2018 Platform Sessions Abstracts

PS1 Is It Time for an Animal Program Price Index?

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

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

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The Yale Animal Resource Cost and Benchmarking survey, conducted in U.S. academic research institutions about every other year since the late 1990s, was modified to capture similar thematic information from League of European Research University (LERU) member institutions from Denmark, England, Finland, France, Germany, Ireland, Italy, Netherlands, Scotland, Spain, and Switzerland. Preliminary data analysis suggests that: a) like U.S. programs, most LERU programs have mice and rats, but fewer programs have monkeys, b) LERU vivaria have about equal amounts of housing and procedure space, while U.S. facilities tend to have twice as much housing as procedure space within the vivaria, and c) per diem rates have similar compositions, with ~50% covering salary and fringe, followed by supplies (~25%), facility costs (~10%) and other expenses (~15%). Unlike some U.S. programs, the LERU programs tend not to over-recover mouse care costs, but ~60% of both US and LERU programs under-recover mouse care costs. However, while the vast majority of U.S. programs under-recover medium NHP care costs, in LERU programs the split is more even between break even and under-recover. On average LERU programs have a small positive net-operating balance, while U.S. programs average a large deficit. In LERU programs less than 50% of institutions cover an animal program deficit, while almost 100% of such deficits in U.S. programs are covered by the institution. Deficits not covered by institutions in LERU programs tend to be allowed to accumulate, are covered by program reserves, or are covered by a loan to the program. In setting per diem rates, LERU programs rely more on cost accounting, care more about having competitive rates with peer institutions, and are less influenced by animal user groups than U.S. programs. Outsourced services are similar, with virology and serology services being most frequently outsourced. The full analysis details how the European and U.S. financial environments compare and elucidates the various financial pressures under which animal-based research operates in the 2 environments. Preliminary conclusions are: a) LERU programs are more accountable to their budgets and b) diversity amongst programs is more institution to institution and the state/country matters less.

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

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

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

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*Betta splendens* have become a model for understanding the genetic and neurological basis for aggression. While a mainstay in the aquarium hobby, Betta fish have rarely been used in a laboratory setting. Unlike most other laboratory fishes, they present a number of physical challenges for large cohorts as they must be singly housed to prevent fighting and require static water for breeding. Maintaining appropriate water quality required buffering RO water to the correct pH and conductivity as well as twice weekly 100% water changes to compensate for frequent feedings, resulting in reduced ammonia levels. These are challenges not seen as commonly in recirculating systems used for zebrafish because there is often no establishment of a bio filter within static tanks so water quality can quickly reach dangerous levels. Since the colony is breeding, feeding practices over a variety of life stages were developed, including the use of paramecia, brine shrimp, and several commercial dry feeds. Similar feed structuring is used for growing fish in our Zebrafish Core, with brine...
shrimp being a mainstay for all species. Upon seeing another betta, males show acute aggression, which can wane if they become accustomed to viewing each other. Since part of the research includes an assessment of aggression, housing of this species required the placement of dividers between tanks to ensure that they can only see forward and not develop that acclimation. Furthermore, due to the lack of professional vendors, laboratory bettas are purchased from a variety of breeders which can bring unwanted pathogens with them. While other laboratory fishes are often kept in groups of the same strain, individual bettas are particularly more valuable so steps are taken to ensure they do not succumb to the various diseases seen. Multiple treatment regimens for commonly seen conditions were developed, requiring the stocking of an aquarists’ pharmacy to include a broad spectrum treatment for the control of diseases caused by *Ichthyophthirius* (ich), *Costia*, *Trichodina*, *Chilodonella*, *Oodinium*, and fungal infections (malachite green and formalin); neomycin sulfate; minocycline; and a nonantibiotic treatment for bacterial and fungus infections. We currently house more than 100 adult bettas and have a thriving breeding program.

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

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

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

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At our institution, socially compatible large animals are socially housed by default, but often become separated temporarily at some point during their study due to anesthesia or surgical procedures. It is not uncommon for a single animal to undergo multiple separations and/or be grouped with different animals during the study. Our medical record system and social housing records have traditionally been paper-based. One of the challenges of paper-based social housing records is the administrative burden of documenting grouping and separating animals. This typically requires completing duplicative information on multiple animal records. Paper-based systems do not easily lend themselves to viewing of a group of animals and in our experience were not effective for managing the dynamic nature of social housing. Over the past few years our department has been developing electronic recordkeeping methods for a variety of animal facility operations. To address this, we added an element to our digital large animal clinical case tracker that adds the functionality of scanning animal barcodes or selecting animals to add them to groups, buttons to separate them from their cohort, and data entry options to document single-housing rationale. Single-housed animals are flagged in the system which prompts periodic reevaluation. We added social group information to our dashboard view of each holding room which indicates which animals are grouped and which are individually housed. Migration from paper-based social housing records to this digital method has greatly improved accuracy, consistency, simplified our social housing documentation.

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

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

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

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

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

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

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

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

Platform Sessions

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

GLAS: Yes
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Light and lighting protocols for laboratory animal facilities, as outlined in the Guide, are important to both biomedical researchers and animal care personnel. Light entrains the master circadian pacemaker located in the suprachiasmatic nucleus (SCN) of the brain, which controls all metabolic, physiologic, and neurobehavioral processes of the body, particularly the nighttime circadian melatonin signal, in a near 24-h circadian manner. Previously we demonstrated in rats that blue-enriched light (460-480 nm) from light-emitting diode (LED) lighting at daytime (bLAD) increases the amplitude of the nighttime circadian melatonin signal by 7-fold compared with broad-spectrum (300-700 nm) cool white fluorescent (CWF) lighting, resulting in improved animal health and wellbeing. Here we tested whether adult male and female nude mice (Crl:NU(NCr)Foxn1nu; n=6 per group), an important model in cancer and metabolism studies, exposed to bLAD, compared to CWF, lighting amplifies the circadian nighttime melatonin signal. Animals in an IACUC-approved protocol were
maintained in an AAALAC-accredited facility for 8 wk on a common lighting regimen 12L (300 lux; 123 µW/cm²; lights on 0600 h):12D (0 lux) on either CWF (control) or bLAD (experimental) lighting, and were assessed for arterial blood acid/gas, metabolic, and neuroendocrine hormone levels at 6 circadian time points. Results revealed that adult mice maintained in bLAD vs. CWF lighting had lower \( (P < 0.001) \) dietary (-15.4 ± 0.3%) and water intake (-13.1 ± 0.1%), and body growth rates (-10.1 ± 0.4%). Plasma nighttime melatonin levels were over 5-fold higher in the bLAD- vs. CWF-exposed mice, while integrative mean levels of plasma total fatty acids, glucose and lactic acid during the 24-h day were significantly lower by as much as 21.7 ± 0.2% \( (P < 0.001) \), consistent with a more healthful phenotype. The present findings suggest that daytime exposure to high-blue emission LED light, compared to CWF light, has a marked positive impact on the circadian regulation of neuroendocrine, metabolic, and physiologic parameters associated with the promotion of animal health and wellbeing that may influence scientific outcomes.

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

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

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

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

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

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

**PS15 Measuring Immune System Perturbations associated with the Use of Buprenorphine in Laboratory Mice**
Buprenorphine (Bup), both in regular and sustained-release (SR) formulations, is one of the most commonly used analgesics for laboratory mice. There is often concern by investigators that buprenorphine use may alter the host immune response which could influence research results. To assess the immune perturbations associated with SR-Bup, 20 6 to 8-wk-old female CD-1 mice were immunized with ovalbumin (OVA) on day 0. They were placed in groups of 5 and treated with saline, SR-Bup (0.6 mg/kg every 48 h), Bup (0.5 mg/kg daily) or SR-vehicle (0.5 ml every 48 h) for 18 d. Mice were immunized again on day 18 with OVA and euthanized 3 d later. Blood was collected for serum antibody titers to OVA, and spleens were collected for isolation. Splenocytes were isolated and stimulated with OVA and cultured for 72 h. TNF-α, IFNγ, and IL-10 production were measured in cell supernant by enzyme-linked immunosorbent assay. Serum antibodies to OVA were also measured by ELISA. The cytokine levels were significantly elevated in the saline, SR-Bup, and SR-Bup vehicle-treated mice compared to unstimulated splenocytes. The Bup stimulated splenocytes were only slightly elevated compared to unstimulated splenocytes. All treatment groups produced a robust serum OVA antibody response. These findings suggest there is minimal impact on the host immune response as SR-Bup has a similar immune response as saline treatment, and Bup may have a suppressive effect on cytokine production, but that does not impact antibody responses. These findings should be considered when deciding an appropriate postoperative analgesic regimen.
mouse model is more tolerant of whole body radiation than a model with the SCID mutation. Here we describe a study examining chemotherapeutic tolerability of common DNA damaging oncology drugs including 5-fluorouracil (5-FU), doxorubicin (Doxo), and cyclophosphamide (CTX) (n=10 per group) in female R2G2 mice. 5-FU was given at 30, 60, or 100 mg/kg, intraperitoneally, twice weekly for 5 wk. Doxo was given at 2 or 5 mg/kg, intraperitoneally, once weekly for 3 wk. CTX was given at 100 or 140 mg/kg intraperitoneally, once weekly for 3 wk. Body weight and survival were recorded weekly and complete blood count and clinical chemistry were performed at the end of the study. Results show that the R2G2 mouse model tolerates higher doses of these chemotherapeutic drugs than doses found in the literature for SCID models. A separate study was completed to examine estrogen tolerance. Exogenous estrogen tolerance is another common concern in oncology research as some immunodeficient mouse models cannot tolerate the subcutaneous estrogen pellets, developing negative secondary effects resulting in removal from study. We performed an estrogen pellet dose-response study in female R2G2 mice using 4 doses of 60-d release 17-β estradiol pellets at 0.18, 0.36, 0.72, and 1.7 mg/pellet (n=10 per group). Body weight and survival were recorded weekly for 60 d, and complete blood count and clinical chemistry were performed at the end of the study. R2G2 mice show dose-dependent effects of estrogen on morbidity and mortality. These data will allow researchers to determine the optimal dose for use in the R2G2 model. In conclusion, these data support that the R2G2 mouse model may be a good alternative to SCID models when administering DNA damaging chemotherapies or when estrogen supplementation is required for xenograft growth.

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

J Naden*

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We describe growth of multiple patient-derived (PDX) and tumor cell line xenografts and allo-grafts in the B6;129-Rag2<sup>tm1Fwa</sup>IL2rg<sup>tm1Rsky</sup>/DwIHsd (R2G2) immunodeficient mouse model. The PDXs examined include colorectal and head and neck cancers. The CDXs studied include esophageal (OE33 and FLO1) and stomach cancer (AGS). Tumor growth data was also collected from 2 allografts of murine colorectal cancer (CT26) and B-cell lymphoma (A20) cells. Colorectal PDX tissue was subcutaneously implanted bilaterally into 5 male R2G2 and 5 male NSG mice. Growth was comparable between the R2G2 and the NSG mouse models, however the standard error was much lower in the R2G2 strain. Head and neck PDX 626 and 635 was transplanted in 2.2 mm<sup>2</sup> tissues into 4 sections of each of 2 R2G2 mice each
(n=2/PDX), and 100% of mice developed either 1 or 2 tumors. The human esophageal adenocarcinoma OE33 cells were implanted into the left and right flanks of 3 each of R2G2, athymic nude, and SCID mice. There was a 100% take rate in R2G2 mice, 0% in SCID mice, and 17% in athymic nude mice. The human esophageal adenocarcinoma FLO1 cells were examined in 2 studies. In both studies, cells were injected into both flanks of R2G2 and SCID mice. Study A also examined growth in athymic nude mice. In study A, no tumor growth was seen in athymic nude or SCID mice, whereas the take rate was 100% in R2G2 mice. In study B, the take rate was 100% in both the R2G2 and the SCID mice, although differences were seen in growth rate. Human gastric adenocarcinoma AGS cells were implanted in both flanks of 4 each of R2G2 and SCID mice. The take rate was 75% in R2G2 mice and 0% in SCID mice. Head and neck squamous cell carcinoma SQ20b cells were implanted in 20 R2G2 mice and take rate was 90%. Growth of 2 allogeneic tumor lines was also examined. The mouse colon carcinoma CT26 cells were implanted in ten R2G2 mice and took in 100% of the mice. Mouse B-cell lymphoma A20 cells were implanted in ten R2G2 mice and take rate was 100%. Both allografts grew to 1000 mm³ by 13 d post-implantation. These data provide evidence that the R2G2 mouse model is a valuable tool for oncology programs including cell line tumor models research, with high take rates and quick growth of allogeneic models.

**PS18 Early Life Oral Cholera Toxoid Vaccine Lowers Risk of Cancer**

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During the past 40 y, there has been an inexplicable increase in chronic inflammatory disorders including cancer in humans. Earlier studies in mice showed disrupted host gut microbes and immunity associated with westernized living practices led to higher risk for cancer of lymphatics and other nonintestinal tissues in later generations, highlighting the potential roles for gut microbiota and the need for health remedies to counteract such heritable risks of modern lifestyles. Here we tested whether a safe and simple oral vaccination strategy with an immune adjuvant during infancy is sufficient to counteract heritable cancer risk associated with a carcinogenic microbiome. As predicted, CD1 stock mice harboring the carcinogenic microbiome spontaneously developed a high frequency of lymphoma in 100% (10/10 males and 10/10 females) of mice examined, plus hepatocellular carcinoma in 80% (8/10) of males, and mammary carcinoma in 60% (6/10) in females, upon necropsy at 9-mo old. In contrast, we found that feeding 10µg of sterile *Vibrio cholerae* exotoxin subunit B for a total of 3 times every other week via gastric gavage starting at 4 wk of age was
sufficient to significantly inhibit cancers (lymphoma, liver, and mammary, \( P < 0.05 \))
development when compared with matching controls at 9-mo of age in our mouse models. Beneficial effects were transplantable to other animals using purified lymph node cells alone, confirming an immune-mediated mechanism. Taken together, we concluded that oral vaccination with cholera toxin B during early life helps stimulate health-protective immune responses and counteract cancer development later in life in host animals. In summary, immune adjuvants derived from bacteria may serve as potential vaccines to lower risk of cancer later in life.

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

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One in 10 newborns are born premature and receive an average of 14 +/- 4 painful procedures daily in the hospital. The developing neonatal nervous system readily alters in response to sensory stimuli due to neuroplasticity and immature descending inhibitory mechanisms. These early-life, injury-induced alterations can lead to increased severity of subsequent painful events. The effect on chronic osteoarthritis (OA) pain is unknown. The objective of this study was to assess the impact of early repetitive needle prick (RNP) injury on subsequent OA pain during adulthood.

Sprague Dawley rat pups were placed into early life injury (n=18 male, 16 female; PND1)/no injury (n=16 male; 14 female; PND1) groups at birth. Each animal received a series of RNP or tactile (T) stimuli from postnatal d 1 to 7. Gait assessments, reflexive tests, and behavioral assays were performed at regular intervals. At 17 wk, OA (RNP+OA; T+OA, n=9 male, 8 female, 17-wk-old) and control (RNP+C, n=9 male, 8 female, 17-wk-old; T+C, n=7 male, 7 female, 17-wk-old) groups were created. OA was induced using 2mg monoiodoacetate (MIA) injected into stifle joint. During 6-wk period following OA-induction, RNP+OA animals had reduced ipsilateral limb use, compared to others, characterized by: decreased standing weight distribution on the ipsilateral limb \((P < 0.0001)\), reduced maximum contact area \((P < 0.0001)\), reduced intensity \((P < 0.0001)\) and longer swing phase \((P < 0.0001)\) during walking. Mechanical hypersensitivity was greater in RNP+OA groups when compared to all other treatment groups \((P < 0.02)\). RNP+OA animals showed less horizontal exploration \((P < 0.05)\) and spent less time in the center of the open field area \((P < 0.02)\) compared to controls. On the elevated plus maze, RNP animals spent more time...
on open arms. We have shown that early RNP injury appears to heighten pain due to OA-induced by MIA, over MIA alone in mature rats, as defined by clinically relevant limb use, with similar trends reflected in reflexive behaviors and complex behaviors measured. Future studies should assess the underlying mechanisms responsible for these chronic effects of RNP on later chronic pain.

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

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Intervertebral disc degeneration (IVDD) is a leading cause of back pain and disability. There are no known regenerative treatments. Etiopathogenesis of IVDD is poorly understood and appears to be multifactorial. Inflammatory and degradative disease mechanisms are consistently associated with it. However, specific pathways and potential interactions between annulus fibrosus (AF) and nucleus pulposus (NP) have not been fully elucidated, and valid animal models that closely resemble human IVDD are lacking. Dogs develop spontaneous IVDD similar to humans, and clinical IVDD is another significant canine health concern. This study was designed to test the hypothesis that co-culture of AF and NP would be associated with significantly higher levels of inflammatory and degradative metabolite production compared to separate AF and NP mono-cultures. With ACUC approval, we evaluated IVDs from non-chondrodystrophic dogs (n=6, euthanized for reasons unrelated to this study) to elucidate interactions between AF and NP. Lumbar IVDs were collected aseptically and 4mm explants were created from each AF and NP. Explants were assigned to co-culture or mono-culture. Cultures were maintained for 21 d; media were collected and refreshed every 3 d. Media were tested for inflammatory and degradative metabolites using commercially available assays. Production levels were compared for statistically significant ($P < 0.05$) differences. Significant differences in IL-6, KC, MMP2, NO, and PGE$_2$ levels were noted between co-culture and NP monoculture. Significant differences in IL-8, KC, MMP2, MMP3, NO, and PGE$_2$ levels were noted between co-culture and AF mono-culture. IL-6, MCP1, MMP2, and MMP3 were significantly lower in co-culture while KC and PGE$_2$ were significantly higher in co-culture. Significant differences in IL-6, IL-8, MMP2, MMP3, NO, and PGE$_2$ levels were noted between AF and NP mono-cultures. Collectively, these data suggest that key metabolites known to be involved in IVDD are preferentially produced by AF or NP, and that the interactions between tissues significantly influence levels of production. Ongoing translational studies will be aimed at further elucidation of these important
interactions towards understanding and addressing IVDD mechanisms.

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

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

**Platform Sessions**

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

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

**PS23 Voluminous Regurgitation and Inappetence in a Golden Retriever with Duchenne’s Muscular Dystrophy**

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A 6-mo-old, male, intact Golden Retriever dog, affected with Duchenne’s Muscular Dystrophy (DMD), was reported for an acute bout of voluminous regurgitation and inappetence of 18 h duration. Ten wk prior to presentation, the patient was on a Tamoxifen study and was discontinued 6 wk into the study due to exacerbation of the
DMD phenotype (severe bilateral carpal hyperextension, severe bilateral hind limb plantigrade stance and continuous paraphimosis with penile trauma). The patient was also reported for intermittent regurgitation and nonproductive retching once a week for the 2-3 wk prior to presentation. The clinical manifestations of DMD and the reported clinical signs were managed with supportive care. At presentation, the patient was quiet, but alert with referred upper airway noise on auscultation. Abdominal palpation revealed no pain or abnormalities. Differential diagnoses for regurgitation for this patient were megaesophagus, foreign body, intussusception, or other GI obstruction. Differential diagnoses for the abnormal lung auscultation was aspiration pneumonia, pain, or panting. Three-view thoracic radiographs confirmed severe megaesophagus. The thoracic radiographs also showed alveolar-bronchiolar opacities in the cranioventral and caudodorsal portions of the chest with an ill-defined diaphragm. Euthanasia was elected due to the severity of clinical signs and poor prognosis. Gross necropy confirmed severe megaesophagus and atelectasis of the left caudal lung lobe. A gastroesophageal intussusception was discovered immediately cranial to the diaphragm. The mesenteric blood vessels were engorged with diffuse congestion of the small intestines. Megaesophagus and aspiration pneumonia are common sequelae of DMD in Golden Retrievers. However, gastro-esophageal intussusception is a very uncommon condition in this, or any, population of dogs. Repeated bouts of regurgitation and nonproductive retching in a DMD dog should not be ignored as a complication of megaesophagus and radiographs or other advanced imaging should be considered for rapid diagnosis and institution of treatment as deemed fit.

**PS24 White Spots on the Liver**

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

**PS25 Ataxia and Disorientation in a Yucatan Minipig**

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

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

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

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

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A 4-y-old, female rhesus macaque (*Macaca mulatta*) presented with abdominal distention during access for a routine research procedure. Physical exam revealed the abdomen was moderately distended and soft. The animal was thin, with a body
condition score of 2.5/5. Prior to presentation, the animal had a normal attitude and fecal production, although appetite was variable and the animal was underweight for its age. Abdominal radiographs revealed significant gas distention of the large intestines, specifically the proximal colon. A red rubber catheter was passed per rectum to attempt to relieve the gas distention but was unsuccessful in reducing air volume. Diagnostics including complete blood count, serum chemistry, blood culture, urinalysis, fecal exam, and culture were collected and were unremarkable. The animal was prescribed simethicone 20mg PO SID and monitored for fecal production and quality. Recheck abdominal radiographs showed increased large intestinal gas distention. A barium study was performed but was inconclusive in ruling out mechanical obstruction. The animal was prescribed milk of magnesia 10mg/kg PO SID, meloxicam 0.1mg/kg SQ SID, and the simethicone dose was increased to 40 mg BID as continued supportive care. A saline enema was administered followed by colonoscopy, which was ultimately unsuccessful and inconclusive. Due to poor prognosis, the animal was euthanized and submitted for necropsy. Gross pathology findings revealed 2 strictures affecting the proximal colon, approximately 3.4 cm apart. The colon proximal to the strictures was distended with gas and some liquid feces, while the distal colon contained normal, formed feces. The strictures acted as a partial mechanical obstruction allowing for passage of liquid feces which solidified in the distal colon and obstructed passage of gas leading to abdominal distention in this animal. Histopathology showed multiple, chronic ulcers with abundant granulation tissue, fibroblasts, and collagen, as well as neutrophilic inflammation, within examined sections from the stricture. Chronic cicatrizing colitis, resulting in ulceration and fibrosis, is a condition that can affect rhesus macaques and appears to be the cause of the colonic strictures and clinical signs in this case.

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

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

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

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An adult 1.6 kg male, singly housed, Southern Giant Pouched Rat (*Cricetomys ansorgei*) presented with right-sided facial swelling. The rat was housed in an AAALAC-accredited institution and was part of a colony used for reproductive and behavioral research. Under general anesthesia with isoflurane, a 3 x 2 cm swelling was palpated in the musculature at the level of the ear and jaw, and copious, putrid, yellow-white pus exuded from the ear canal. The rat was started on meloxicam (2mg/kg PO SID) and amoxicillin/clavulanic acid (20 mg/kg PO BID). Differentials included a primary otitis media, cheek pouch abscess, and cheek tooth root abscess. Two d later, the swelling had not improved and the rat was not consuming his oral medications. The rat was anesthetized for medical CT, which showed severe lysis and periosteal reactions along the entire length of the right mandible, apparently
originating from the right lower incisor with secondary involvement of the cheek teeth. An oral exam was unremarkable. Although the rat was maintaining weight and appeared appetent, the animal seemed too painful to eat. The rat was transitioned to injectable meloxicam (1 mg/kg SC SID) and long-acting cefovecin (8 mg/kg SC). Due to poor prognosis associated with surgical debridement and marsupialization, and the preexisting mandibular instability, euthanasia was elected. After euthanasia, nano-CT was performed, which confirmed changes seen on medical CT. Necropsy showed a 1 x 1 cm encapsulated nodule that extended caudally from the right mandibular third molar, with severe inflammation in the muscle, fascia, periodontal ligaments, and bone. A pathologic fracture was visible. Anaerobic and aerobic cultures grew many *Fusobacterium necrophorum* and *Bacteroides fragilis* group, as well as a gram-positive branching rod resembling *Actinomyces*. These bacterial species are anaerobic oropharyngeal commensals commonly found in abscesses due to oral trauma. *Actinomyces bovis* is known in cattle to cause lytic regions of osteomyelitis surrounded by new periosteal bone and fibrous tissue, colloquially known as lumpy jaw. To our knowledge, this is the first time that an odontogenic infection or abscess resembling lumpy jaw has been reported in a Southern Giant Pouched Rat.

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

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

Platform Sessions

PS31 Moving Beyond 3Rs in IACUCs

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In every IACUC in the U.S., there are 2 constants: discussion on harm/benefit of a study design and how the 3Rs can decrease the harm. Each institution has a mechanism to record discussions at IACUC meetings and in many instances use forms, templates, or checklists. These tools are developed to facilitate a robust review and provide documentation satisfying USDA and federal funding requirements as well as institutional policies. There are recognized benefits to using forms, templates, or checklists; they compel completion of obligatory documents or information. Based on a recent review of the top 20 U.S. institutions receiving NIH funding, any prompts for ethical discussions were often limited to the 3Rs and harm/benefit analysis. Except for 1 institution, no other directions on ethical theory, such as telos, were found in the templates. However, there is a potential harm that arises to the nonhuman animal subjects from lack of substantive guidance on ethical principles or values to be considered. One solution would be consideration of voluntary institutional or departmental mechanisms for discussions employing ethical principles. Better integration of ethicists into review and debate of study protocols, even before submission to the granting body and IACUC is yet another possible solution. As practiced today, forms, templates, and checklists benefit IACUCs in assuring compliance. These same forms, templates, and checklists do not prompt rigorous ethical discussion beyond harm-benefit analysis and the 3Rs. For those of us who have accepted the charge by society to ensure humane, necessary, and ethical research, we must continue to seek a solution. Because if not by us, then by whom?
PS32 How to Build an IACUC from Scratch

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

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

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

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

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

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

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

**PS36 Working with Universities to Support and Encourage Engagement with the 3rs**

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

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

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

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

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

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

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

PS40 Whose Line Is It Anyway? Establishing an In-House Rodent Registry

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

Platform Sessions

PS41 Impact of Partial Cage Division on Aggression and Behavior on Long-term Housing in Co-housed Male C57bl/6 Mice

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

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

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

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

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

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

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

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

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

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

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We evaluated breeding efficiency in C57Bl/6J mice in a novel individually ventilated
cage (IVC) design (product A) compared to a traditional IVC design (product B).
Product A introduces an airflow from below the level of the bedding, creating a
plenum in the cage where airflow is diffused through the bedding. One outcome of the
design modification is this product can operate at a low-velocity airflow (e.g. 30 air
changes per hour (ACH)). As mice often use scent cues in normal behaviors such as
breeding, a low airflow was hypothesized to increase breeding efficiency. Further, the
product A design keeps the bedding dry, preventing ammonia accumulation and
affording a longer cage change interval. Thus, allowing the nest to go undisturbed
until weaning, potentially increasing reproductive efficiency. Eight monogamous
breeding pairs were housed per each cage system and were allowed to breed for 6 mo.
Cages were changed on day 1, the day after pups were observed in the cage. Using the
manufacturer recommend settings, a 14-d cage change at 60 ACH in product B was
compared to a 21-d cage change at 30 ACH in product A. Various reproductive
parameters, such as litter size, number of litters, pup weaning weight, and cage
performance were compared. Product B averaged 6.4 pups per litter and had 27 litters
total while product A averaged 7.3 pups per litter and had 33 litters total. Average pup weight on d 28 was 14.7g (n=17) in product B and was 12.9g (n=23) in product A. In regards to cage performance, ammonia levels were measured by an electrochemical sensor and by wireless metal-oxide semiconductor sensors. Other cage performance observations found that product A reduced flooding incidences due to automatic watering valve failures. This study finds that product A, with a significantly modified design concept, to be a suitable environment for mouse breeding on a 21-d cage change schedule.

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

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

**PS48 The Art of Wild Rat Wrangling**

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Domesticated, standardized, pathogen-free research animal colonies have shaped standards of care, housing, health surveillance, and safe handling. Recently, questions
have arisen about the relevance of clean, lab-reared rodents versus pet store or wild-caught animal models of human disease. An IACUC-approved research protocol queried whether results from the spontaneous Sprague Dawley epileptic seizure model would be recapitulated in wild Brown Norway rats. We were tasked with trapping wild rats on campus and housing them in our vivaria for behavioral-test studies. This posed several obstacles such as zoonotic adventitious pathogen exposure, adequate housing, and safe handling. Working closely with the campus IACUC, occupational health nurse, environmental health and safety officers, integrated pest management officer, and attending veterinarian, we developed a protocol for live capture of contaminated rodents for which we normally keep out of our vivaria. Using an aromatic bait and Sherman-like catch and release traps, we set at various locations across campus in the evenings when Colorado nighttime lows were > 40 degrees F. After donning approved PPE, traps were checked in the mornings. Challenges we faced while trapping included escapees, bait consumption without capture, aberrant species, predation, and capture of pregnant females. Upon successful capture, animals were transported to ABSL-2 quarantine, anesthetized, and samples were taken for a global panel of 50 pathogens of which 19 pathogens were detected. All animals were treated for multiple internal and external parasites. After stabilization and treatment, the lab was then cleared to perform surgeries and behavioral testing within the ABSL-2 suite. Since domesticated strains are very intelligent, the “street smarts” of the wild rats and the unique hurdles they present were not underestimated. Successful introduction of wild-caught rodents into our vivaria resulted in reevaluation and refinement of our animal husbandry practices for the safety of the animals, the safety of our staff, and the protection of our colony animals.

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

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

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

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Preterm infants have been shown to have metabolic and developmental deficits within their first postnatal week of life leading to an increase in neonatal morbidity. Our goal was to design an effective model using Yorkshire swine that would resemble preterm infants and allow the investigative group to carry out a nutritional support study. Three groups of preterm piglets with 8, 12, 13 piglets, respectively at gestational ages 105-107 d) were delivered via Caserean section, resuscitated, and allowed to recover in a controlled environment. Each piglet was provided a group of caregivers, including members of the NICU staff, a veterinary technician, and direct oversight of the NICU physician and veterinarian. Once the piglets were stabilized clinically based on predetermined parameters they underwent an anesthetic event, placement of a central line and placement of feeding tube within hours of the Caserean. The piglets were again allowed to recover from these procedures and remained on study for 7-10
d. During the study length many challenges were faced in the medical management of premature piglets that we do not face with animals of a full gestational age. The care of the piglets underwent a great evolution over 3 litters through trial and error. The final major alterations were made for the third litter, which was the most successful. New recovery units were purchased which allowed us to control environmental conditions and eliminate piglet crowding to give us a better visual on each piglet. The Caserean and recovery process was modified to maintain a controlled environment. We eliminated the need for an anesthetic event and central line placement on the first day of the procedure by use of umbilical catheterization. The veterinarian determined critical monitoring parameters, and were posted identifying when intervention was deemed necessary. The parameters included albumin, blood glucose, temperature, SpO2 and mentation. Finally, nutritional support was changed by the group to support the growing piglets. The parameters that we changed gave us 100% survival to end point of the final litter. We determined that these changes were imperative to the success of the study and overall care and welfare of the piglets. Not only was this study incredibly challenging from a medical and husbandry aspect it also had a profound emotional impact on everyone involved in the study.

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

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

Platform Sessions

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

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

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

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*Corynebacterium bovis* is the causative agent of *Corynebacterium*-associated hyperkeratosis (CAH) in immunocompromised mice. The resulting skin pathology can be profound and may be associated with severe wasting, potentially making the animals unsuitable for research. The administration of antibiotics is effective in resolving disease, but does not eradicate the bacterium; however, antibiotic use may be contraindicated as it can affect tumor growth. Lytic enzymes (lysins) obtained from bacteriophages are being investigated as potential novel antimicrobial agents for a variety of bacterial diseases. The advantage of a lysin is its target specificity without affecting the host or engrafted tumor. The goal of this study was to identify *C. bovis*-specific phages and isolate their lysin to test as a potential therapeutic. *C. bovis* isolates, obtained from 1 human and 2 mice, were treated with mitomycin C to induce phage replication. All samples showed turbidity reduction over time, which suggested potential lytic activity. However, when plate-lysis assays were conducted no bacterial lawn clearance was observed. Thus, the presence of phage could not be confirmed by this method or by electron microscopy. Subsequently, using whole-genome sequencing technology, the genome of 20 *C. bovis* isolates obtained from humans, cows, and rodents were sequenced and analyzed for phage. No intact phage sequences were identified in any of the isolates. Thus, despite the ubiquity of phages in almost all bacteria, it appears that *C. bovis* does not have any associated phage.
Genomic analysis further revealed that all isolates had at least 1 confirmed clustered regularly interspaced short palindromic repeats (CRISPR) system, which may explain why no phages were found as CRISPRs serve as an adaptive immune system for prokaryotes. Additionally, toxin-antitoxin systems, shown to mediate defense mechanisms against phage infection, were detected in the majority of the isolates. Although highly unusual, C. bovis does not appear to have a phage system, making lysin therapy more difficult. However, lysins from phage of closely related organisms could have an effect on C. bovis, but this has not yet been explored.

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

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Astroviruses, a genetically diverse group of RNA viruses, are known to infect a wide range of mammalian and avian species. The first astrovirus in laboratory mice was described in 1985. Advances in molecular genomics and increased population surveillance has led to the identification of various astrovirus strains in feral and laboratory rodent populations, the significance of which is currently unknown. A common astrovirus infecting mice, murine astrovirus 1 (MuAstV1), is endemic in many research and production laboratory mouse colonies. We recently identified a novel astrovirus genetically distinct from MuAstV1 using metagenomics. This virus, murine astrovirus 2 (MuAstV2), is most closely related to astroviruses isolated from 2 feral rat species in China and a virus recently identified in feral mice in New York City. Our search for the virus was initiated following false positive results to MTLV in a multiplex immunofluorescence assay that used MTLV antigen generated in an immortalized murine AKR T cell line that was subsequently found to be infected with a closely related virus. MuAstV2 is readily transmitted by the fecal-oral route, and immunocompetent mice infected during the initial outbreak remained asymptomatic. Histopathologic evaluation of all organs submitted from these mice showed no significant morphologic changes. However, mild to moderate levels of viral nucleic acid were detected by in situ hybridization (ISH) in the enterocytes of the small and large intestine, and within mononuclear cells of Peyer’s patches and mesenteric lymph nodes. We report on studies conducted to characterize the infectivity, tissue tropism,
and pathology of this virus following oral inoculation of young adult C57BL/6NCrl and NOD-Prkdc<sup>em26Cd52Il2rg<sup>em26Cd22</sup>/NjuCrl (NCG) mice. The magnitude and duration of viral shedding was characterized by performing fecal qRT-PCR. Gross and histopathological changes, as well as viral tropism identified using in situ hybridization were examined at various time points post-inoculation. These findings as well as the potential impact of MuAstV2 on research will be discussed.

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

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

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

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*Demodex musculi* infestations are rarely reported in laboratory mice. However, the sharing of genetically engineered mouse strains among institutions has led to the inadvertent introduction of *D. musculi* into many colonies. Until recently, detection methods were unreliable and testing for the parasite was not routinely performed as its presence in laboratory mice went unrecognized. We estimate a prevalence of approximately 10-30% in academic colonies based on PCR data from animals imported into our institution. While infestations are clinically inapparent in most mouse strains, *D. musculi* burdens are increased and clinical signs have been reported in several strains of immunodeficient mice. Though the immune phenotypes of animals in these reports were known and likely the cause of increased susceptibility, what, if any, effect *D. musculi* has on the immune system of immunocompetent mice is unknown. We hypothesized that infestation with *D. musculi* modulates innate and adaptive immune responses and the mite burden would be dependent on the immunophenotype of the infested strain. As Th2-mediated immune responses are known to increase resistance to parasitic infections, Th1-dominant strains will likely be more susceptible to infestation. We characterized the mite burden and immunologic changes in naïve Swiss Webster (outbred), C57BL/6NCrl (Th-1 skewed immune response), and BALB/cAnNCrl (Th-2 skewed immune response) mice following exposure to *D. musculi*. *Demodex*-infested NSG mice from a previously established *Demodex*-positive colony were cohoused with naïve Swiss Webster, C57BL/6NCrl, and BALB/cAnNCrl mice. Age-matched mice (n=5) were euthanized for sample collection 14 and 28 d later. Mite burden was determined by PCR and comprehensive skin histopathology. CD4 and CD8 cell counts, T-cell activation markers (CD44, CD25, CD69, Ly6C) were evaluated using flow cytometry, and
complete blood counts were performed. Not surprisingly, highly immunocompromised NSG mice had the highest mite burdens, which correlated with high PCR copy numbers, although no evidence of clinical disease was noted. No significant differences between infested and naive animals in terms of T-cell activation or complete blood counts were observed in Swiss Webster mice.

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

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Buprenorphine (BUP) and buprenorphine SR (BupSR) are used as preemptive analgesics in mice, potentially affecting anesthetic requirements. The purpose of this study was to determine the effects of BUP and BupSR on the minimum alveolar concentration (MAC) of isoflurane and on heart and respiratory rates. We hypothesized that BUP and BupSR would significantly decrease MAC, heart, and respiratory rates in isoflurane anesthetized mice. Forty-seven male and female C57BL/6 (6-15 wk of age) mice received either 0.2 mL of saline (control), BUP (0.1 mg/kg), or BupSR (1.2 mg/kg) subcutaneously 10 min before induction of anesthesia with isoflurane. Mice were maintained at 3 randomized, increasing, isoflurane concentrations and depth of anesthesia was assessed by response to a noxious stimulus after 10 min at each isoflurane concentration. A surgical plane of anesthesia was defined by the loss of hind limb withdrawal. MAC for each injection was calculated using quantal and bracketing techniques. A 1-way ANOVA at the MAC for each injection was used to compare MAC, heart, and respiratory rates between the injections. Significant differences were observed between BUP and BupSR on MAC ($P < 0.001$). BUP reduced MAC by 18% in females (1.72±0.08% control; 1.41±0.09% BUP) and 26% in males (1.82±0.03% control; 1.33±0.15% BUP). BupSR reduced MAC by 16% in females (1.45±0.11%) and a 22% MAC reduction in males (1.42±0.13%). Sex did not significantly affect MAC. The heart rate of the mice receiving BUP was significantly lower ($P < 0.001$; 359±24 bpm BUP;) than the mice receiving the BupSR and control injections (455±68 bpm BupSR, 499±48 bpm saline) at similar planes of anesthesia. There were no significant differences in the respiratory rate (126±21 bpm BUP; 132±13 bpm BupSR; 122±22 bpm control). This study demonstrates that BUP and BupSR act similarly in male and female mice and decrease isoflurane requirements to maintain a surgical plane of anesthesia in mice.
PS58 Female-induced Ultrasonic Vocalizations in Male C57BL/6J Mice as a Proxy Indicator for Acute Pain

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Recognizing pain objectively in rodents is challenging, yet it is an essential component to minimizing pain and distress in these animals. Mice produce ultrasonic and audible vocalizations to communicate with conspecifics and this behavior has been studied as a modality for pain recognition with mixed results. Female-induced ultrasonic vocalizations (FiUSV) are ultrasonic vocalizations produced by adult males when presented to adult females or their urine. This is an affiliative behavior that may be reduced if the mice are in pain or distress. To determine if FiUSV can be used as a proxy indicator for pain recognition, we compared ultrasonic vocalizations produced by male C57BL/6J mice in response to female urine at baseline, 1h, and 3h post administration of a sublethal dose of lipopolysaccharide (LPS; 12.5 mg/kg IP) or equal volume of saline. Pain was assessed by orbital tightness, posture, activity, and piloerection immediately after ultrasonic measurement. We hypothesized that painful or distressed male mice would have a decreased inclination to mate and therefore would produce fewer FiUSV. At baseline, 32 out of 33 mice produced FiUSV (149 ± 127 USV/2 min). There was no change from baseline at the 1- or 3-h time points in the saline-treated mice, whereas LPS-treated mice demonstrated significantly fewer FiUSV than baseline ($P = 0.0078$), producing 0 USV at both time points. Mice treated with LPS showed signs of pain at 3 h but not 1 h according to orbital tightness, posture, activity, and piloerection. These findings show that FiUSV can be used as a proxy indicator for acute pain and a change from baseline can be detectable prior to onset of visual clinical signs.

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

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A significant concern in laboratory animal medicine is contamination due to pathogen outbreaks and how to adequately address the issue of small equipment decontamination. Many factors play a role in the selection of the decontamination method including cost, efficacy, and personnel time and safety. Chlorine dioxide (ClO2) gas is an effective method, but commercially available systems which include a large-scale ClO2 gas generator and specialized air-tight exposure chamber can be costly and impractical in some situations. The goal of this study was to create and validate an effective, small-scale, decontamination method utilizing ClO2 gas, which is affordable, efficient, safe, and reproducible. First, we identified a product where 2 dry reagents react to produce ClO2 gas. To find an affordable exposure chamber, we evaluated the ability of 4 household totes with gasket-seal lid systems to retain ClO2 gas and relative humidity (RH). Validation of the efficacy of decontamination was assessed by using 2 different biological indicators (BI) concurrently, *Bacillus atrophaeus* (*B.a.*), and *Geobacillus stearothermophilus* (*G.s.*). ClO2 gas concentration, total exposure dose, and RH were measured using a commercially available photometer and data logger. Our results confirm that ClO2 gas production is reliable and scalable. All household totes evaluated held sufficient gas and RH for a 15-h cycle (overnight), providing adequate exposure to inactivate both BI evaluated. Our results suggest that a total exposure dose of 71 ± 42 ppm-h and 1.17 ± 0.14 mg of ClO2 gas over 15 h at > 90 % RH is adequate to inactivate both *B.a.* and *G.s.* While there was no statistical difference in the 2 BI as indicators for decontamination (n=460 BI, P = 0.362), for the end user, *B.a.* was more consistently inactivated. In conclusion, we have successfully brought together a variety of low-cost materials to establish an effective, small-scale method to decontaminate small laboratory equipment. Depending on the size of the tote and whether BI are used, the upfront cost is approximately $75-$750. This method is roughly 100 times less expensive than large-scale ClO2 gas generators used with specialized air-tight exposure chambers.

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

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

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

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The *Guide* indicates that pharmaceutical-grade chemicals “should…be used, when available, for all animal-related procedures” and “[t]he use of non-pharmaceutical grade chemicals or substances should be described and justified in the animal use protocol…” For vision research in isoflurane-anesthetized mice, the nonpharmaceutical grade sedative chlorprothixene has been widely used to reduce spontaneous eye movement in pupillary light reflex imaging, and to lower the isoflurane to a level that does not substantially weaken light-evoked responses in electrophysiological recordings. However, data is lacking to justify the use of
chlorprothixene. This study evaluated whether pharmaceutical-grade sedatives would be appropriate alternatives. Male 15-wk-old B6129SF2/J mice were IP-injected with 1 mg/kg chlorprothixene (n=5), 5 mg/kg acepromazine (n=5), 10 mg/kg chlorpromazine (n=4), or saline (n=5), then induced with isoflurane after 1 min of sedation. Anesthesia maintenance was 0.5% and 1% isoflurane for mice administered sedatives and saline, respectively. A 16.0 photons cm\(^{-2}\) s\(^{-1}\) 470 nm light stimulus was applied to the right eye, and the left eye was imaged for consensual pupillary constriction and involuntary pupil movement. Induction parameters measured were time to immobilization and loss of righting reflex, and physiologic parameters were assessed during anesthesia. ANOVA analyses showed no significant differences in baseline pupil diameter, pupillary light reflex response, and induction parameters, while mean heart rate in the saline group was significantly lower. Substantial involuntary pupillary movement was observed in 2/5 mice in the saline group, moderate movement in 1/4 mice in the chlorpromazine group, and slight movement in 1/5 mice in the acepromazine group. Full recovery, as defined by purposeful movement, response to tactile stimuli and full alertness, was not achieved in any sedative group during the 1.5-3 hour postanesthetic period due to continued substantial sedation. In conclusion, acepromazine is likely a suitable pharmaceutical-grade alternative to chlorprothixene, but lower dosages may need to be further investigated for use in survival procedures given the lack of full recovery in the experimental groups.

Platform Sessions

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

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

Adapting acupuncture to research animals can be facilitated by adjusting traditional methodology to research requirements, diagnosis, animal temperament, anatomy, and size.

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

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Gene therapy is a promising area of drug development for a number of diseases, including neurological disorders. Cynomolgus macaques (*Macaca fascicularis*) show high nucleotide sequence homology with humans and need to be used where test article cross-reactivity occurs. An IACUC-approved regulatory toxicity study required bilateral administration of a gadolinium-labeled viral vector into 4 locations into the striatum (caudate nucleus and putamen) with temporarily implanted catheters. Magnetic resonance imaging (MRI) was used for calculation of trajectory and for surveillance of test article delivery during convection-enhanced delivery. Twenty-four cynomolgus macaques (4 groups, 12 M/12F) were prepared for a 6-8 h anesthesia including presurgical analgesia, induction of anesthesia with ketamine/medetomidine, intubation, clipping, disinfection, and placement in a stereotactic frame. Inhalation anesthesia was conducted with isoflurane. Animals were transferred to the MRI to obtain data for calculation of position and angle and depth for catheter placement, assuring that the trajectory will not cross blood vessels or ventricles. Following
transfer to the surgical suite, the stereotactic frame was used to ensure correct placement of the catheters based on the coordinates determined by MRI. The skin was reclined from the skull and holes were carefully drilled. Catheters were cut to specific length and inserted along the calculated trajectories to administer the viral vector to 4 different locations into the striatum. Catheters were connected to infusion lines containing the gadolinium labeled test article. Infusion (rate 0.3mL/h) was monitored in the MRI for 80 m to verify correct administration into the striatum. Thereafter, animals were transferred back to the surgical suite and catheters were explanted. Animals recovered from the 6-8 h anesthesia within 15–30 min and were treated with analgesics and antibiotics. Two animals presented laryngeal swelling were treated with corticosteroids successfully. No animal showed neurological abnormalities. Histopathology results will be presented. Using convection-enhanced delivery, 96 catheters were successfully implanted with fast and uneventful recovery from a 6-8 h isoflurane anesthesia. There was successful intracranial administration into the striatum.

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

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

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

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

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

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

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

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Opioids are essential for rhesus macaques (Macaca mulatta) requiring multimodal analgesia or those unable to receive NSAIDS as part of their pain management plan. The current opioid epidemic has universally limited the availability of these vital analgesics, compelling clinicians to investigate other options including new opioid formulations or different dosing regimens. A long-acting, highly concentrated formulation of buprenorphine is available as a single dose, injectable solution that provides therapeutic plasma concentrations lasting 24 h in cats (Felis catus). We hypothesized that this highly concentrated buprenorphine solution (HCBS) would achieve therapeutic concentrations (≥ 0.1 ng/mL) for at least 24 h in rhesus macaques similar to the cat. The objective of this study was to evaluate the pharmacokinetic profile of a single subcutaneous dose of HCBS in rhesus macaques at 0.24 and 0.72 mg/kg and then compare them to each other and the cat. Six healthy, adult rhesus macaques (3 male and 3 female) were included in a randomized, 2-period, 2-treatment crossover study. Plasma buprenorphine metabolite concentrations were determined prior to and for a maximum of 120 h after administration, measured using liquid chromatography-tandem mass spectrometry and pharmacokinetic analysis was performed. The low dose achieved a maximum plasma concentration of 19.1 ± 5.68 ng/mL at 19.6 ± 4.02 h with an AUC of 236.4 ± 22.5 h/ng/mL and a terminal elimination half-life of 19.6 ± 4.02 h; for the high dose, these parameters were 65.2 ± 14.7 ng/mL at 0.034 ± 0.004 h, 641.3 ± 79.4 h/ng/mL, and 20.6 ± 2.30 h, respectively. The mean concentration at 24 h post-injection was significantly (P < 0.01) above the therapeutic threshold for both dosages in macaques. One animal showed mild pruritus at both doses and another animal showed mild somnolence at both doses. These findings support the use of HCBS in rhesus macaques for once daily dosing without problematic adverse effects and represent a potential new alternative.
PS68 Enucleation and Temporary Tarsorrhaphy for the Treatment of Unilateral Exophthalmia in Cotton Rats (Sigmodon hispidus)

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Twenty-five cotton rats (Sigmodon hispidus) 8-13-mo-old presented with acute, unilateral exophthalmos over a 6-mo period. The cotton rats otherwise appeared healthy and had no previous clinical signs of disease. The majority of affected cotton rats were retired breeders and were not exposed to experimental manipulations such as retroorbital blood collection or anesthesia. Differentials for exophthalmia in cotton rats included traumatic proptosis, infectious agents such as adenovirus, cardiomyopathy with secondary retroorbital thrombi, or a retroorbital mass. Diagnostic samples collected from these animals included histopathology (n=14), corneal bacterial culture (n=9), and human adenovirus PCR panel (n=2). The bacterial cultures grew multiple strains of opportunistic bacteria and the adenovirus PCR panel was negative. Interestingly, ocular histopathology identified marked keratitis and conjunctivitis along with occasional retrobulbar fibrin thrombi in some of the samples. Additionally, histologic evidence of cardiomyopathy and pulmonary thromboemboli was observed in some of the rats. Together these findings suggest that exophthalmos was likely caused by retroorbital thrombi secondary to cardiomyopathy. This case study demonstrates the potential for high incidence of ocular issues in older colonies of cotton rats. Clinical cases were managed with systemic analgesics and prompt enucleation due to rapid clinical progression to severe keratitis and secondary infections. In order to reduce the number of enucleations performed, a simple temporary tarsorrhaphy procedure was designed for mild cases detected early in malady. All animals thrived postoperatively; therefore, based on severity of the lesion, enucleation and temporary tarsorrhaphy are potential surgical interventions for cotton rats presenting with exophthalmia.

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

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

Platform Sessions

PS70 Ethanol Overdose as a Refinement to CO₂ for Euthanasia of Chickens (Gallus gallus domesticus)

GLAS: Yes
According to the AVMA Guidelines for Euthanasia of Animals, injectable pentobarbital and inhalant CO₂ are 'acceptable' and 'accepted with conditions' methods of euthanasia for avian species. However, barbiturates are controlled substances and challenging to use in the field and laboratory setting. Additionally, there is limited literature on the use of CO₂ in avian species. CO₂ also has been reported to induce anesthesia and euthanasia at inconsistent time intervals and is cited by users to be visibly distressful to birds. Recent studies demonstrated that intraperitoneal ethanol overdose is a novel euthanasia agent in mice, but is inconsistent in rats. Ethanol is easily accessible and its pharmacological properties suggest that it could induce a humane death. Thus, we sought to determine if intracoelomic (IPc) ethanol could be used as an alternative euthanasia agent in chickens. To evaluate this, we compared IPc ethanol to IPc pentobarbital. Chickens weighing approximately 2.0kg where fitted with ECG, capnography, and doppler. Chickens were randomized into 3 groups: 20ml of 100% ethanol, 20ml saline, or 0.5ml of pentobarbital IPc. Loss of consciousness was assessed by intubation time, capnography was used to confirm respiratory arrest, and ECG to confirm cessation of cardiovascular function. Time to intubation was significantly different in chickens receiving ethanol (n=6, 494.56s +/- 330.11s) and pentobarbital (n=9, 250s +/- 96.70s) by 2-sample t-test (P = 0.049). The time to loss of respiration was markedly different between the ethanol (n=5, 964.8s +/-200.2s) and pentobarbital (n=5, 342.2s +/- 11.14s) groups by 2-sample t-test (P = 0.0005). Time to reach asystole in chickens was significantly different between the ethanol (n=4, 1052.60s +/- 200.01s) and pentobarbital (n=5, 772.2s +/- 54.67s) groups by 2-sample t-test (P = 0.03). There was a total of 8 chickens (4 per group), who failed to reach asystole. No overt signs of pain or distress were observed. There were no significant histological changes and there was no degradative effect on RNA extraction. Further, there were no significant aberrations on ECG recordings between groups. We conclude that 20mL IPc ethanol and 0.5mL IPc pentobarbital induces euthanasia inconsistently; demonstrating that ethanol as an alternative euthanasia method in chickens requires further investigation.

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

GLAS: Yes
Euthanasia methods for avian species outlined in the 2013 AVMA Guidelines for Euthanasia of Animals are extrapolated from methods used in mammals, with injectable pentobarbital and inhalant CO₂ as the ‘acceptable’ and ‘acceptable with conditions’ methods for euthanasia of avian species. The objective of this study was to determine if intracoelomic (IPC) injection of 0.5mL of 100% ethanol is an efficacious means of euthanasia in zebra finches (*Taeniopygia guttata*). Adult cull zebra finches were block randomized and received an IPC injection of 0.5mL of saline (n=21), 0.5mL of 100% ethanol (n=22), or 0.05mL sodium pentobarbital (n=21). Finches were placed in an observation box and video recorded. Time to loss of the righting reflex (LORR) and cessation of all movement (CAM) was recorded. Tissues were submitted for histopathology scoring and postmortem blood glucose was performed. Videos were blinded and randomized for retrospective analysis of pain and distress. LORR following IPC ethanol (72.73s +/- 55.17s) was not significantly different from the IPC pentobarbital (48.38s +/- 20.73s) or the IPC saline (59.05s +/- 19.68s) groups (\( P = 0.09 \)). There was no significant difference in CAM following ethanol (183.45 s +/- 161.37), pentobarbital (183.86 s +/- 146.07), or saline + CO₂ (100.05 s +/- 21.11) groups using 1-way ANOVA (\( P = 0.13 \)). Retrospective behavioral scoring was not significantly different between groups. There was a significant difference in the blood glucose levels with birds euthanized with 100% ethanol exhibiting significantly lower blood glucose levels (369.55 mg/dL +/- 78.63) than birds euthanized with CO₂ following saline injection (n=21, 445.57 mg/dL +/- 97.16) using 1-way ANOVA (\( P = 0.02 \)). Histopathologic scoring showed no significant difference between the groups. Another group of cull zebra finches were randomized and used to determine an effective dose 50% (ED₅₀) of 100% ethanol via the Dixon up-and-down method. This information was then used to develop a linear regression model. The ED₅₀ of 100% ethanol IPC was 0.023 mL/g (standard error = 0.004 mL/g). We conclude that 100% ethanol, dosed at 4 x the ED₅₀ (0.09ml/g), is an efficacious and novel method of euthanasia in zebra finches.

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

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

**PS73 Enrichment Preferences of Singly Housed Zebrafish (*Danio rerio*)**

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Despite the increasing popularity of zebrafish (*Danio rerio*) as an animal model, environmental enrichment preferences of this species have been largely unexplored. We sought to determine enrichment preferences of mature female zebrafish that were singly housed with or without access to 1 of 10 inanimate forms of enrichment that recapitulated species-specific behaviors. Enrichment items were rotated every 7 d until the completion of all 10 conditions. Place-preference, as indicated by fish location within the tank, was observed by video recording. All subjects showed a
preference for the front of the tank when caretakers entered the room, demonstrating an effect of human presence on tank location \((P < 0.00001)\). Out of the 10 conditions tested, subjects showed the strongest preference for the back of the tank when housed with mirrored paper on the side of the tank when compared to the barren half of the tank \((P < 0.0005)\). Fish were also observed interacting with 3 of the other items in species-specific behaviors. These included PVC pipe, marbles, and tulle. Given that enrichment imitating social interaction had such a prominent effect, we set up a second study to assess the value of visual exposure of conspecifics in adjacent tanks. The subjects were then provided 1 of 3 conditions: a singly housed neighbor fish, group-housed neighbor fish, or an empty neighbor tank. All zebrafish housed next to neighboring fish showed a preference to be on the side of the tank nearest to the other fish \((P < 0.05)\). Overall, our data indicate that singly housed zebrafish prefer enrichment items which promote social behaviors, either in the form of self-visualization or neighboring fish. Thus items such as mirrored paper or housing next to conspecifics should be strongly considered as enrichment strategies for singly housed zebrafish. Items such as PVC pipes, marbles, and tulle should be considered as alternative enrichment strategies if opportunities for visual imagery of fish are not available.

PS74 Evolution of a System for Video-based Automated Assessment of Activity Measures and Behavior of Mice in Ventilated Racks

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In recent years there has been increased interest within the laboratory animal science community in the usefulness of automated profiling of rodent activity in their home cages. Activity data may reflect health, welfare, phenotype, and/or effects of toxicity. Video-based systems offer distinct advantages in being able to recognize discreet behaviors, and some video-monitoring functions have been designed into specialized mouse racks. Wide-spread use of this technology may be achieved by retrofitting existing high capacity racks with video-monitoring capability and developing data management tools. We demonstrate a mechanical design that uses 3-D printing technology and inexpensive electronic components. Custom-designed printed circuit boards are used to provide constant near-infrared illumination, allowing consistent image acquisition with no inference with photoperiod. Our newest system utilizes
commercially available IVC racks, and we have developed software to assess activity and behavior measures during prolonged experiments. Algorithms are being developed to provide detailed analysis of rodent activity within their home cages on ventilated racks.

**PS75 Real-time Monitoring of Animal Welfare through a Smart Animal Alert System**

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A primary goal in animal research is respectful and responsible care aimed toward minimizing stress and discomfort while enhancing collection of accurate and reproducible scientific data. A major challenge in monitoring animal welfare is that most cage-side observations are attempted during the light cycle when rodents are predominately asleep, making it difficult to discern potential health conditions. Alternatively, animals are handled to uncover health conditions but handling can induce stress which may increase the inter- and intrasubject variability. We investigated how technology can complement existing means of monitoring animal health by capitalizing on noninvasive, cage-level video monitoring, and computer vision-derived metrics of animal motion and breathing rate. We analyzed historical data collected from rodents that represent a diverse range of therapeutic models including inflammation, respiratory, oncology, metabolism, and aging disorders, and identified abnormal patterns that were associated with sick animals, but absent in healthier controls. Currently, this alerting system can detect abnormal physiological and behavioral changes associated with decreases in motion, changes in breathing rate, and abnormal circadian rhythms. The performance of the alert system was evaluated using the results of a survey taken by 5 scientists with a combined 10+ years of using the metrics and measured the value of proposed alerts at specific time points. The alert system accurately identified 83.3% of desired alerts, while only falsely identifying 6.6% of alerts. An animal alerting system improves animal welfare by enhancing the ability of vivarium staff and researchers to automatically identify animals that 1) require more careful observation, 2) need medical attention, or 3) should be euthanized, without the need to handle the animal. This alert system effectively utilizes near real-time physiological metrics tied to vital life functions and normal animal behavior and represents a critical advancement toward merging traditional animal housing with technology such that critical health conditions are not missed and the utility of every subject in a study is maximized to drive responsible scientific advancement.
PS76 Assessing Technical Proficiency in a Germ-free Facility

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A university started a 4,533-sq.-ft. a germ-free facility that consists of 80 flexible film isolators that house approximately 300 mice and 100 rats. Six technicians carry out the day-to-day husbandry and technical procedures within the facility. Since research staff is not permitted to perform technical procedures for ongoing studies, it is imperative to have an effective and successful proficiency training and assessment program. This is essential so that we can provide documentation to researchers and contractors requesting our services, demonstrating that the staff has been deemed proficient to perform the requested technical procedures needed for their studies. We have set up a training plan covering technical procedures for proper restraint, injections (ID, IP, IV and SQ) and blood collection (maxillary vein) in mice and rats. Checklists are used to assess the 6 different technical skills for mice and rats. Trainees deemed as proficient are capable of consistently and accurately performing the specific techniques with an 80 to 100% pass rate. Each year the technicians are reassessed and deemed proficient. Reassessment is vital in verifying that the technician’s technical skills are sufficient and helps in evaluating the current training needs.

PS77 Using 3D Measurement and Machine Learning to Quantify Subcutaneous Tumors and Improve Animal Welfare and the 3rs in Cancer Research

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The standard method of using handheld callipers to measure tumor growth and response to drug therapy in preclinical oncology trials results in operator and interoperator bias. Length and width measurements are used to estimate volume under the assumption that tumors are spherical, leading to further inaccuracies for nonuniformly shaped, flat, and small tumors. Overall this can lead to repeat studies, resulting in more mice being used and longer and more costly trials. We developed a novel 3D scanning system to capture and automatically detect and quantify the length, width, and volume of tumors in mice. 3D surface images are captured using stereo and photometric imaging and are analyzed, segmented, and characterized. Machine
learning has been used to train and independently validate a dataset of 2197 3D mice scans consisting of 19 tumor types and 6 mouse strains which were compared against measurements from callipers. We demonstrated that it is possible to record tumor measurements in a rapid, minimally invasive, morphology-independent, and human bias-free way, removing interoperator variability while providing full reproducibility, transparency, and traceability of data used in studies. With callipers only 9% of measurements are within 25% of the measured excised tumor mass compared with 60% using our 3D scanning method. Machine learning will drive further improvements. Animal handling is minimized as tumors can be measured in under 5 s and sedation is not required, an advantage over alternative calliper replacement technologies. Our results show much promise for reducing the handling and use of mice and decreasing the cost and duration of cancer drug development. This digital method demonstrates better welfare in adherence with the 3Rs and provides greater confidence in when to stop testing and could enable systematic topical symptoms to be recalled and exploited as surrogate endpoints for early diagnosis of undesired effects.

**PS78 Epigenetics and the Inheritance of Acquired Characteristics in Laboratory Animals**

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Much has been written recently regarding the lack of reproducibility between different experiments involving rodents in the laboratory setting. In order to produce consistently meaningful results, animal characteristics must be kept as consistent as possible. However, anyone who has worked with these manipulated species for any length of time can recount instances of purportedly “identical” animals producing widely varying results. We discuss 1 of the chief culprits responsible for this conundrum, epigenetics. In short, while genotype may be the same from 1 animal to another (as in monozygotic human twins or inbred isogenic mouse strains), recent findings have clearly demonstrated the disproportional impact that nongenetic variables, such as environment (both pre-and postpartum), diet (both of the animal and of the animal’s ancestors), and social structure can have on their ultimate phenotype, and thus, their performance in a given experiment. We provide a general primer on epigenetics, introducing those new to the field to its basic concepts, as well as providing further information relevant to the laboratory animal community regarding epigenetic effects on animal research outcomes and the medical, husbandry, and welfare issues associated with these animals.
PS79 An International Crowdsourcing Data Project to Investigate Aggression in Group-housed Laboratory Male Mice

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The UK’s National Centre for the 3Rs (NC3Rs) led a crowdsourcing data project for animal technicians to collect data on the prevalence and potential triggers for aggression in laboratory mice. Aggression in group-housed mice is a serious welfare concern, but so too is housing mice singly. Further understanding of the causes of mouse aggression could have a significant positive welfare impact on a large number of animals worldwide. The crowdsourcing approach allowed data to be collected from multiple institutions and, to our knowledge, is the first time such an approach has been applied to a laboratory animal welfare problem. During a consecutive 4-w period over October and November 2017, technicians observed group-housed, male mice during daily routine cage checks and recorded incidents of aggression-related injury and the actions taken to minimize further aggression. Each facility also completed a questionnaire about their standard husbandry practices and submitted an injury log. Data gathered included mouse characteristics, weaning ages, stocking densities, enrichments, and experimental procedures. Participants continued to observe the cage over a 7-d period to record if the remedial action taken prevented further injury to mice in the cage. In total, 44 facilities from 9 countries participated in the study. Data was collected by 143 animal technicians who developed their skills in data collection and recording of behavior. A total of 788 incidents of aggression-related injuries were reported across a sample population of over 130,000 mice. The mean prevalence of aggression-related incidents reported across facilities was 2.66% of total mice held during the collection period (ranging from 0-48%). The additional information provided was collated and used to identify patterns and potential triggers of mouse aggression. The results will be used to generate a published evidence base to inform and support best practice to minimize aggressive behavior in group-housed, male mice.

PS80 A Comparison of Laboratory Rat Behavior and Welfare in Standard Enriched and Larger Enriched Tower Cage Housing Conditions

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The design of any captive animal housing system should aim to promote welfare by encouraging species-specific behaviors, however, many laboratory animal cages fail to meet the needs of the animals, providing either insufficient space and/or complexity to allow the expression of full behavioral repertoire. Over a 10-wk period we studied 12 groups of 4 to 12-wk-old male Sprague-Dawley rats housed in either standard laboratory rat cages (58cmL, 38cmW, 22cmH) containing a shelter, tunnel, nesting material and aspen chew block, or larger (“tower”) cages (73cmL, 58cmW, 46cmH) with multiple shelters, perches, nesting material and locations, tunnels, and the aspen chew block. Behavioral data were collected from video during periods of relatively high activity in dark phase, with activity, posture, and location compared between the 2 housing systems. Rats in tower cages groomed less ($F_{1,50}=55.80$, $P = <0.001$) than rats in standard cages and were more likely to show tactile social behaviors ($t=2.60$, $P = 0.026$) such as placing a paw on another rat. Rats in tower cages also performed fewer antagonistic behaviors such as a prolonged pin-down of a subordinate rat, without allowing escape, ($t=-2.86$, $P = 0.017$), and fewer bouts of sleep ($F_{1,50}=27.83$, $P = <0.001$) than those in standard cages. The increase in positive affiliative behavior, reduced aggression, and reduced self-directed behavior suggests that rats housed in tower cages have improved welfare compared to rats in standard cages even when both systems feature extensive cage enrichment. All animal studies were ethically reviewed and carried out in accordance with Lincoln University Ethical Review Procedure, and the GSK Policy on the Care, Welfare and Treatment of Animals, which determined that the work did not constitute a procedure under A(SP)A 1986.

Platform Sessions

**PS81 Hyperemic Interdigital Webbing in a Group of *Xenopus laevis***

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Three cohoused adult female South African clawed frogs (*Xenopus laevis*) used for egg harvesting developed acute onset of redness of the interdigital webbing. One wk prior to presentation, a faulty gasket was noted in the recirculating water system and was replaced 2 d later with no evidence of clinical abnormalities observed in the colony. The ammonia, pH, conductivity, and temperature parameters were all within normal range. Physical examination revealed hyperemia and necrosis of the hind limb,
interdigital webbing, erythema of all 4 limbs, depigmentation of the skin, and buoyancy compromise. One frog also had discoloration of the right front limb and had difficulty extending the affected limb. Inspection of the other tanks connected to the recirculating water system, revealed 6 other frogs with minor erythema of their hind limbs. Necropsy of the 3 clinically affected frogs revealed micro and macro bubbles around the webbing of the feet and subcutaneous emphysema in the hind limbs. Culture of the skin was positive for *Aeromonas hydrophilia* for all 3 frogs and *Bacteroides uniformis* was cultured from one of the frogs. The history of compromised recirculating water system and the pathognomonic lesions of gas bubbles in the interdigital webbing and subepidermal regions of the hind limbs in the frogs are diagnostic for gas bubble disease. All other frogs recovered with no additional morbidity or mortality noted in the colony. Gas bubble disease is an acute disease that occurs due to oversaturation of dissolved gases, such as nitrogen and argon into the water, commonly caused by defects in the piping of the aquatic system. The animals in this case presented 5 d after issues in the recirculating water system were repaired suggesting that gas bubble disease can present even after restoration of the facility recirculating water system.

**PS82 Scaly Skin and Lethargy in a Nude Mouse**

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A 13-wk-old male athymic nude mouse (Crl:NU(NCr)-*Foxn1nu*) enrolled in a subcutaneous heterotopic human pancreatic tumor study was placed on report for hyperemia and a severe generalized hyperkeratotic dermatitis. The mouse was otherwise bright, alert, and responsive and in good body condition, but by the next day had become lethargic. Due to the clinical presentation and lethargy, the mouse was euthanized for colony health surveillance. A gross necropsy was performed and was within normal limits except for the diffuse severe hyperkeratosis and mild splenomegaly. The differential diagnosis list included *Corynebacterium bovis*, *Staphylococcus xylosus*, and chemical exposure. Three samples (1 skin swab, 1 oral swab, and feces) were submitted to a commercial laboratory for culture and PCR of *C. bovis*, *S. xylosus*, and *Corynebacterium* spp. (HAC2); all test results were negative. The mouse was then submitted for full histopathologic evaluation. Skin changes included marked acanthosis, hyperkeratosis, and parakeratosis with mild dermal fibrosis and lymphocytic/neutrophilic infiltrate. Few scattered gram-positive coccobacilli were identified within layers of the thickened stratum corneum with more diffuse bacterial colonization on the head. Histopathology suggested infection with
a *Corynebacterium* spp. so dry swabs (head, body) of 3 cage mates and 1 other animal in the experimental cohort were collected and submitted for culture. *Corynebacterium mastitidis* was isolated from all 4 submitted samples. Although *C. mastitidis* was not isolated directly from the affected mouse, it is the most likely etiologic agent based on the histopathology in addition to the high sensitivity of the PCR tests ruling out infection with *C. bovis, S. xylosus,* and *Corynebacterium spp.* (HAC2). *C. mastitidis,* which can be found in cell lines and has been isolated from preputial gland abscesses in mice, should also be considered as a differential diagnosis for generalized hyperkeratotic dermatitis in nude mice.

**PS83 Acute Neurologic Presentation of a Rhesus Macaque (*Macaca mulatta*)**

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A 13- y-old, 12.8 kg Chinese-origin rhesus macaque (*Macaca mulatta*) individually housed indoors since import at 1 y of age presented with acute onset left-sided hemiparesis. The animal had exhibited self-injurious behavior at 7 y of age following experimental amphetamine treatment between 2.5-4 years of age. Cognitive testing batteries during his juvenile and sub-adult periods revealed consistently poor performance, with an average percentile rank of 37.0% across several cognitive tasks and below median performance in each task. Awake physical exam at acute presentation revealed a mentally appropriate attitude, normal visual tracking and menace responses, intact limb withdrawal reflexes, and deep pain present. He exhibited difficulty manipulating and chewing food on the right side of his mouth. Sedated physical exam revealed normal vital signs and mild muscular atrophy of the left limbs. Differential diagnoses at this time included a vascular infarction, neoplasia, trauma, intervertebral disk herniation, fibrocartilaginous embolism, and infection. Cerebrospinal fluid cytology, thoracic and skull radiographs, complete blood count, blood chemistry, coagulation panel, and D-dimers were unremarkable; euthanasia was elected. Necropsy revealed generalized hydrocephalus and microcephaly, a right-sided focal cortical depression with corresponding subdural mineralization, and signs of chronic diffuse cortical degeneration (such as gliosis, Rosenthal fibers, hemosiderin-laden macrophages). Chronic cortical changes secondary to an early-in-life insult, such as bacterial meningitis or trauma, are suspected to be the proximate causes of subnormal intelligence. No clear cause of acute paresis was identified via pre- or postmortem diagnostic procedures; it is possible that the chronic changes noted acutely exceeded the ability of the animal to compensate.

**PS84 A Suspicious Mass in a Lab Rat**
A 1-y-old, male CD rat exposed to an EEG/EMG telemeterized, experimental nerve agent presented with an approximately 5 x 4 cm mass with focal necrosis on the left distal thorax under the subcuticular telemetry device. The animal appeared normal otherwise. Surgery was performed to relocate the implant and excise the mass. The firm, infiltrative mass had multifocal cysts containing serosanguinous fluid. The surgeon elected to relocate the implant and sought advice before excising the mass. One wk later, a focal area of necrosis occurred over the new implant site. A second surgery involved relocating the implant and fully excising the mass. The mass appeared 2 times larger and engulfed the wires and the telemetry device. The rat recovered without complications and survived to the study endpoint. Grossly, the excised mass was 5.5 x 4.0 x 2.5 cm, firm, pale, and multilobulated. Histologically, the mass was densely cellular and composed of neoplastic spindle to strap-like cells forming streams and bundles on a fibrovascular stroma. Neoplastic cells had pale eosinophilic cytoplasm and elongate nuclei with granular chromatin and often blunt, cigar-shaped ends. There was marked anisokaryosis and occasional multinucleated cells. Mitoses averaged 5/HPF. Multifocally, there were extensive areas of necrosis and hemorrhage. With Masson’s trichrome stain, the neoplastic cells stained red. These histologic findings are consistent with leiomyosarcoma. Leiomyosarcomas are malignant neoplasms that belong to a group of soft tissue sarcomas and derive from smooth muscle tissue. They most commonly occur in hollow organs with smooth muscle but can form in any place with smooth muscle including skin (arrector pilae). Foreign-body carcinogenesis from the local inflammation and extensive fibrosis associated with the subcuticular telemetry device is the most likely factor involved in the formation of this rat’s sarcoma. Interestingly, several other implanted rats in this study had extensive fibrosis and occasionally other types of implant-associated sarcomas.

PS85 Abdominal Mass in a Female Baboon

A 15-y-old female baboon on an IACUC-approved protocol was examined for a firm swelling in the left lower quadrant of the abdomen. The animal had no abnormalities noted on prestudy physical examination 7 mo prior, or during study-related
procedures 2 mo prior to the mass being observed. Technical staff performing observations did not note any signs of pain or distress and the animal moved around normally in her enclosure. The palpable mass measured 13cm in diameter. Radiographs demonstrated irregularity of the pelvic bones from the pubic symphysis to the obturator foramen, a flattened left femoral head, and acetabular irregularity. Ultrasound showed a well-defined, firm, echogenic soft tissue mass. On bloodwork the animal was anemic, and urinalysis was normal. A fine needle aspirate was nondiagnostic. Due to the poor prognosis, euthanasia was elected and a complete necropsy was performed. On gross examination, the mass extended from the pelvic symphysis, infiltrated the pelvic bones, and extended along the abdominal wall displacing the abdominal organs laterally with regional adhesion to the urinary bladder. The mass was firm, solid, mottled yellow-tan to dark red, and bulged on cut section. The cortex and medulla of the pelvic bones and the abdominal wall tissues were effaced and replaced by dense sheets of large polygonal cells with interspersed deposits of osteoid and cartilage alternating with areas with dense collagen with streams and bundles of spindle cells and minimal supporting stroma. Areas with large multinucleated polygonal cells had frequent mitotic activity ranging from 0-6 per high powered field. Histologic features were consistent with an osteosarcoma, most likely originating from the pelvic bones. This is an unusual presentation of osteosarcoma.

**PS86 Hypothermia in an Immunosuppressed Yucatan Pig**

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As part of a xenotransplant study, a 7-mo-old castrated male, 33 kg, Yucatan miniature pig received a myocardial infarction, jugular catheter, ECG telemetry implant, and daily amiodarone, an anti-arrhythmic. Two wk after infarction, stem cells were injected at the infarct site, and daily immunosuppressive medications including cyclosporine and methylprednisolone were administered. The pig also received antibiotic prophylaxis with cephalixin and trimethoprim sulfamethoxazole. Two wk after cell injection, routine blood chemistry screening revealed elevated GGT (93 U/L) and AST (100 U/L), and hypercholesterolemia (378 mg/dL). Three wk after cell injection, free T4 levels (1.2 ng/dL) and total T4 (2.1 µg/dL) were elevated with suppression of TSH (0.08 ng/mL), and elevated BUN (61 mg/dL). Known side effects of amiodarone include hepatopathy and hyperthyroidism. After a 50% reduction of the amiodarone dose, AST and thyroid values returned to baseline. Five wk after cell injection the pig was examined for hypothermia, and presented as bright, alert and responsive, euhydrated, with normal respiration, yet was hypothermic (rectal temperature 99.7°F) and mildly tachycardic (130 bpm). Heart failure secondary to
myocardial infarction was a primary differential. However, clinical pathology supported an acute inflammatory insult of infectious etiology, with neutropenia (448/µL) with a left shift and moderate toxic changes, and reactive lymphocytes. Thrombocytopenia (67,000/µL), hypoproteinemia (4.5g/dL) and marked hyperglycemia (artifact of glucose-containing catheter lock solution) were also noted. Due to progressive hypothermia and inappetance, euthanasia and necropsy were elected. Pleural effusion, pulmonary edema, and hemorrhages on the serosal surface of the small intestine were noted grossly. Histopathologically, there were cytomegalic cells with intranuclear inclusion bodies in the lungs and liver, consistent with cytomegalovirus. Additional findings included bacterial pneumonia, severe hemorrhagic nephritis, serosal hemorrhagic enteritis, and systemic intravascular thrombi. Tapering of immunosuppressive drugs as well as antiviral prophylaxis were successful in preventing opportunistic CMV infection in the remaining pigs from this experimental cohort.

**PS87 Coelomic Distention and Erect Scales in a Zebrafish**

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An adult wildtype zebrafish presented with marked generalized coelomic distention and erect scales. Swimming patterns, buoyancy, and system water quality parameters were within normal limits (temperature, pH, conductivity, ammonia, nitrite, nitrate, alkalinity, and total hardness). The fish was euthanized in buffered MS-222 and submitted for necropsy and histopathology to help elucidate a cause for the coelomic distention. Differentials included renal damage or failure, coelomic cavity mass/tumor, systemic infection(s), and branchial abnormality. At necropsy, approximately 0.3 ml of clear fluid was removed from the coelomic cavity via syringe and needle. The carcass was fixed in Bouins’ fixative. On histopathology, a large, well-demarcated coelomic mass was noted, composed of densely cellular basophilic spindle cells arranged in sheets and whirls within a fibrous stroma. There were also numerous inflammatory cells (lymphocytes and macrophages) present throughout the stroma, and cholesterol clefts were noted within the caudal aspects of the mass. There was local invasion of the mass into surrounding organs, including ovaries, spleen, and liver. Within the spinal cord, a focal microsporidium xenoma with numerous oval shaped spores was observed. Special stains were submitted to further characterize infectious (Fite’s acid fast, gram stain) and neoplastic (pancytokeratin, vimentin) etiologies. Positive staining for epithelial cell marker (pancytokeratin) was noted in the mass, and a mild, diffuse, systemic, bacterial infection (gram-negative bacteria) was noted in most tissues outside of the mass, including liver, spleen, and kidney. The
histologic appearance, staining, and location of the mass were most consistent with a peripheral nerve tumor. Zebrafish are more predisposed to developing neoplasia of the nerve sheath of the peripheral and cranial nerves compared to mammals. The bacterial infection noted is most likely secondary to the primary neoplastic mass and may be associated with immunosuppression secondary to its chronicity. Microsporidium spores within the spinal cord were consistent with \textit{Pseudoloma neurophilia}.

**PS88 Rash and Lethargy in Castrated Male Miniature Yucatan Pig**

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A 9-mo-old castrated male miniature Yucatan pig presented for acute onset dry, nonpruritic nodular rash over the hind end and dorsum. The pig was on a xenotransplant study, and had received daily oral immunosuppressives (cyclosporine, methylprednisolone) and prophylactic antibiotics (cephalexin) 1 mo prior to presentation. The pig was otherwise normal on initial exam. Over the next the 3 d it became progressively lethargic and inappetant, and was administered oxytetracycline IM. Seven d after initial presentation, the pig was euthanized for endpoint. The major gross findings in addition to the dermal lesions included mildly enlarged, pale kidneys with cortical petechia and multifocal to coalescing; multifocal moderate chronic-active pneumonia; focal mild granulomatous hepatitis; and mild mesenteric lymphadenopathy. Key histopathologic findings included systemic segmental necrotizing vasculitis predominantly in the kidney and skin accompanied by hemorrhage and necrosis. Additional findings included focal lymphoblastic lymphoma in the liver (consistent with reports of cyclosporine-induced lymphoma), and severe mixed bacterial pneumonia with abscessation. The clinical signs and lesions noted in this case are consistent with porcine dermatitis and nephropathy syndrome (PDNS), which is commonly associated with porcine circovirus 2 (PCV2) and other infectious and noninfectious causes, including bacteria and drugs. The underlying pathogenesis of PDNS is still ill-defined, yet there is evidence for an immune-mediated component. Lymphoma has been reported in immunosuppressed mini pigs leading to their proposal as a model of posttransplantation lymphoproliferative disease. This case demonstrates the impact of immunosuppression in mini pigs and has management implications for future studies.

**Platform Sessions**
**PS89 Circulation of Multidrug-resistant *Campylobacter* Species in Nonhuman Rhesus Monkeys (*Macaca mulatta*)**

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Antimicrobial resistance (AMR) is an ongoing public health issue as antimicrobial usage for human and in animal production continues. As one of the important zoonotic agents, *Campylobacter* causes Campylobacteriosis, the most common foodborne disease worldwide. Every year over 1.3 million people in the United States are affected with *Campylobacter*. Two *Campylobacter* species, *C. jejuni* subsp. *jejuni* and *C. coli*, are major zoonotic pathogens causing gastroenteritis worldwide. Nonhuman primates are also susceptible to *Campylobacter* infections. A total of 155 rhesus monkeys (*Macaca mulatta*) were screened and 14 bacterial isolates were preliminarily identified as *Campylobacter* species based on morphological characteristics using gram-staining, scanning electron microscopy (SEM), and biochemical assays. Further speciation was performed to differentiate *Campylobacter* isolates at a species level using multiple biochemical and molecular tools. However, because *Campylobacter* isolates are biochemically inert and share a high level of DNA sequence identity, automated biochemical identification system (Vitek2C), fatty-acid profile-based identification system (MIDI), and 16S rDNA sequencing method failed to differentiate *C. jejuni* subsp. *jejuni* and *C. coli*. In contrast, a MALDI-TOF-based identification system was successfully able to differentiate the 14 *Campylobacter* isolates. The subsequent whole genome sequence revealed the Biotyper system correctly identified them at the species level. Of the 14 isolates, 7 isolates (50%) were identified as *C. coli*, 6 (43%) as *C. jejuni* subsp. *jejuni*, and 1 (7%) as *C. fetus* subsp. *vernerealis*, respectively. In addition to intrinsic antimicrobial resistance, 10 (71%) were ciprofloxacin-resistant and/or tetracycline-resistant, 7 (50%) were ciprofloxacin-resistant and tetracycline-resistant, 2 (14%) were ciprofloxacin-resistant, and 1 (7%) was tetracycline-resistant. Our findings indicate the necessity of active surveillance programs to early detect and correctly identify circulating multidrug-resistant *C. jejuni* subsp. *jejuni* and *C. coli* in nonhuman rhesus monkeys to protect research and animal care professionals.

**PS90 Gentle Handling Benefits Animal Welfare without Disturbing Gut Microbiota in a Rat Model of Alzheimer's Disease**

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Gentle handling and socialization of research animals are important for improving animal welfare. Rats, one of the most commonly used research animals in the United States, have been shown to respond positively to gentle handling and “tickling” by humans, which simulates play behavior. This refinement decreases anxiety-like behavior and makes these animals easier to handle. However, handling could affect the gut microbiota (GM), an important factor in research reproducibility that can be influenced by a variety of environmental factors. F344-AD rats, a model of Alzheimer’s disease, startle easily and can be difficult to work with. Their GM also differs from their wild-type littermates and may be involved in disease pathogenesis. We set out to determine whether routine gentle handling of this model would 1) increase positive interactions with the handler, and 2) affect the GM composition. Transgenic and wild-type rats were allocated to handling groups that received daily gentle handling for 3 wk, or control groups that were only handled during cage changes. Fecal and cecal samples were collected at the end of the study and bacterial populations were characterized via targeted 16S rRNA sequencing. Video testing of rats’ response to handling was conducted at the end of the study. As anticipated, differences in cecal GM were noted between transgenic and wild-type rats (P < 0.05). However, no significant differences were seen in GM between handling groups. Rats in handled groups voluntarily spent more time with the handler and exhibited more rearing behavior, indicating increased comfort with their environment (P < 0.05). These results support the implementation of a handling program to improve the welfare of rats at our institution without risking major alterations in GM, and the role of GM in the pathogenesis of Alzheimer’s disease remains worthy of further study.

PS91 Effect of Pelleting, Irradiation and Autoclaving on Mouse Parvovirus and Mouse Norovirus Infectivity In Rodent Feed

GLAS: Yes
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Mouse norovirus (MNV) and mouse parvovirus (MPV) are among the most common adventitious viral infections in laboratory mice and emerge in barrier facilities despite rigorous biosecurity programs. Extensive research has been done on pathogenesis, monitoring, and eradication of the virus but very few have evaluated the source of viral entry into facilities. Some have implicated nonirradiated feed as a source of
MPV in rodent facilities but none have conclusively documented viral particles in the feed. We hypothesize that both viruses can resist the pelleting process but not subsequent irradiation or autoclaving, thus revealing a potential source of outbreaks in rodent facilities. To test this hypothesis, we contaminated powdered feed with 10-fold increasing concentrations of MNV and MPV and fed it to both Swiss Webster (SW) and C57BL/6 (B6) mice to determine a ‘powdered ID$_{50}$’ based on seroconversion over a 28-d period. We repeated the experiment using powdered feed contaminated with 10-fold multiples of the powdered ID$_{50}$ which was subsequently pelleted and determined a pelleted ID$_{50}$. We finally looked at the effect of irradiation and autoclaving on contaminated pellets using the same experimental design. The powdered ID$_{50}$ was relatively low and identical in both mouse strains (2.51X10$^2$ pfu) for MNV but higher in B6 (3.2X10$^6$ cp) than SW (2.5X10$^4$ cp) for MPV. As hypothesized, mice were effectively infected by contaminated rodent feed despite the pelleting process. Indeed, pelleting resulted in a 1-2 log increase in ID$_{50}$ in both strains for MNV and MPV. On the other hand, irradiation and autoclaving effectively prevented seroconversion of mice exposed to high doses of MNV contaminated pellets while 1 mouse seroconverted at the highest dose for MPV. These data suggest that conditions reproducing the pelleting process for rodent chow does not inactivate MNV and MPV and that nonirradiated rodent chow might be a source of viral outbreaks. On the other hand, autoclaving and irradiation of the feed mitigate most of the risks of viral contamination.

**PS92 Comparison of Antibody Titer and Seroconversion Kinetics of Outbred Heterozygous Nude and Swiss Dirty Bedding Sentinels to Murine Norovirus**

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The production of outbred athymic nude mice requires breeding heterozygous nude females with homozygous nude males. This breeding scheme results in 50% heterozygous nude offspring that do not have a common use in research. An alternative use for these excess mice is as dirty bedding sentinels, as they are outbred mice that are reared in isolators with a similar health status as some outbred Swiss mice such as the Crl:CD1(ICR)-Elite (CD1-E). Heterozygous nude mice have a thymus, but there are reports of decreased thymic size and decreased bone marrow stem cells compared to control background strains suggesting that they might not be immunologically normal. The aim of this study was to compare the antibody titer and seroconversion kinetics of heterozygous nude Crl:NU(Ncr)$^{\text{Foxn1nu/+}}$ (Het-nude) and CD1-E dirty bedding sentinels to murine norovirus (MNV). Sixteen Het-nude and 16
CD1-E female dirty bedding sentinels were exposed to 100% dirty bedding from MNV positive colonies every 1 or 2 wk (depending on housing location) during the quarter. Blood was collected for serology at 3 and 9 wk post dirty bedding exposure, and at the end of the quarter (14-19 wk post dirty bedding exposure). There was no significant difference between antibody titers to murine norovirus between Het-nude and CD1-E mice. There was a significant relationship between weeks of exposure and titer levels ($P < 0.001$) with an increase in titer over the testing time period. At 3 wk post exposure, only 21% of mice seroconverted, at 9 wk 75% of mice seroconverted, and by the end of study 100% seroconverted. This study demonstrates the possible utility of Het-nude mice as dirty bedding sentinels as they have an equivalent antibody response to MNV as CD1-E mice. In situations where dirty bedding sentinels may be utilized for 9 wk or less (e.g. quarantine), the addition of fecal PCR or direct colony testing may be necessary to increase MNV detection rates.

**PS93 Comparing Shedding Profiles and Detection of Corynebacterium bovis in Immunocompetent and Immunocompromised Mice**

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*Corynebacterium bovis* (*C. bovis*) is a gram-positive bacterium that causes hyperkeratotic dermatitis in immunocompromised mice, negatively impacting both animal welfare and research outcomes. Facility outbreaks are difficult to control and eradicate due to environmental persistence and fomite transmission. Although immunocompetent mice do not show clinical signs of *C. bovis* infection, it is unknown whether immunocompetent mice become subclinically infected and shed infectious material. To investigate the bacterial loads of *C. bovis* in immunocompetent and immunocompromised mice, we co-housed C57BL/6NCrl (B6) (n=10), nude (Crl:NU(NCr)-*Foxn1*<sup>nu</sup>) (n=5), and NOD.Cg-Prkdc<sup>scid</sup>Il2rg<sup>tm1Wjl</sup>/SzJ (NSG) (n=5) mice with a clinically infected NSG mouse overnight (day 0). Skin was swabbed on days 3, 5, 7, 10, 14, 21, 28, and 35, and samples were processed for quantitative polymerase chain reaction (qPCR) analysis. To compare detection sensitivity of different sampling sites, buccal mucosa was also swabbed in nude and NSG mice. At the observed timepoints, B6 mice did not exhibit quantities detected by qPCR above noise level, similar to negative controls. Nude mice exhibited bacterial shedding at the earliest measured time point (day 3), continued to rise through day 7, and plateaued at day 10. While NSG mice also exhibited bacterial shedding at day 3, bacterial load continued to increase throughout the study. Skin swabs and buccal swabs were
comparable in sensitivity in both nude and NSG mice. Our results suggest that B6 mice do not actively amplify *C. bovis*. These data provide information for management decisions and diagnostic testing during a *C. bovis* outbreak.

**PS94 Assessing Genotypic Differences between Isolates of the Opportunistic Pathogen *Corynebacterium bovis* Obtained from Various Hosts**

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*Corynebacterium bovis* is an opportunistic bacterial pathogen that has been shown to cause eye and prosthetic joint infection and abscesses in humans, mastitis in dairy cattle, and skin disease in immunocompromised laboratory rodents. At present, little is known about the genetic characteristics and genomic diversity of *C. bovis* as only 1 draft genome has been sequenced. The aim of this study was to sequence, characterize, and compare the genome of *C. bovis* isolates obtained from different host species and, with respect to murine isolates, different geographical locations and time points. Whole genomic sequencing was conducted for 20 *C. bovis* isolates (6 human, 4 bovine, 1 rat, and 10 mice origin). Sequences were analyzed using various comparative analysis tools. Sequencing generated high-quality scaffolds with an average size of 2.53 Mbp and the number of coding DNA sequences (2,174) was similar among all isolates. A neighbor-joining tree for the *Corynebacterium* genus revealed *C. falsenii* as the genetically closest species to *C. bovis*. Interestingly, genome relatedness indices showed that isolates were grouped according to the pathogen's host with human and bovine isolates clustering together and the rodent isolates forming a separate group. Furthermore, the average number of putative genomic islands and virulence factors were significantly higher in rodent isolates compared to the human/bovine isolates. The *C. bovis* pan-genome (total number of nonredundant genes) contained 3,067 genes and of these 1,354 were core genes (genes shared by all isolates). The core genome showed a large number of genes related to metabolism and information storage and processing. However, the highest proportion of genes were classified as function unknown or unclassified and a large number of
virulence factors were only classified as toxins, highlighting the need to characterize proteins with unknown functions to shed light on bacterial pathogenicity. In conclusion, the genomic diversity of *C. bovis* was greater than previously expected with human/bovine isolates and rodent isolates forming 2 distinct clades with different pathogenicity characteristics.

**PS95 Helicobacter saguini Causes Multigenerational Inflammatory Bowel Disease in C57/129 IL-10−/− Mice**

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Inflammatory bowel disease (IBD) is an idiopathic disease in which family history is a significant risk factor. Cotton-top tamarins (CTTs) develop multigenerational chronic IBD that resembles human IBD. We isolated a novel *Helicobacter* species, *H. saguini*, from CTTs with chronic colitis. As *Helicobacter* species are associated with intestinal inflammatory diseases, we hypothesized *H. saguini* infection has the pathogenic potential to induce IBD. IL-10−/− specific-pathogen free (SPF) mice orally inoculated were not colonized with *H. saguini*; however, *H. saguini* mono-infection in germfree IL-10−/− mice developed IBD. Using this model, we found monoinfection of *H. saguini* could naturally transmit and colonize 4 sequential generations (F1–F4) and result in significant inflammatory lesions in the large intestine. Fluorescent in situ hybridization confirmed *H. saguini* persistently colonized the mucosal surface after 40 wk of infection. Additionally, immunohistochemistry for γ-H2AX, a histone marker for DNA damage, was significantly higher in the cecum of infected mice than age-matched controls. Representative isolates from F2-4 generations cultured for whole-genome sequencing analysis revealed host- and generational-dependent increases in single nucleotide polymorphisms in genes responsible for environmental signaling, suggesting *H. saguini* underwent genetic adaptions to a murine host during multigenerational infection. SPF IL-10−/− mice (9 controls, 10 infected, equal genders) were then orally inoculated with the *H. saguini* F4 isolate to test if a mouse-adapted strain could colonize conventional mice. *H. saguini* was detected by PCR of fecal samples at 4wpi in infected mice, but not sham-dosed controls. By 10wpi, 3/10 infected male mice developed rectal prolapse. Histology of the colon revealed moderately severe colitis with mild epithelial hyperplasia and dysplasia in 5/10 infected mice (4 males, 1 female). Controls appeared clinically and histologically normal. Together, our findings indicated that *H. saguini* mono-infection persistently colonized and induced multigenerational IBD in germfree IL-10−/− mice. Overall, this data provides evidence that specific microbial infections can play a role in the
etiology and pathogenesis of IBD in CTTs and humans.

**PS96 Murine Astrovirus 2: A Novel Virus Infecting Laboratory Mice**

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We previously reported on the detection of an unknown virus in sentinel mice in a research mouse colony during routine colony health monitoring. Multiple soiled bedding sentinels in 4 adjacent rooms apparently seroconverted to MTLV on a multiplex immunofluorescence assay (MFIA) using a novel antigen produced in a murine AKR T cell line. The MTLV assay antigen had previously been obtained from thymocytes harvested from MTLV-infected neonatal mice. Sero-conversion was also observed in these mice when their sera was used in an IFA employing the uninfected AKR T cell line. The cell line was subsequently found to be positive by Mouse Antibody Production test using the same MFIA. Using metagenomics, we identified a novel murine astrovirus in feces from Swiss Webster mice (Tac:SW), placed as sentinels, which had recently seroconverted in the novel MFIA. The astrovirus was genetically highly divergent from MuAstV1 commonly present in research mice, yet closely related to viruses isolated from feral Norwegian and Sikham rats in China. Using a PCR assay developed to detect both the Chinese rat and our mouse astroviruses, the T cell line was confirmed to be infected with a closely related astrovirus. Interestingly, an astrovirus similar (89% genome identity) to those found in our colony and the cell line was recently identified in feral mice in New York City. The PCR assay was subsequently used to implement a test and cull eradication plan in our research colony. We focus on 2 key issues related to this novel astrovirus: how a virus contaminated cell line and research mice serendipitously led to the identification of a new virus and how the virus was eradicated from the research colony.

**PS97 MagPlex MFIA: A Next Generation Multiplexed Fluorometric Immunoassay for Serodiagnosis of Rodent Infectious Diseases**

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Multiplex immunoassays including multiplexed fluorometric immunoassay (MFIA) based on Luminex polystyrene (PS) beads have been in use for more than 15 y for routine serosurveillance of laboratory rodents. A new MFIA using the next generation magnetic MagPlex microspheres was developed. MagPlex beads have several advantages over PS beads, including no pre-filtration of samples, no leaky expensive filter plates, and improved washing efficiency. MagPlex is quick and easy to separate from solution using a magnetic separator. Antigens for several common infectious agents in lab mice and rats, including mouse parvovirus, mouse hepatitis virus, adenovirus, CAR bacillus, *C. piliforme*, *E. cuniculi*, lymphocytic choriomeningitis virus, pneumonia virus of mice, reovirus-3, rotavirus-A, and Sendai virus were part of the 33- and 28-member mouse and rat MFIA bead panels. Whole virus or purified recombinant antigens were individually coupled to different color-coded bead sets. In addition, several system and sample suitability controls including tissue control beads to determine the sample related nonspecific antibody binding, species-specific IgG and anti-IgG beads, were added to respective panels to validate individual runs of the MFIA. Efficacy of this next generation MagPlex MFIA was compared to PS MFIA in a validation study using 16 known positive sera from naturally or experimentally infected rodents (mice and rats each) for 1 or more of the above mentioned infectious agents. A similar number, 16 known negative sera for each species were used from specific pathogen free colonies. All samples were tested by 2 different technicians on 3 different days for a total of 6 runs. A total of more than 6,000 assays were performed and analytical performance of the rodent MagPlex MFIA assay including selectivity and limit of detection was found to be comparable to or better than those obtained by PS MFIA. Overall diagnostic sensitivity of rodent MagPlex MFIA was >97% compared to >98% for PS MFIA. Diagnostic specificity of both MagPlex and PS MFIA were nearly 100%, suggesting that MagPlex MFIA is an acceptable alternative assay for serodiagnosis of adventitious infectious agents of laboratory rodents.

**PS98 Evaluation of Exhaust Air Dust Testing Using an Inline Collection Device on an Individually Ventilated Cage Rack without Cage-level Air Filtration**

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Exhaust air dust (EAD) PCR testing is a sensitive tool in screening for rodent infectious agents on individually ventilated cage (IVC) racks. Previous reports for EAD testing using an inline filter for this rack type proved the detection of MNV. Our study demonstrates the ability of this inline filter, as well as pooled swabs IVC rack and air handling unit (AHU), to detect many agents including viruses, bacteria,
parasites, and protozoa. We compared detection by EAD sampling using the inline filter and pooled IVC/AHU swabs to traditional soiled bedding sentinel testing. Inline filters were placed in the exhaust plenum just prior to the AHU and in front of the prefilter within the AHU. Pooled swabs were collected from plenum, exhaust hose, prefilter, and stainless steel drawer in the AHU. Baseline swab testing by PCR prior to the start of the study demonstrated the absence of rodent pathogen nucleic acid on the rack and AHU. Pet shop mice simulating a 15% prevalence were used to provide pathogen nucleic acid. Testing was performed at monthly intervals for 3 mo. EAD samples were tested by PCR only while soiled bedding sentinels, contact sentinels, and control mice were evaluated by PCR, serology, pathology, parasitology, and bacteriology. A total of 23 agents, including viruses, bacteria, protozoa, fur mites, and pinworms were detected in the pet shop mice upon arrival using PCR testing of antemortem sample types. K virus, MCMV, *Giardia, Campylobacter*, and *CAR bacillus* were identified in the pet shop mice, but were not transmitted to sentinels nor were they detected by EAD sampling. In general, more infectious agents were detected by EAD than by soiled bedding sentinel testing. Agent detection rates for bedding sentinels were 33% for traditional testing and 35% for PCR testing. Combined pooled swabs of the rack and AHU detected 74% of the infectious agents, while overall the inline collection devices detected 70% of the agents. Mouse adenovirus, Rotavirus, *M. pulmonis, P. pneumotropica, S. moniliformis, Cryptosporidium, S. muris*, and *Tritrichomonas* were detected by EAD only. This study supports that EAD testing using either an inline collection device or combined pooled rack/AHU swabs is a viable alternative to traditional sentinel monitoring.

**PS99 Effectiveness of Aerosolized Hydrogen Peroxide in Simultaneous Decontamination of a Laboratory and a Biological Safety Cabinet**

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The use of aerosolized hydrogen peroxide (AHP) in laboratory decontamination is becoming prevalent due to a need for safe, complete surface disinfection. AHP is a proven method of spore inactivation within sealed rooms by combining liquid and vapor phases. Achieving similar outcomes within the plenums and filters of a biological safety cabinet while simultaneously treating the laboratory would provide a safe means of comprehensive decontamination. Operational biosafety cabinets filter out 99.99% of aerosols suggesting any type of aerosol decontamination may be compromised, thus the efficacy of AHP in simultaneous treatment of a cabinet and laboratory was studied. A 3000-cubic foot lab was sealed and equipped with an AHP
generation system and biosafety cabinet. The generator controlled the injection of 7% hydrogen peroxide and pulse phases. Three decontamination times and 6 repeatable treatments were tested. The cabinet operated at normal, reduced, and no flow conditions. In each test, vapor monitors and a minimum of 30 *Geobacillus stearothermophilus* biological indicators (BIs) ≥ 1 x 10^6 were placed in critical locations in the laboratory and cabinet. A gaseous phase resulted from the cabinet’s internally re-circulated airstream that exhausted back into the laboratory. Gaseous concentration depended on the evaporation rate of the lab aerosol and liquid phase collected on the cabinet’s filters. Photographs demonstrated a reduced aerosol concentration in the lab when the cabinet was on. While the biosafety cabinet was operational, all 184 BIs were successfully inactivated, signifying spore sterilization. The only exceptions across all 6 tests were 9 BIs in the cabinet’s internal plenums when it was off. In conclusion, perceived efficacy challenges with filters engaged were proven unfounded. Biosafety cabinet operation, while reducing aerosol concentration, had no significant effect on gaseous concentration and did not compromise decontamination. Outcomes demonstrated AHP is a viable solution to simultaneous decontamination of a laboratory and its contents, including typically challenging areas within the internal plenums and filters of a biosafety cabinet, provided the cabinet is operational.

**Platform Sessions**

**PS109 Assessing the Efficacy of Intranasal Midazolam in Sedation Of Young Pigs (*Sus Scrofa*)**

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Due to their energetic nature, young pigs in research often require anesthesia for noninvasive procedures such as echocardiography and bandage changes. However, drugs currently available for anesthesia in swine are often detrimental to the cardiovascular system and are not ideal for use in animals involved in cardiopulmonary research. Midazolam is a fast-acting benzodiazepine frequently used in veterinary medicine that causes muscle relaxation, hypnosis, and has little cardiovascular effects. The intranasal (IN) administration of midazolam is often used to sedate human pediatric patients for noninvasive procedures, eliminating injection-associated stress in the recipients. The purpose of this study was to evaluate the efficacy of IN midazolam in young pigs for noninvasive procedures. Transgenic
domestic piglets (*Sus scrofa*) with targeted disruptions of the RBM20 gene were selected for this study. Thirteen piglets, between 7 and 9 d of age, were separated into 3 IN midazolam dose groups, 0.25 mg/kg (n=4), 0.50 mg/kg (n=5), 1.0 mg/kg (n=4). To assess sedation, we adapted a sedation scoring system previously used in dogs. Adequate sedation (AS) was defined as tolerance of a nonnoxious stimulus (application of moderate pressure to the thorax for 30 s to mimic an echocardiogram) while in a sling at any time during the study. Using a 1-sided trend test, we found that 10/13 pigs reached AS (50%, 80%, and 100% in the 0.25 mg/kg, 0.5 mg/kg, and 1.0 mg/kg groups, respectively). Regardless of dose, AS was affected by ease of IN administration, with AS reached in 100%, 60%, and 50% of pigs that, following administration, did not sneeze, sneezed slightly, or sneezed significantly, respectively. These results suggest that IN midazolam at 1.0 mg/kg can be reliably used to sedate young pigs for nonnoxious procedures, and ease of administration affects adequate sedation in pigs of this age group.

**PS110 Ultrasonographic Anatomy of the African Clawed Frog (*Xenopus laevis*) and Sexing of Juveniles**

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The African clawed frog (*Xenopus laevis*) has been an invaluable research tool for developmental biology, electrophysiology, biochemistry, and neurobiology for almost a century, yet literature on amphibian medicine and diagnostics is underrepresented. Because of this, disease in laboratory frogs is not well understood, nor are clinicians prepared to utilize and interpret diagnostics, such as imaging. Additionally, *X. laevis* is a sexually dimorphic species, however, phenotypic signs of sexual maturation can take 1 to 2 y to develop. The ability to sex juvenile frogs has the potential to improve colony management and reduce the number of animals used in research. Ultrasonography provides an easily accessible, non-invasive platform useful for both research and diagnostics, and has recently become more popular in aquatic medicine. Traveling easily through water and the external slime coat of the frog, ultrasound waves allow for easy identification of the majority of *X. laevis* coelomic organs. Needing only light anesthesia with tricaine methanesulfonate (MS-222), or gentle manual restraint, the normal size, echogenicity, and echotexture of heart, lungs, major arteries and veins, liver, gallbladder, stomach, gastrointestinal tract, kidneys, urinary bladder, and gonads were recorded in 4 adult males and 4 adult females. Half of the females were also imaged before and after hormonally induced ovulation, a non-invasive technique commonly used for egg harvesting in biomedical research.
Electronic calipers were used to measure oocyte diameter and results reflected the asynchrony of oogenesis in this species. Furthermore, juvenile animals (15-22g) not yet old enough to be sexed by phenotypic characteristics, were imaged with ultrasound before being euthanized and necropsied to correlate the images with sex. This is the first report documenting baseline ultrasonographic anatomy of adult male and female X. laevis, with assessment of oocytes before and after ovulation, providing clinically relevant data for veterinarians and X. laevis investigators. These results also demonstrate the potential use of ultrasonography as an early and non-invasive way to sex juvenile X. laevis.

**PS111 Ocular Pharmacokinetics of Insulin-loaded, Thermoresponsive, Biodegradable Nanogels for the Treatment of Diabetic Retinopathy**

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Diabetic retinopathy (DR) affects approximately one-third of the estimated 422 million people with diabetes mellitus and is a leading cause of vision loss worldwide. DR is associated with retinal neurovascular degeneration, and studies indicate that systemic, subconjunctival, or intravitreal injection of insulin may reduce the risk of DR onset and progression. However, insulin has a short half-life, and the risk of hypoglycemia limits patients’ ability to take enough insulin systemically to prevent and treat DR. The purpose of this project is to develop thermoresponsive and biodegradable nanogels for sustained release of insulin to the retina after subconjunctival injection to treat DR. Thermoresponsive and biodegradable nanogels containing N-isopropylacrylamide, Dextran-lactate-2-hydroxyethyl methacrylate, and acrylic acid were synthesized by surfactant-free emulsion polymerization. Insulin at 15 wt% was loaded into the nanogels during nanogel synthesis. The nanoparticles were characterized with respect to size, zeta potential, yield, and insulin loading efficiency and capacity. The in vitro release kinetics of insulin from the nanogels over two weeks were studied by using dialysis method with the released insulin quantified by ultra performance liquid chromatography. Fluorescent-labeled nanogels alone and insulin-loaded nanogels were subconjunctivally injected in the left eyes of Sprague Dawley (SD) rats (5/group) at 10 mg/ml and 20 mg/mL. The ocular pharmacokinetics of the nanogels and insulin released from the nanogels at 1 and 7 d post-injection were investigated by using fluorescent reader and liquid chromatography-mass spectrometry, respectively. The results showed that the yield of the nanogel synthesis was >68%, and the sizes of the nanogels were 100-200 nm. The nanogels could load
insulin with extremely high loading efficiency of >98%. The nanogels were able to sustain the release of insulin in vitro for at least 7 d and cross the sclera, choroid, and retinal pigment epithelium to reach the retina after subconjunctival injection in SD rats. The insulin-loaded nanogels showed promise for the future of effective therapies for the prevention and treatment of DR.

**PS112 Genotoxic E. coli Strains Encoding Colibactin, Cytolethal Distending Toxin, and Cytotoxic Necrotizing Factor Colonize Laboratory Rats**

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While many *E. coli* strains are considered commensals in mammals, strains encoding the cyclomodulin genotoxins are associated with clinical and subclinical disease in the urogenital and gastrointestinal tracts, meningitis, and inflammatory disorders. These genotoxins include the polyketide synthase (pks) pathogenicity island, cytolethal distending toxin (cdt), and hemolysin-associated cytotoxic necrotizing factor (cnf). These *E. coli* strains are not excluded from rodents housed under specific-pathogen free (SPF) conditions in academic or vendor facilities. Our recent publications have noted a high incidence of pks+ *E. coli* colonization in mice and clinical disease associated with pks+ *E. coli* in immunocompromised mice. The aim of this study was to isolate and characterize genotoxin-encoding *E. coli* from laboratory rats obtained from 4 different academic institutions and 3 different vendors. Sixty-nine distinct *E. coli* were cultured from fecal, rectal swab, or extraintestinal regions of 52 different rats and biochemically characterized. PCR for pks genes, cdt genes, cnf genes, and phylogenetic group was performed on all 69 isolates. Forty-five of 69 isolates (65%) were positive for pks, 20/69 (29%) were positive for cdt, and 4/69 (6%) were positive for cnf. Pks was the sole genotoxin identified in 21 of 45 pks+ isolates (47%), whereas cdt or cnf was also present in the remaining 24 isolates (55%). Cdt or cnf was never present together or without pks. All genotoxin-associated strains were members of pathogen-associated phylogroup B2. Select *E. coli* isolates were characterized by *HeLa* cell in vitro cytotoxicity assays, serotyped, and whole genome sequenced by Illumina MiSeq. All clb, cdt, and cnf-encoding isolates induced megalocytosis in *HeLa* cells. Serotypes corresponded with vendor origin and cyclomodulin composition, with the cnf+ serotype representing a known human uropathogen. Whole genome sequencing confirmed the presence of complete pks, cdt, and hemolysin-cnf pathogenicity islands. These findings indicate that genotoxin-encoding *E. coli* colonize laboratory rats from multiple commercial vendors and academic institutions and suggest the potential to contribute to clinical disease and
introduce confounding variables into experimental rat models.

PS113 Mammary Tumor and Mastectomy Synergistically Promote Chronic Neuroinflammation in a Breast Cancer Survivor Model

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Even after treatment for breast cancer ends, many women experience mental sequelae including depression and anxiety that can last for years. Understanding the cause of these cognitive deficits is essential for developing targeted treatment plans and improving quality of life for breast cancer survivors. Microglial priming results in heightened responses to homeostatic disturbances thereby exacerbating neuroinflammation and neurodegeneration, and offers a potential mechanism for this cognitive dysfunction. This study examined whether mammary gland tumors prime microglia and augment the inflammatory profile and behavior of mice. To test this, we injected nonmetastatic mammary tumor cells (67NR) orthotopically into BALB/c mice, allowed them to grow for 16 d, and then removed the tumors via mastectomy. Following a 14-d surgical recovery, we challenged the mice with LPS, and then evaluated central and peripheral inflammation, anxiety, and depressive-like behavior (n=10-15/group). Open field test assessed anxiety, whereas forced swim and sucrose anhedonia tests evaluated depressive-like behavior. Here we show that after 16 d of tumor growth (the time of mastectomy surgery), there were no differences in inflammatory markers in the hippocampus or serum apart from increased serum CXCL1 concentrations in tumor-bearing compared to non-tumor-bearing mice. Similarly, mastectomy surgery alone did not significantly affect major central or peripheral inflammatory markers. Interestingly, hippocampal mRNA expression of major proinflammatory cytokines IL-1\(\beta\) and TNF\(\alpha\) along with microglial scavenger receptor CD68 was increased following surgical recovery in mastectomy tumor removal animals relative to controls. This means that tumor growth nor surgery alone resulted in a proinflammatory response, but together they led to neuroinflammation persisting past surgical recovery. Nonetheless, after LPS administration there were no exaggerated immune responses or behavioral changes to substantiate microglial priming in the tumor removal group. In summary, these data demonstrate that tumor-bearing together with mastectomy surgery promotes neuroinflammation and microglial activation; however, immune challenge with LPS did not elucidate this inflammation as maladaptive for the host.

PS114 Recurrent Laryngeal Nerve Transection in Mice Results in Translational
Upper Airway Dysfunction

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The recurrent laryngeal nerve (RLN) is responsible for normal vocal fold (VF) movement. The RLN is at risk for iatrogenic injury during anterior neck surgical procedures in human patients, resulting in subsequent VF paralysis that may contribute to swallow, voice, and respiratory dysfunction. Unfortunately, treatment for RLN injury does little to restore function, as no treatments can truly promote regeneration of the injured nerve. Thus, we sought to create a mouse model with translational functional outcomes to further understand spontaneous RLN regeneration and investigate therapeutic interventions. To do so, we performed a ventral neck surgical procedure in 21 C57BL/6J male mice. Mice were divided into 2 groups: unilateral RLN transection (n=11) and sham injury (n=10). Furthermore, mice underwent the following assays to determine upper airway function at multiple time points prior to and following surgical manipulations. Transoral endoscopy was utilized to assess VF motion. Videofluoroscopic Swallow Studies were used to quantify swallow function. Vocal function was assessed using ultrasonic vocalization assays, while whole-body plethysmography was used to assess respiratory function. Results revealed that RLN transection created ipsilateral VF paralysis that did not recover by 12 wk after surgery. Furthermore, there was evidence of significant vocal and respiratory dysfunction in the RLN transection group, but not the sham injury group. However, no significant differences in swallow function were found between the 2 groups. In conclusion, our mouse model of RLN injury provides several outcome measures to increase the translational potential of findings in preclinical animal studies. We aim to utilize this mouse model and our regimen of behavioral assays to assess various treatment options to promote RLN nerve healing.

PS115 The Role of Type II Alveolar Epithelial Cell Injury in Development of Idiopathic Pulmonary Fibrosis in Murine Models

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Idiopathic pulmonary fibrosis (IPF) is a debilitating, progressive, and fatal lung
disease. It is characterized by excessive extracellular matrix deposition in the pulmonary interstitium which blocks gas exchange. IPF affects greater than 5 million people in the world and can be as high as 400 cases per 100,000 people over 65 years old. Lung transplantation remains the only therapeutic option that can prolong survival in patients with IPF. A better understanding of the pathogenesis of IPF in animal models will hasten the development of antifibrotic drugs. Numerous lines of evidence, from our group and others, support the idea that alveolar epithelial cell (AEC) injury and apoptosis is important for the development of fibrosis, but the mechanism by which AEC injury/death promotes fibrosis is unknown. We hypothesize that progressive pulmonary fibrosis is driven by a phenotypic change in alveolar macrophages that is induced after their engulfment of apoptotic AECs, a process termed efferocytosis. Murine AECs were isolated from both wild-type (WT) and SPC-GFP mice, and treated with UV light to induce cellular apoptosis. Coculture of apoptotic AECs with alveolar macrophages showed that macrophages could uptake apoptotic AECs, and then upregulate expression of profibrotic genes such as arginase and TGF-β. In order to understand if fibrosis is mediated by efferocytosis, we repeatedly administered apoptotic bodies derived from type II AECs or a mouse lung epithelial tumor cell line (MLE-12) to the lungs of WT mice and mice which lack CD36, an important efferocytosis receptor. CD36 null mice demonstrated less fibrosis on histology and by hydroxyproline assay of lung tissue. Bronchoalveolar lavage fluid in CD36 null mice contained significantly lower amounts of TGF-β than in WT mice by ELISA. Overall, these studies support a novel mechanism of how AEC injury initiates progressive fibrosis, and reveal CD36 as a potential therapeutic target against fibrosis.

**PS116 Modeling Crohn’s Disease: Identifying Environmental Triggers in a Genetically Susceptible *Atg16l1* Rat Strain**

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Crohn’s disease (CD) is 1 of 2 chronic inflammatory bowel diseases (IBD) that affect the lining of the gastrointestinal (GI) system. CD is a multifactorial disorder caused by a combination of genetic susceptibility and environmental factors. Numerous susceptibility loci have been linked to CD, 1 of which lies in the autophagy-related 16-like 1 (ATG16L1) gene. The mechanism by which the ATG16L1 variant (T300A) causes increased susceptibility to CD is still incompletely understood, and current mouse models harboring this variant do not properly express the clinical inflammation noted in human Crohn’s patients. Our laboratory generated a knock-in rat using
CRISPR-Cas9 technology in a Fischer 344 background strain to determine whether the rat could serve as a more characteristic model of the human Crohn’s phenotype. To validate this rat strain as a model for CD, it was necessary to identify appropriate environmental triggers of disease. Two experimental groups of heterozygous (HET) rats carrying the T300A susceptibility allele and their wildtype (WT) littermates were chronically exposed to 1 of 2 different known environmental triggers of CD: either a low-dose nonsteroidal anti-inflammatory (NSAID; diclofenac, 1.25 mg/kg/PO) or ad libitum Western diet formulated rodent feed. Each group, including a control group, contained 24 animals cohoused by sex and experimental group (mixed WT/HET housing) with equal numbers of sex and genotype per group. We found that HET rats in both the oral NSAID and Western diet groups had increased inflammation and changes to the mucosal epithelium of the ileum and colon like that seen in patients with CD as determined by blinded histopathologic assessment. It was also found that HET rats given oral NSAID had a significantly altered microbiome profile compared to WT rats and heterozygous controls when serial fecal samples were collected under sterile conditions and analyzed by 16S rRNA sequencing against the SILVA database at the OTU level. These results show that, unlike in current mouse models of CD, known environmental triggers of CD can cause histopathology like human CD patients in rats harboring the human ATG16L1 T300A variant.

**PS117 Lytic Enzymes: A Novel Antimicrobial Treatment for Methicillin-resistant *Staphylococcus aureus* in Instrumented Nonhuman Primates**

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Methicillin-resistant *Staphylococcus aureus* (MRSA) can cause fatal human and animal infections. Alternative therapies are needed due to antibiotic resistance. Lytic enzymes encoded by bacteriophages (lysins) and bacteria (bacteriocins) are the source of novel antimicrobial agents as they can degrade bacterial cell wall resulting in hypotonic lysis. A colony of nonhuman primates (*Macaca mulatta*) with permanent intracranial implants showed persistent cutaneous MRSA carriage. We considered whether lytic enzymes are an effective treatment method for topical MRSA colonization. Treatment animals (n=2) received 5 doses every other day of the bacteriocin lysostaphin (5 mg/mL) active against our MRSA isolate in vitro. Control animals (n=2) received buffer. Treated areas included the cranial implant margin (3mL/animal), nostrils, and perirectal area (both as ointment formulation), shown to
be MRSA positive. All animals and their environment underwent a 5-d
decontamination to decrease cross-contamination. During animal decontamination,
the implant margin was cleaned/debrided with sterile saline and the head and face
were wiped with a 2% chlorhexidine solution followed by saline and then dried.
Mouth was rinsed with 3 ml dilute chlorhexidine oral rinse. The animals were bathed
with a 2% chlorhexidine shampoo and dried. The environment was decontaminated
with ACCEL TB. On day1, day 3, and day 5, the animals were placed in new
decontaminated cages (mechanical cage wash with exposure to 180°F and detergent)
after bathing. Based on pilot studies, all animals received clindamycin 10 mg/kg IM to
treat MRSA systemically. Lysostaphin treated areas were swabbed before, during, and
after treatment to determine MRSA colony forming units (CFUs). The CFU count
decreased below detectable levels (100 CFU/mL) in the nostrils and peri-rectal area in
all animals after decontamination and antibiotic treatment. The CFU count from the
cranial implant margin decreased (4-5 log) after decontamination and antibiotic
treatment in all animals. A week after local lysostaphin treatment, MRSA decreased to
below detectable levels (25–125 CFU/mL) in the treatment but not the control group.
Three wk after lysostaphin treatment CFU counts in the cranial implants of all animals
showed an increasing trend suggesting that topical lysostaphin treatment coupled with
decontamination and systemic antibiotics may be effective at decreasing MRSA
colonization but failed to eradicate MRSA leading to increased bacterial colonization.
We suspect this failure was a result of the cranial implant restricting the ability to
administer the bacteriocin into all colonized areas. Nevertheless, lysostaphin shows
promise for treating colonized monkeys topically as long as the sites of colonization
are accessible.

PS118 Water-soluble fenbendazole: A possible alternative to fenbendazole
medicated feed for treatment of pinworm infections (oxyuriasis) in laboratory
rodents.

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Fenbendazole (FBZ) has been extensively studied and used as an anthelmintic
medication for several species of animals, including rodents. Fenbendazole has
demonstrated activity against pinworms, but FBZ has low water solubility (0.9 ug/ml),
and to our knowledge, there are no commercially available water-soluble formulations
that might be used in drinking water for treatment of pinworms. Fenbendazole has
been formulated as a medicated rodent feed with demonstrated success treating
pinworms. More recently, a FBZ oral suspension became available for delivery in
water to use in swine and chickens. Unfortunately, this new product must be mixed fresh daily or requires agitation to prevent precipitation of the active ingredients. This study’s new water-soluble formulation of fenbendazole (FBZ-QS, 1 mg/ml) may provide a cost effective, non-precipitating, liquid alternative to medicated feeds. Our current hypotheses tested FBZ-QS’s palatability in common strains of laboratory rodents. FBZ-QS was tested at several concentrations in tap water: 0 ug/ml (control tap water), 0.00075 ug/ml, 0.0075 ug/ml, 0.075 ug/ml, and 0.75 ug/ml (n = 16 per treatment group) in a cross over design over the course of eight weeks. Four boxes of four mice each were rotated one week at a time through the treatments above, with a washout week in between each FBZ-QS concentration. Body weight and hydration status were monitored daily. Fluid consumption was recorded weekly, including washout weeks. All other husbandry guidelines followed the institution’s standards for rodent care. As hypothesized, FBZ-QS was readily consumed at all concentrations tested, by C3H/HeN mice. No significant differences were detected between control and any other medicated water treatment (214.62 ml/kg/day ± SEM 5.59). At the highest FBZ-QS treatment, mice consumed 158.64 ug/kg/day ± SEM 7.70. No evidence of dehydration was detected and mice gained weight as expected throughout the study. Results for Balb/c mice (Balb/cAnNHsd), Wistar (Hsd:WI), and Sprague-Dawley rats (Hsd:Sprague Dawley) are pending. A positive outcome for this study would provide an alternative and possibly a more cost effective method for treatment of pinworms in rodents.

Platform Sessions

PS100 Clinical Management and Pathologic Findings in Immunosuppressed Yucatan Swine

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We report common pathologic findings and subsequent medical management of a cohort of immunosuppressed Yucatan swine. The 4-8 mo-old, castrated male pigs, were on longterm immunosuppressive regimes (greater than 2 wk) as part of a xenotransplant study. Animals were obtained from a vendor that routinely vaccinates and test negative for the most common porcine pathogens. Pigs were immunosuppressed with an oral regimen of methylprednisone and cyclosporine, as well as a twice monthly IV dose of Abatacept, a T cell modulator, up to endpoint (2-8 wk). Animals were administered IV antibiotics during surgical procedures and were on daily oral prophylactic cepalexin postoperatively. Throughout the study, several
pigs presented clinically with vomiting, inappetence, and diarrhea. These animals were usually managed with fluid therapy, oral rehydration solutions, gastroprotectants, and a variety of antibiotics; however, most animals were poorly responsive to therapy and had progression of clinical signs, so early euthanasia was required. Consistent necropsy findings included wet congested lungs, gastric ulcerations, and renal hemorrhages. Histologically, multiple animals had rampant pneumocystis pneumonia, evidence of bacterial infections, and severe renal hemorrhagic medullary nephritis with variable fibrinohemorrhagic glomerulonephritis and arteriolar lesions. One animal had early histologic changes to the liver consistent with lymphoma. Additionally, at least 1 animal had evidence of porcine circovirus-associated porcine dermatitis and nephropathy syndrome and 2 had severe disseminated porcine cytomegalovirus infection. After histopathologic review of approximately 20 necropsied animals, the prophylactic drug regimen was modified to include an oral antiviral, sulfamethoxazole/trimethoprim, and probiotics. Once these medications were started, reduced pneumocystis burdens were noted histologically, and fewer animals presented with clinical gastrointestinal disease, and to date, all animals have made it to the planned endpoint. The clinical and necropsy findings in this cohort illustrate the power of clinical care informed by pathology and subsequent prophylactic treatment for the management of opportunistic infections in profoundly immunosuppressed pigs.

**PS101 Respiratory Signs in Swine Vaccinated against Porcine Reproductive and Respiratory Syndrome Virus**

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Ten of 14 newly arrived Yorkshire-cross pigs (*Sus scrofa*) (38–80 kg) presented with upper respiratory signs. Clinical signs ranged from sneezing alone to crackles on thoracic auscultation with accompanying lethargy, decreased appetite, and fever. Pigs were experimentally naïve, and had received a modified-live porcine reproductive and respiratory syndrome virus (PRRSV) vaccine administered by the vendor. Differential diagnoses included *Mycoplasma hyopneumoniae*, porcine circovirus-2, swine influenza virus, *Pasteurella multocida*, *Bordetella bronchiseptica*, *Actinobacillus pleuropneumoniae*, porcine respiratory coronavirus, *Salmonella enterica* ser. *Choleraesuis*, *Haemophilus parasuis*, and *Streptococcus suis*. Pigs presenting with decreased appetite and increased expiratory effort received an injection of long-acting tulathromycin 2.5 mg/kg as empiric therapy for primary or secondary bacterial respiratory pathogens. Within a week of arrival at our facility, pigs underwent
terminal surgical procedures. Following euthanasia, a postmortem examination was performed by the veterinary staff for 3 pigs, 2 of which had received tulathromycin. The lungs of all pigs were grossly unremarkable, and lung tissue was sent to a diagnostic laboratory for further testing. The pig who had not received tulathromycin tested PCR positive for *Mycoplasma* spp. All pigs were PCR positive for PRRSV North America strain and PCR negative for swine influenza, porcine circovirus-2, and PRRSV Europe strain. In an attempt to differentiate a positive result due to PRRSV vaccination versus natural infection, histopathology of lung tissue from 2 pigs was performed. Histologic findings were suggestive of natural PRRSV infection, so lung tissue was submitted for DNA sequencing, which revealed PRRSV wild-type virus. PRRSV is an economically devastating virus, causing reproductive disease in gestating gilts and sows and causing respiratory disease in growing pigs, as seen here. This case series underscores the importance of a careful workup and that even when animals are vaccinated against a pathogen, that pathogen should remain a differential until proven otherwise.

PS102 Multiple Cases of Otitis Media in Immunocompromised Mice Linked to *Burkholderia gladioli*

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Over a 9-mo period, a marked increase in the number of mice presenting with a head tilt was observed in numerous holding rooms within 1 of our 3 barrier vivaria. Mice affected represented several immunocompromised strains, including CB17.Cg-PrkdcscidLystbg-J/Crl, NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ, C57Bl/6J-Rag1tm1Mom, and B6.129S6- Rag2tm1FwaN12, and were housed in rooms shared with immunocompetent mice. Nine affected mice were submitted for complete necropsy. Grossly, all mice had tympanic bulla empyema, which was evident histologically as marked supplicative bacterial otitis media. Mice also had hepatitis (7/9) and encephalitis (3/9). Bacteria isolated from the bullae via aerobic culture were identified as *Burkholderia* spp. in 7/9 mice. In the remaining 2/9 mice, intralesional and intracytoplasmic gram-negative rods were seen in the bullae, with *Burkholderia* spp. isolated from hepatic and brain lesions in 1 mouse. A subset of these isolates (6/9) were further speciated by MALDI-TOF as *Burkholderia gladioli*. *B. gladioli* is historically known as a plant pathogen, but is of growing concern as a cause of severe respiratory tract infections in cystic
fibrosis patients, particularly following lung transplantation. To date, only one other account of *B. gladioli* infection in laboratory mice has been reported, when an outbreak of otitis media in a facility housing immunocompromised mice was caused by *Burkholderia gladioli* in 2004. The pathogen was found in oropharyngeal swabs of affected mice and believed to cause otitis via colonization of the Eustachian tube. Following identification of *B. gladioli*, increased surveillance for this bacterium was undertaken in our colonies. *Burkholderia* spp. was subsequently isolated in another barrier from the gastrointestinal tract of a cohort of NOD.Cg-Prkdc<sup>scid</sup>Il2rg<sup>-</sup>im1<sup>Wj</sup> mice presenting with *Clostridoides difficile*-associated diarrhea. This result suggests this bacterium may be more prevalent in laboratory mice than previously known and may be poised to become an emerging opportunistic pathogen in research colonies using immunocompromised mice.

**PS103 Methods of Microbial Surveillance and Aseptic Maintenance to Reduce Contamination of Germ-free Mouse Derivations**


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Embryo transfer (ET) for derivation of germ-free (GF) mice is performed in biosafety cabinets using standard surgical gowning, gloving, and aseptic technique. We discovered that many projects exhibited bacterial growth postderivation during routine weekly microbial surveillance of flexible film isolators used to house the ET recipients. To investigate the stage in the process at which the ET recipients were becoming contaminated, a microbial surveillance program was implemented using daily RODAC plating of gloved hands and various surfaces touched by surgeons and assistants during the ET procedure, as well as ATP sanitation monitoring of surfaces inside the biosafety cabinets. Results of the monitoring showed growth of specific organisms on gloves and surfaces which often matched those identified later as contaminants in animals that underwent surgery the day of the environmental monitoring. Surfaces at high risk for contamination were identified and procedures to optimize asepsis were implemented, such as use of disposable covers for microscope knobs, 70% isopropyl alcohol wipes for disinfection of surfaces, and thorough disinfection of the room, equipment, and biosafety cabinet the week before GF derivations are performed as well as supplemental training of all support staff and surgeons. After implementation of the daily monitoring and procedural changes, the rate of contamination in GF derivations postprocedure was significantly reduced.

**PS104 Immune Mediated Hemolytic Anemia in Humanized NOG Mice**
Sixteen 4-mo-old female hGM-CSF/hIL3-NOG mice were shipped from a vendor to a heightened biosecurity facility after receiving irradiation and bone marrow transplant of human myeloid cells. The mice were kept in sterile housing while participating in a vaccine study. At the age of 7 mo all mice exhibited varying degrees of pale extremities, hypothermia, weight loss, and hunched posture. After a physical exam, supportive care was initiated in the form of a high-calorie diet gel. Empiric medical intervention was avoided to reduce confounding experimental factors. Mouse weights were tracked for 2 wk. At that time 2 mice reached weight loss endpoints, 1 of which received multiple experimental vaccinations and the other received sham vaccinations. These animals were euthanized and submitted for further diagnostics. Differential diagnoses included infection, graft vs host disease (GvHD), anemia secondary to xenographic bone marrow displacement, and graft failure. Noninvasive biologic samples were collected from all mice for a sentinel PCR panel. All 44 murine pathogens tested were negative. Gross necropsy revealed pale livers with multifocal pinpoint hemorrhages and pale kidneys. PCV and CBC revealed a severe macrocytic anemia and lymphopenia. Chemistry was unremarkable with mild hyperalbuminemia, hyperglycemia, and electrolyte imbalances consistent with hemolysis. Tissues were submitted for histopathology which revealed diffuse collections of iron-positive, hemosiderin-laden macrophages and multinucleated giant cells in the lungs and liver. Collections of lymphocytes were noted in lungs, liver, pancreas, and salivary gland interstitium. These findings are characteristic of immune-mediated hemolytic anemia (IMHA) in which macrophages react against MHC1 mismatched hematopoietic tissues. IMHA is a major contributing factor of morbidity in both GvHD and graft rejection. Humanized mice are at risk for IMHA despite extensive measures taken to prevent adverse graft/host reactions and these conditions should always be considered differential diagnoses despite advances in xenograft technologies.

**PS105 Novel Focused Ultrasound Technique to Identify Duodenal Ulceration in Common Marmosets (Callithrix jacchus)**

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Common marmosets (*Callithrix jacchus*) are a new world primate species commonly used in biomedical research with a documented predisposition to a spectrum of gastrointestinal diseases. At our facility, non-invasive methods to evaluate the gastrointestinal tract are limited to radiography with or without the use of intra-oral barium. Recently, several group-housed marmosets developed vomiting and weight loss. Following necropsy confirmation of duodenal ulceration and perforation in several of these animals, a more sensitive noninvasive monitoring technique was desired. In collaboration with veterinary radiologists, a protocol for focused ultrasound assessment of the cranial abdomen of marmosets was developed. The result was a simplified technique that could be easily taught to staff veterinarians and a 6-item checklist for assessment of the peritoneal space, hepatobiliary system, and proximal gastrointestinal tract. This technique was incorporated into biannual health screenings of the marmoset colony, with particular attention paid to any symptomatic animals. A total of 38 animals was evaluated using this technique. Ultrasound findings of proximal duodenal abnormalities ranging from mucosal irregularity to deep ulceration were observed in 6 animals. Of those animals with duodenal ulceration, 1 also had evidence of duodenal perforation at the time of ultrasound examination. Affected animals were treated with antiulcer and antimicrobial therapy or euthanized as appropriate for each individual case. Necropsy confirmed duodenal ulceration and/or perforation in the 2 animals euthanized to date with sonographic evidence of these lesions. Noninvasive diagnosis and early detection are essential for proper management and long-term follow-up of duodenal ulceration and perforation in common marmosets. This focused ultrasound technique is easy to incorporate with routine health assessments of a colony and normal ranges generated in this study will be clinically useful for further studies of gastrointestinal disease in this species. We expect the technique of focused cranial abdominal ultrasonography may be adapted for use in other nonhuman primate species.

**PS106 Clostridioides difficile Typhlocolitis Resulting from Amoxicillin Treatment in Highly Immunocompromised Mice**

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*Clostridioides difficile* is an important opportunistic pathogen affecting both humans and animals. Although its ability to cause naturally occurring disease in mice is
extremely rare, *C. difficile* has been described in a few case reports. Little is known about the clinical significance of this bacteria in highly immunocompromised mice. We investigated an outbreak of diarrhea in NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup>tm1Wjl</sup>/SzJ (NSG) and related strains. Affected mice were previously treated with a 14-d course of 0.12% amoxicillin impregnated feed to control a spike in *Corynebacterium bovis* infection. The vast majority of cases were detected, on average, 20 d after the provision of amoxicillin-compounded feed ceased. Most mice (~93%) had been implanted with human xenografts while the remaining (~7%) were naïve. Affected animals exhibited 3 clinical syndromes: 1) peracute death; 2) severe diarrhea leading to death or euthanasia; and, 3) mild to moderate diarrhea followed by recovery. All of these mice could be found within a single cage, occasionally alongside clinically unaffected cage mates. Transfaunation with feces from healthy NSG mice and subcutaneous fluids were administered to some mice with limited efficacy. Fifty-three sick or dead mice were submitted for bacterial culture and histopathology. *C. difficile* was isolated from the cecum or colon in approximately 70% of these cases. The presence of both *C. difficile* toxins A and B were confirmed in 3/4 cases. Antimicrobial sensitivity of 5 isolates revealed 3 different profiles with all isolates having at least intermediate sensitivity to ampicillin, the surrogate for amoxicillin. Histopathological lesions included fibrinonecrotizing and neutrophilic typhlocolitis with characteristic ‘volcano’ erosions or pseudomembrane formations of varying severity. Four samples from distinct mouse colonies were submitted for whole-genome sequencing to assess strain type, epidemiologic relatedness, and virulence factors. This outbreak was unexpected as we have administered amoxicillin to mice for years without incident.

**PS107 Hemolytic E. Coli as a Probable Cause of Reproductive Failures in a Specific-Pathogen Free Cat Colony**

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A specific-pathogen free (SPF) breeding colony of approximately 25 cats presented with multiple cases of resorbed pregnancies, stillbirths, and pyometras over the course of 6 mo. Litters that did go to term were typically small (1-3 kittens), and these kittens were often of low to low-normal birth weight. The top differential was decreased fertility due to inbreeding of the colony for over 30 y. Other differentials included cystic endometrial hyperplasia and infectious causes such as *E. coli*, *Streptococcus*, or viral infection. One pyometra was successfully managed medically, with Clavamox (62.5 mg BID) and Lutalyse (0.25 mg/kg daily for 5 d). This queen became pregnant and underwent a gravid ovariohysterectomy for experimental reasons, at which point
abnormal fetuses were recovered. Two other pyometras were managed surgically by ovariohysterectomy, with uneventful recovery of the queens. A pregnant queen then presented in dystocia after passing 2 stillborn kittens, so a Caesarean section was performed to retrieve the third nonviable fetus. The placenta, fetal tissues, and full-thickness uterine biopsy were submitted for histopathology. There was fibrinosuppurative endometritis in addition to a mild neutrophilic placentitis, consistent with bacterial endometritis. Hemolytic *E. coli* was cultured from the placenta and fetal tissues, implicating this agent as the cause of the stillbirths. Preputial cultures from the 2 intact males in the colony were also positive for hemolytic *E. coli*, as well as light mixed growth consistent with normal preputial flora. The 2 intact males and 2 pregnant females were treated with orbifloxacin (7.5 mg/kg daily) for 2 wk. One wk after completing treatment, follow-up preputial cultures of the intact males revealed no hemolytic *E. coli* growth. The 2 queens that were treated with orbifloxacin had litters of 1 and 2 live kittens. Breeding has been on a temporary hiatus since these litters were born, with no further cases of pyometra in the colony.

**PS108 Opportunistic Infections in 2 Cohorts of New Zealand White Rabbits**

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Shortly after shipping from the vendor, 7 rabbits from 2 independent cohorts presented with inappetence and diarrhea. Each cohort consisted of both male and female New Zealand White rabbits that were ordered based on weight (>2.50 kg) and arrived at the facility in February and April 2018. The February cohort had 4 rabbits present with inappetence, and 2 with concurrent diarrhea. Physical examination and blood work revealed mild (5-7%) dehydration, perineal fecal staining, and scant fecal pellets, which were often small and misshapen. Fecal samples were collected for fecal flotation and aerobic and anaerobic culture. Empirical treatment with oral enrofloxacin (10 mg/kg BID) and subcutaneous electrolyte solution (20 mL/kg Q8h) was initiated. Flotation did not reveal evidence of intestinal parasites, but *Clostridium perfringens* was identified on culture. As a result, oral metronidazole benzoate (32 mg/kg BID) was initiated. Despite 3 d of treatment, the rabbits continued to show progressive weight loss and dehydration. Euthanasia was elected. Similarly, the April cohort had 3 rabbits present with inappetence and marked diarrhea. Because these rabbits were intended to undergo infection studies, the possibility of confounding factors led to the decision to euthanize rather than initiate treatment of any kind. Necropsy of the affected rabbits demonstrated both gross and histopathologic findings consistent with severe enterocolitis and bacterial dysbiosis. Cultures and molecular
diagnostics from affected rabbits identified *C. perfringens* type A, which the literature supports the role of this bacteria as an opportunistic pathogen secondary to bacterial dysbiosis. Following discussions with the vendor, it was discovered that the rabbits were fed a low-fiber diet at the vendor’s facilities prior to shipment, thus predisposing them to bacterial dysbiosis. New practices, such as increasing the fiber content of the diet upon arrival, were set in place to prevent potential future cases of dysbiosis following shipment.

**Platform Sessions**

**PS119 Vitamin D Toxicity in a Cohort of *Smad3^tm1Par/J (Smad3^-/-)* Mice**

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A cohort of female *Smad3^tm1Par/J (Smad3^-/-)* mice (n=9, 5-12-w-old) received subcutaneous slow release vitamin D$_3$ pellets as part of a study seeking to evaluate the effects of systemic vitamin D supplementation on the gut microbiome. The pellets were purchased from a commercial vendor and were formulated to contain 10 mcg vitamin D$_3$ per pellet that would be released over a 21-d period. One, 2 or 3 pellets were implanted subcutaneously over the dorsal trunk of each mouse (n=3 per dose) under isoflurane anesthesia using aseptic surgical technique, formulated to release ~0.5 – 1.5 µg (~ 20 – 60 IU) vitamin D$_3$/day/mouse. Mice received a single preoperative dose of buprenorphine (0.05 mg/kg) subcutaneously to provide postoperative analgesia. Two days following surgery, all 3 mice that had received 3 subcutaneous pellets presented as lethargic, hunched, and markedly dehydrated with an unkempt hair coat and poor body condition. They also displayed increased tail tone and intermittent tremors when moving about the cage. The surgical incisions remained clean, dry, and well opposed with no evidence of swelling, erythema, or discharge. Over the next 12 h, similar clinical signs were observed in the rest of the cohort. Animals were euthanized via CO$_2$ asphyxiation and tissues and blood were collected for clinical and histopathological examination. Differentials for the clinical presentation included sepsis or vitamin D toxicity. Necropsy findings and serum chemistry data were consistent with vitamin D toxicity, revealing moderate multifocal to coalescing acute tubular necrosis of the proximal renal tubules and significant elevations in serum calcium (20.4-23.3 mg/dL) and serum phosphorus (15.2-20.2 mg/dL). Analysis of the pellets by HPLC found that each pellet contained approximately 2.5 mg of vitamin D$_3$, resulting in doses ~250 x those indicated by the formulation. This case emphasizes the importance of verification and validation of
concentrations for commercially available compounds and describes clinical and pathologic findings associated with vitamin D₃ toxicity in mice.

**PS120 Increased Frequency of Sterile Struvite Urolithiasis in a Colony of Research Dogs**

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Struvite urolithiasis is rare in dogs without a concurrent urinary tract infection. However, in 2017, dogs (n=2, males, about 3-y-old) maintained as part of a veterinary research colony on unrelated protocols were diagnosed with sterile struvite urolithiasis. To further investigate if urolithiasis was endemic throughout the colony, free-catch urine samples were assessed from a random selection of dogs across research protocols. Samples with evidence of crystals permitted targeting of dogs (n=22; about 1-6-y-old.; n=9 males, n=13 females, variety of breeds) from whom urine was then collected via ultrasound-guided cystocentesis. Samples were submitted within 2 h of collection for complete urinalysis, aerobic culture, and mycoplasma culture. The average urine pH of the examined animals was 7.65, higher than the pH range of 5.7 to 6.9 reported in clinically healthy dogs. Struvite crystalluria was observed in 36% (8 of 22) of samples. The prevalence of bladder material/stones, combined with high levels of struvite crystalluria seen on urinalysis, was 14%, higher than the reported 0.5% frequency of urolithiasis in the general pet population. Urine cultures were negative in 21 of 22 dogs, and the one positive culture (*Escherichia coli* and *Lactobacillus*) was deemed a likely contaminant by collaborating pathologists. As diet may have contributed to the sterile urolithiasis, feed was changed to a formulation with decreased levels of magnesium, phosphorous, and protein for the 2 dogs that presented with hematuria. Dissolution of uroliths and heavy debris within the bladder was confirmed via ultrasound 5 mo following diet transition, and repeat urinalyses showed improvements in urine pH and crystalluria in 1 affected animal. This study identified unexpected risk factors for struvite urolithiasis in our research colony, including diet, alkalotic urine, and a possible genetic component; future colony management practices will include scheduled urinalyses and continued review of dietary interventions.

**PS121 Clinical Presentation of Spontaneous Cardiomyopathy in a Cynomolgus Monkey (Macaca fascicularis)**

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A 5-y-old, 9.1kg male cynomolgus macaque (*Macaca fascicularis*) was sedated for an experimental surgical procedure with ketamine (2mg/kg IM) and dexmedetomidine (0.03mg/kg IM). Anesthesia was induced with propofol (0.4mg/kg IV) to facilitate intubation, and isoflurane anesthesia was initiated. Auscultation of the thorax revealed tachycardia and the presence of a gallop rhythm, as well as moderate, coarse bilateral crackles on inspiration. A moderate amount of blood-tinged fluid was observed in the endotracheal tube, and the animal displayed prolonged capillary refill time (2-3 s), but no sign of ascites or other abnormalities. Right lateral and ventrodorsal radiographs were performed, showing an interstitial unstructured lung pattern, bronchial narrowing, and cardiomegaly with right displacement of the heart axis. The animal was presumed to have pulmonary edema due to an underlying heart condition. Treatment was initiated with furosemide (2 mg/kg PO) twice daily until further diagnostics could be performed. After 10 d, the patient was sedated again with the same regimen. On auscultation, heart rate and rhythm were normal, but occasional wheezing and mild crackles were still present. Upon repeat radiography, the lung fields were clearer and the bronchi appeared normal. The heart still appeared enlarged but less displaced compared to the previous examination. Electrocardiography showed a sinus arrhythmia and marked right axis deviation with normal P waves. Right ventricular enlargement with normal right atrial size was suspected. Due to the absence of a murmur, pathology of the tricuspid or pulmonic valve was not suspected. An echocardiogram confirmed right ventricular enlargement with no evidence of valvular regurgitation. Based on the clinical presentation and the globoid cardiac silhouette, the diagnosis was determined to be congestive cardiomyopathy.

**PS122 Novel Approach to Optimize the Laying Hen Preclinical Model of Ovarian Cancer**

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No appreciable improvements in incidences and mortality rates of women with ovarian cancer have been made over the last 40 y; this fact alone indicates that scientists and clinicians lack the adequate tools to conquer this deadly disease. The American Cancer Society estimates that more than 22,000 women will be diagnosed with ovarian cancer this year, and over 14,000 deaths will be attributed to this disease.
This translates to 1 out of every 75 women in the U.S. being diagnosed with ovarian cancer, and of those diagnosed, over 60% will die from the disease. Lack of a more predictive animal model has been an obstacle to progress in ovarian cancer research. It is hypothesized that laying hens, though not fully characterized, could be an optimal animal model for the study of human ovarian cancer initiation, progression, therapy, and relapse. Domestic laying hens (Gallus gallus domesticus) spontaneously develop ovarian cancer at a high incidence. In effort to better characterize ovarian cancer in laying hens, a surgically implantable, biocompatible port was created using 3D printing technology, allowing for repeat access to the ovary for laparoscopic serial sampling, observation, and imaging. The ability to follow laying hens via easily accessible ports throughout their lifespan may pave the way for discovery of early diagnostic techniques for this disease. The fact that laying hens are a spontaneous ovarian cancer model with a high incidence of disease suggests their usefulness as a preclinical animal model. Little is known about tolerability and efficacy of chemotherapeutics in the laying hen or in avian species in general. A cohort of 10 4.5-y-old laying hens suspected for ovarian cancer was administered a 6-wk course of paclitaxel to assess chemotherapeutic efficacy. Magnetic resonance imaging (MRI) and positron emission tomography–computed tomography (PET/CT) were used to identify cancerous laying hens as well as to assess changes in tumorigenesis throughout treatment. Results are indicative of chemotherapeutic tolerability and efficacy, as well as the value of using a noninvasive method for diagnosis of cancer within the coelomic cavity, further suggesting the potential of the laying hen as an animal model for preclinical research.

**PS123 Septicemia and Pneumonia in a Rhesus Macaque with an Indwelling Catheter**

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A 16-y-old, singly housed, female rhesus macaque (Macaca mulatta) with a clinical history of chronic dysmenorrhea and heavy menses was re-examined due to dyspnea and recurring bruising on the ventral abdomen during menses. She was on a longterm cocaine neuropharmacology study requiring a surgically placed vascular access port and jugular catheter. Physical examination revealed a fever, abdominal bruising, and an enlarged uterus. Diagnostics included a complete blood count (CBC) which revealed mild neutrophilia with a mild left shift and a moderate thrombocytopenia. A blood chemistry analysis had the most striking abnormality being a moderately
elevated BUN and a moderate to severely elevated BUN/creatinine ratio. A coagulation panel demonstrated elevated fibrinogen levels but all other parameters were within normal limits. Septicemia was suspected and blood cultures were drawn. Ultrasound evaluation of the enlarged uterus indicated a large, cystic structure on the serosal surface. Chest x-ray results revealed lobar consolidation of the left cranial lung field, indicating pneumonia. Due to a poor prognosis, the animal was euthanized. Pathological examination detected jugular vascular calcification consistent with an indwelling catheter. A vegetative growth was found on the serosal surface of the uterus and there was a firm mass adjacent to the uterus composed of fibro-fatty tissue that on sectioning contained a cystic space filled with dark brown fluid. Histological evaluation of both these lesions were consistent with endometriosis. There was cranioventral consolidation of the left cranial lung lobe. The left caudal lung lobe had embolic foci, indicative of bacterial showering which was confirmed on histological evaluation. Bacteriology cultures collected from the affected lungs and the blood isolated *Kluyvera cryocrescens*. *Kluyvera* species are gram-negative, rod-shaped nosocomial bacteria occasionally cultured from human bacteremias. To the author’s knowledge, this is the first reported case of *Kluyvera cryocrescens* in a rhesus macaque with an indwelling peripheral intravenous catheter.

**PS124 Developmental Dysplasia of the Hip and Associated Long-Term Malformations in New Zealand White Rabbits: A Model for the Comparison of Late Human Analogies**

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Hip instability derived from hip dysplasia is a common finding in human medicine where its genetic role has been widely discussed, since in infants suffering from this syndrome shows an array of early incapacitating lesions and if untreated late dislocation. The study of this condition has been well documented in several animal species and recent insights have demonstrated a strong genetic role and candidate genes for congenital splay leg in piglets, which translates into gross physical and radiographic abnormalities in affected animals. In this study, a pair of rabbits (*Oryctolagus cuniculus*) with a unknown genetic load and carriers of splay leg syndrome were mated resulting in an offspring of 5:2 dysplastic animals, studied for a period of 180 days to determine its influence over morphologic and skeletal deviations further compared with normal rabbit and children hips. The results showed drastic
impaired locomotion in the animals, limited abduction and progressive bilateral
dysplastic acetabular involvement, including early osteoarthritic changes analogous to
human cases, where asymmetric thighs or buttock creases as well as pronounced
shortness of legs were clinically and radiographically determined. Finally, this
radiographic study illustrated that the induced condition in the rabbit shared similar
patterns of sex inheritance towards hip dysplasia. Interspecies differences were
disclosed and notorious short-term evolutive maldevelopment such as femoral
anteversion and coxa valga and lesions resembling femoroacetabular impingement
(FAI) were revealed. Of late, this has resulted in femoral shaft torsion in this model, a
suitable animal for the study of human hip dysplasia. Genetic involvement needs
further research in this species.

PS125 Lameness in a Yucatán Mini Pig

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A 1-y-old female spayed Yucatán mini pig presented with nonweight-bearing
lameness of the left hindlimb. There was severe swelling proximal to the coronary
band on the fourth digit of the left hindlimb. Differential diagnoses include trauma
versus infection. Pain and swelling were managed with NSAIDs and opioids.
However, these were insufficient and a local liposomal bupivacaine ring block was
performed. Radiographs of the limb showed mineral and gas in the soft tissue swelling
and moderately widening of the distal interphalangeal joint with focal osteolysis. While
awaiting final radiographic interpretation, antibiotics were administered. The final
radiology reports supported the high suspicion of septic arthritis. To retain a valuable
research animal, digit amputation of the fourth digit was elected. The pig became
partially weight bearing on the affected limb immediately upon recovery. NSAIDs,
antibiotics, and opioids were continued for the first week postamputation, and
bandages were changed thrice weekly until 20 d postamputation. During this time, the
pig was comfortable and weight bearing. The amputation site healed completely 5 wk
postprocedure and the animal continued to do well. She continued to gain weight until
her weight plateaued at week 11 postprocedure. The pig regained full use of her hind
limb, was able to be used in a study, and was euthanized at 15 wk post procedure.
This case demonstrates that toe amputation can be successfully used to manage pain
secondary to septic arthritis of the digits in swine.

PS126 Complications in a Stroke Model in Yucatán Mini Pigs

G Kim*
Stroke was induced in a number of Yucatan mini pigs. As part of the study, reliable vascular access was required to complete study-related blood assays. This was accomplished by surgically implanting vascular access ports (VAPs) and indwelling catheters approximately 2 wk before surgically inducing stroke. A 1.5-year-old female spayed Yucatán mini pig was reported for severe lethargy and lameness on the right hindlimb 4 wk following VAP implantation and 2 wk following stroke. On exam, the pig was laterally recumbent, febrile, and tachycardic. Her right hindlimb distal to the hock was diffusely moderately swollen, warm on palpation, and painful. Differential diagnoses included trauma and infection. NSAIDs were administered, but the pig did not make significant clinical improvement. The next day, physical exam revealed a grade III/VI systolic heart murmur and a delayed capillary refill time. The pig was given intravenous fluid therapy and started on opioids and antibiotics. The animal was euthanized 24 h later. Necropsy revealed severe chronic vegetative endocarditis that grew *Staphylococcus aureus*. Additionally, there was severe cellulitis and tenosynovitis with abscessation in the right tarsus/metatarsus, consistent with septic arthritis. The following week, a second 1.5-year-old barrow Yucatán mini pig presented with lethargy and inappetance. The animal became lame on the left front with a palpably warm carpus. Bloodwork revealed a moderate inflammatory leukogram and NSAIDs, antibiotics, and intravenous fluids were administered. *Staphylococcus aureus* was cultured from the blood. The animal was successfully maintained on NSAIDs, antibiotics, and assisted feedings to reach study endpoint 2 wk after presentation. Endocarditis was confirmed on gross necropsy. Naturally occurring infection with subsequent septicemia and endocarditis in swine is typically caused by *Streptococcus* spp and *Erysipelothrix rhusiopathiae*. In this case, multiple sampling through the VAP likely served to introduce *S. aureus* from the skin directly to the circulatory system resulting in these complications. Retraining staff in aseptic technique and removing the VAP following its use prevented the recurrence of *S. aureus* septicemia in additional animals.

**Platform Sessions**

**PS127 Sexual Maturity Confirmation in Group-housed Female Macaca Fascicularis: A Team Collaboration**

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Determining sexual maturity of female macaques requires daily manipulation of animals to obtain a vaginal swab. Typically, animals are pair-housed in cages equipped with squeeze-backs to facilitate positioning of the animals for this process. The behavior team at our facility offered portable transport chutes with dividers as a convenient means of swabbing group-housed, adult female macaques to assist the veterinary team without social group disruption. Use of the transport chutes in this way required modifications to the transport chutes and to the process, involving a collaborative effort between behavioral, facility, and veterinary personnel. Modifications to the equipment and process included designing a frame to match the openings of the transport chute to the social housing unit, a cable hoist to open and close the chute door from inside the pen, a ratchet strap to secure the transport chute to the housing unit, desensitization of animals to the process, facilitation of moving the animal’s tails away from the divider slots inside the chute, modifying materials used for vaginal swabbing, and the introduction of rods to secure the dividers closed while working animals in the chute. Through communication and these modifications and refinements, our behavior team was able to confirm sexual maturity in group-housed females without disrupting social relationships.

PS128 A Novel Approach to Conducting Nonhuman Primate Metabolism Studies that Allows Animals to be Group-housed, Enhancing Welfare and Science

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Currently, metabolism cages for the purposes of conducting absorption, distribution, metabolism, and excretion (ADME) studies, enabling an excretion balance scientific objective to be met, involve single housing of animals. Even though such metabolism cages have limitations for animal welfare, they have been largely unchanged for 25-30 years. We sought to design and build a new metabolism cage to improve welfare and fulfill requirements on enrichments and cage dimensions for standard housing of Cynomolgus macaques as described in EU Directive 2010/63/EU. The purpose was to investigate to excretion balance data from group and single housing of nonhuman
primates (NHP) in metabolism cages, to demonstrate the suitability of conducting excretion balance studies with a group housing design to improve welfare without compromising the scientific integrity of the study. The assessment on the welfare, in terms of stress and behavior, was also investigated. The excretion balance evaluation has been conducted in metabolism cages with single and group housed NHP, using the radiolabelled test compound, Quetiapine, an anti-psychotic pharmaceutical selected for its suitable excretion profile (including both urine and fecal elimination). Concentrations of radioactivity in blood and plasma were determined by liquid scintillation counting. Cortisol concentrations were determined in serum samples daily. Urine and feces were collected pre- and postdose daily for up to 168 h and the radioactivity was quantified. The overall mean recovery for group-housed animals, 83.2% of the dose, was essentially comparable to that of data from single housed primates, 87.1 ± 10.2%. These data are also consistent with the historical data, ca 83%, generated during the development of this compound. Group-housed NHP for future metabolism ADME studies does not compromise the scientific integrity of the study, and therefore is a major progression in the design of these studies which enhance welfare. A large degree of inter-animal variation was observed in the serum cortisol concentrations with a general trend of lower cortisol levels from 48 h onwards in the pair-housed animals.

PS129 So You Want to Retire your Nonhuman Primates? How to Choose a Reliable Sanctuary

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

**PS130 Social Housing of Pigtailed Macaques Decreases the Immune Impact of Acute Simian Immunodeficiency Virus Infection**

GLAS: Yes
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Macaques may be singly or socially housed depending on the research institution and study parameters. In general, singly housed macaques are thought to have elevated stress compared to their socially housed conspecifics, which may result in immunosuppression. Simian immunodeficiency virus (SIV)-infected macaques are a valuable animal model for the study of HIV pathogenesis, and SIV similarly leads to immunosuppression, marked prominently by the decline in CD4+ T cell counts that is commonly used to monitor disease progression along with viral RNA levels. We hypothesized that socially housed SIV-infected pigtailed macaques would demonstrate less immunosuppression and more control of viral replication compared to singly housed SIV-infected macaques. We compared CD4+ T cell counts and viral loads from 35 singly and 41 socially housed SIV-infected pigtailed macaques (Macaca nemestrina); macaques were either singly housed or socially housed with a compatible conspecific, respectively, during both the pre- and postinoculation periods. CD4+ T cell counts and viral loads were monitored at 3 preinoculation baseline time
points and on days 7 and 10 during acute infection. Singly housed macaques demonstrated a greater magnitude of decline in the number of circulating CD4+ T cells throughout acute infection compared to socially housed macaques ($P < 0.001$). Singly housed macaques furthermore had significantly higher viral loads in plasma and cerebrospinal fluid throughout acute infection compared to socially housed macaques (linear mixed effects regression; $P < 0.001$), and greater variability in plasma viral load data (linear mixed effects regression; $P < 0.001$). These data suggest that single housing of SIV infected macaques may promote stress-induced immunosuppression with the potential to confound the translational nature and reproducibility of this animal model of HIV infection.

**PS131 Enrichment for Nonhuman Primates: Identifying, Trialing, and Implementing Destructible Enrichment in an Operationally Efficient and Practical Manner**

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Destructible enrichment (DE) items that can be manipulated and destroyed are a component of the enrichment program for nonhuman primates (NHPs) at many institutions. We had 6 DE options but all lost their novelty over time, being manipulated only when food treats were present. A larger variety of DE enrichment options was needed. Animal care staff recommended 37 ideas to be evaluated for the weekly intervention rotation, consisting of a wide range of materials including plush toys, paint rollers, coconut discs, manzanita wood, and Greenies. Each item trialed was given “as is” (without treats) to a representative group of rhesus and cynomologus macaques. Interactions with each item were scored during a 15-20-min observation on Day 1 and the item was checked daily until destroyed which took on average 5 days. Items were assessed for their durability, safety, practicality, animal usage, and engagement with a scale range from 1-3 (1: used enrichment, was challenging, retained attention, destroyed; 2: was not challenging, showed no interest, item was still intact; and 3: could not use, had minimal engagement, animal was likely to throw or hit the object away.) Additionally, species-specific behaviors including chewing, shredding, sniffing, grooming, picking, licking, shredding, and sorting were
measured. Items were selected for intervention rotation based on their ability to meet these criteria while supported by the display of positive species-specific behaviors. The project resulted in the addition of 12 novel destructible options, increasing the total to 18. This facility-based initiative doubled the intervention rotation schedule, allowing a 2-wk Monday through Friday rotation with a different type of DE given each day. With the 200% increase in DE options, staff now have an array of safe, cost-effective supplies to create dynamic interventions and increase animal engagement without adding time to daily husbandry routines.

**PS132 Behavioral Effects of Environmental Enrichment for Laboratory Rabbits**

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One of the goals of environmental enrichment is to encourage species-typical behaviors while discouraging abnormal behaviors. Assessing the effectiveness of various enrichment modalities can be a challenging endeavor, particularly for prey species such as rabbits that exhibit freezing responses in the presence of people. We initially constructed an ethogram of laboratory rabbit behaviors. Specifically, we housed New Zealand White rabbits (aged 3-9 mo, ~2-4 kg) in 3 different sized cages and video recorded their behaviors. The 3 housing sizes were our typical rabbit cage (25”x 29.5”x 16”), a recovery cage (28”x 45”x 27”), and a large run (65”x 70”x 96”). Based on analysis of the recordings, ethograms were constructed and behaviors were quantified. The rabbits housed in large runs spent an average of 71.8±12.4% of the time analyzed performing active, exploratory behaviors. By comparison, rabbits housed in the typical rabbit cages spent only 30.7±9.2% on active behaviors. These differences were statistically significant ($P < 0.05$). We hypothesized that rabbits housed in large runs experience a higher degree of well-being than rabbits housed in smaller cages. Unfortunately, space constraints inside research facilities often make it impractical to house rabbits in large runs. Therefore, we decided to explore if enrichment devices could be constructed that would promote the expression of these more active behaviors. We constructed 3 devices: 1) a destructible origami box to stimulate foraging, 2) a wire ball hung high in the cage to encourage rearing, and 3) a bin with substrate to promote digging. All 3 enrichment devices promoted active, exploratory behaviors, paralleling those seen in rabbits housed in large runs.
(77.1±8.3%, 64.9±8.0%, and 70.0±7.4% of time analyzed, respectively). The origami boxes increased foraging behaviors and the wire balls encouraged rearing behaviors. The bins did not promote digging as expected, but they did encourage other active behaviors. Overall, the addition of enrichment devices or provision of larger caging encouraged a broad spectrum of active, species-typical rabbit behaviors. Our future plans include measuring fecal cortisol levels and scoring animals for ease of handling when given access to various enrichment devices.

**PS133 An Anxious Temperament Predisposes New Zealand White Rabbits to Higher Rates of Intraoperative Breath Holding**

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Temperament testing is a behavioral tool used to quantify predispositions in animals. It has a wide range of applications including assessing compatibility for social housing, evaluating personalities, and determining individuals at risk for psychological disorders such as anxiety. Given that anxiety can often impact quality of anesthesia, we sought to determine whether temperament testing (along an anxious to bold continuum) correlated to pre- and intraoperative cardiovascular changes. We examined 31 singly housed, female New Zealand white rabbits scheduled for a brief experimental ophthalmic surgery. One wk prior to surgery, temperaments were assessed using cage-side behavioral observations and a modified human intruder test. Rabbits were induced with ketamine (30 mg/kg)-dexmedetomidine (40 mcg/kg) i/m, intubated, and maintained on 0.8-1.5% isoflurane. In addition to monitoring standard cardiovascular parameters, we recorded ease of intubation (ranked on a 1-4 scale), and frequency of pre- and intraoperative breath holding. Analysis was limited to the first 15 min of surgical anesthesia to eliminate variables associated with extended surgical time. We found that 10 rabbits possessed an anxious temperament and 21 exhibited boldness. Anxious rabbits performed significantly more breath holding (mean=3.5) as compared to bold ones (mean=0) (Z = -2.23, P = 0.02). Temperament had no effect on trends in HR (Z = -1.53, P = 0.17) or RR (Z = -1.26, P = 0.21), and all rabbits exhibited wide variability in HR (136-254) and RR (0-65). Additionally, there was no correlation between temperament and ease of intubation (ρ = 0.10, P = 0.53), age (ρ =
-0.03, \( P = 0.89 \), or duration in single housing (\( \rho = 0.09, P = 0.62 \)). Rabbits were moderately difficult to intubate (mean score=2) regardless of temperament. In sum, our temperament test successfully identified a subpopulation of anxious rabbits that were also prone to intraoperative breath holding. Future research will examine if the addition of a pre-anesthetic anxiolytic will ablate breath holding in anxious rabbits, and investigate further risk factors for the development of anxiety.

**PS134 Swine Training Program Enhances Animal Welfare and Research Efficiency**

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Swine are commonly used in the biomedical industry to evaluate medical devices. They have similar skin characteristics to humans and large surface area, enabling testing of multiple devices per swine leading to a reduction in animals. Studies require general anesthesia and the ability to safely premedicate, transport, and recover animals efficiently while minimizing stress. Recovery areas require frequent cleaning to minimize risk of infection and meet swine natural behaviors. Animal care staff developed a swine training program to provide environmental enrichment and to avoid stress and time commitment required to successfully complete IACUC approved protocols. Newly arrived swine underwent a 7-d acclimation period during which staff sought to understand individual swine motivators (food reward, human interactions, swine-to-swine interactions). Swine were placed in dedicated play spaces with an experienced ‘trainer’ swine that readily demonstrated daily routines for handling with animal care staff, injections, physical exams, and transportation in carts for procedures. Desired behaviors were then positively reinforced using individual motivators and clicker training. The training program led to refinements in routine husbandry and research procedures. Swine readily approached the front of their cages for injections and transportation to procedure rooms, reducing animal handling, stress, and hock injuries. Swine were trained to walk back to animal holding rooms post anesthesia to ensure their complete recovery. This refinement decreased postop complications in animals undergoing multiple anesthesia events for study requirements. Researchers and animal staff have observed reduced vocalizations when
working in animal rooms, more effective husbandry, and improved animal health leading to quality data and science. The swine training program has contributed to a culture of care as described by outside inspectors and a greater level of employee satisfaction. Vivarium space is more effectively used due to social housing success in swine that initially were aggressive and territorial. Experienced research swine continue to contribute to the research program as ‘trainers’ building on the principles of the 3Rs.

**PS135 Targeting to Overshadow Fears and Anxiety in Miniature Swine**

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Targeting refers to social nosing or rooting, which is a natural behavior of miniature swine. We use targeting to replace an unacceptable behavior such as a fear response with an acceptable one that involves a touch in response to the same stimulus. The handler first finds highly prized reinforcers, often a food reward, and gradually teaches the animal that is afraid to interact with people to want to interact with people. In this case, the animal is taught to "touch" a target-stick for a food reward first. It is walked a short way following the target stick and asked to touch the target, then reward. Once the animal can predict a reward is coming when interacting with people, we can condition a miniature swine to cooperate for SOPs such as loading a cart. Gradually over several sessions, the animal will touch closer and closer to the cart. Next, the animal gets in, touches the target, and immediately exits. Eventually, the animal stays in the cart with a target-touch reward interaction coming at about every 3 s. If the animal stays calm and responds to the cue-response reward interaction (CRR) consistently, you can add in distractions such as opening the door or people running around the cart or making distracting noises. Every week, caretakers participate in a performance on cue (POC) tournament with a miniature swine they have trained. Pigs follow targeting cues to navigate through a maze of obstacles leading to a cart loaded with food reward. Fears and anxiety are eliminated because the animal is taught another behavior (touch) that is more enjoyable or pleasant to exhibit in the presence of the stimulus (human interactions, obstacles, and cart) that elicits the abnormal behavior, i.e. a fear response.
PS136 Welfare Management of Pigs Undergoing Gastrointestinal Surgery

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Our institution recently ran its first chronic, translational porcine study. This occurred as a pilot on 3,12-to-18-wk-old gilts that underwent gastrointestinal (GI) surgery, followed by a 2-wk recovery and monitoring period. Various recordings needed to take place over the monitoring period, and cooperation from the pigs was essential. We did not want to give pain relief in an injectable form, and ideally wanted to make use of a transdermal patch. After reviewing literature on analgesic protocols in laboratory pigs (of which very little was available, especially that used transdermal patches) and speaking with experts in human GI surgery, we decided on a multimodal pain relief regime using acetaminophen 30mg/kg PO SID x 5d, meloxicam 0.5 mg/kg PO SID x 5d, a fentanyl transdermal patch 50 mcg/hr/21-24kg x 3d and an incisional block of 2mg/kg bupivacaine. Due to the inherent risk of stomach ulcers in growing pigs and potential stress from the surgery, we also gave omeprazole 20mg PO SID x 7d. A specially tailored post-gastrointestinal feeding regime was instituted and refined over the course of the pilot study. Enrichment and behavioral training were implemented to decrease the chance of stress in the first place. Behavioral training was carried out several times daily, commencing 1 wk prior to the surgery. Behavioral training was accomplished using positive reinforcement techniques. This allowed recordings to occur with minimal restraint and a restraint sling that was purchased did not need to be used. Many enrichment items (both food and non-food items) were trialled, creating a hierarchy of food enrichment and favourite toys for each animal. Training went well, and the pigs learned quickly. Individual pigs expressed personal preferences for certain foods and toys. However, the cohort unanimously enjoyed apples, broccoli, and fruit-flavoured gelatine as food-based enrichment and inflatable balls as toys. The end result was 3 pigs with well-controlled pain who successfully contributed the fields of human and veterinary medicine. Due to the success of the pilot, additional recordings will be able to occur in the main study, further increasing the knowledge gained. Welfare management of the pigs will continue to be refined.