Each euthanasia was digitally recorded from the administration of the euthanasia agent to the exhibition of ataxia to determine the approximate time to loss of consciousness. An observer blinded to treatment scored each video for rearing, freezing, jumping, and grooming behaviors. After achieving a surgical plane of anesthesia, a terminal blood collection was performed. Serum noradrenaline, corticosterone, and glucose were compared between treatment groups. Although there were significant strain and sex differences in the behavioral and physiologic responses of the rodents euthanized with carbon monoxide and those euthanized with pentobarbital, the overall responses were not remarkable. For example, female ICR mice reared more overall, but there were no significant differences in rearing behaviors of female ICR mice between treatment groups. Male rats of both strains engaged in freezing behavior with carbon monoxide, but not with pentobarbital, while female rats of both strains engaged in freezing behavior in all treatment groups. These findings suggest that carbon monoxide may be a potential alternative to the use of carbon dioxide or isoflurane. Additional assessment of the effect of this gas on research outcomes is desirable.

P349 Evaluation of How Social Interactions Influence the Welfare of Rats during Euthanasia

DL Hickman*

Laboratory Animal Resource Center, Indiana University, Indianapolis, IN

Most recommendations of best practices for the euthanasia of laboratory rodents advise that these animals should be euthanized in stable groups. However, there is literature available that suggests that pain and distress are experienced more significantly when with bonded conspecifics as compared to when in the presence of a stranger. This study was designed to assess how behavioral and physiological assessments of welfare were influenced by being euthanized alone, with a "friend," or with a "stranger" rat. The rats used on this study were surplus offspring from a breeding colony of an outbred stock of alcohol preferring rats maintained at our institution. Ten males and 10 females were used for each group. All rats were euthanized with 30% volume per minute displacement rate of 100% CO_2. Some subject rats were individually housed and were euthanized alone ("I-I") or with a stranger ("I-S"). Additional subject rats were pair-housed and were euthanized alone ("P-I"), with their partner ("P-P-F"), or with a stranger rat ("P-P-S"). The rats were digitally recorded during the euthanasia process and the video was scored for time to lateral recumbency and proportion of time spent in contact with the other rat (if present). Additionally, terminal blood samples were taken and assessed for serum corticosterone. The results of this study indicated that previously paired rats who were euthanized with a stranger ("P-P-S") spent a significantly increased proportion of time in contact with each other as compared to the other groups ($P = 0.0273$). This group also demonstrated a significant decrease in their serum corticosterone levels ($P = 0.0244$) as compared to the other treatment groups. There were no differences between sexes. These findings suggest that the introduction of a novel conspecific prior to euthanasia may result in increased exploratory behavior and decreased stress for rats during euthanasia. Additional assessment in other strains and species is warranted to shape future recommendations.

P350 Assessing the Welfare of Mice following Repeat Administration of Anesthetic Agents

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B Skiles*, T Shafer, S King, DL Hickman

Laboratory Animal Resource Center, Indiana University,
Indianapolis, IN

In rodent animal research, avertin is a commonly requested injectable anesthetic compound, especially with transgenic animals. Previously published data consistently describes frequent avertin injections as being contraindicated due to increased chances for peritonitis and/or adhesions presenting in the abdominal cavity. The long- and short-term effects of frequent anesthetic exposure to avertin (120 mg/kg), ketamine/xylazine (xylazine 5–10 mg/kg + ketamine 40–95mg/kg IP, dose variable due to highly variable response to anesthetic induction), and isoflurane (5% inhaled in 1L/min O2) were compared. Mice were exposed to 1 of these 3 anesthetics at a frequency of 3 times weekly (Monday, Wednesday, Friday), once weekly or once monthly. Each group consisted of 4 male mice and 4 female mice of an ICR strain, for a total of 8 mice per group, plus 8 control mice (4 male and 4 female). After 9 wk of dosing, none of the animals in the avertin groups demonstrated any clinical signs of peritonitis (for example, hunched posture, dehydration, weight loss) or drug resistance from the repeated administration. However, in the ketamine/xylazine animal groups, the induction of anesthesia was highly variable. In all animals treated with ketamine/xylazine, regardless of frequency of administration, we observed a decreased amount of time at a surgical plane after administration as well as increased time to reach a surgical plane of anesthesia. In conclusion, this data suggests avertin to be a safe and potent anesthetic for use in laboratory animals for various surgical and procedural applications. It further suggests that ketamine/xylazine cocktails may not be a reliable option for studies that require repeat anesthetic events.