PS1 Is it Time for the Certified Lab Animal Diet Contaminant Standards to be Updated?

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The concept of certified lab animal diet was developed during the late 1970s. Certified lab animal diets guarantee that the concentrations for 5 heavy metals, aflatoxin, polychlorinated biphenyls (PCBs), and 23 pesticides do not exceed set limits. It is the recommended diet for good laboratory practices (GLP) studies. As part of the NIH lab animal diet quality assurance program, heavy metal and pesticide analyses have been performed on 15 lab animal diets for 25 years. The QA pesticide screen included 22 of the 23 pesticides on the certified diet list. Over the 25 years of analyses only malathion, on occasion, exceeded its minimum detectable concentration (.01 ppm). Since 1990 14 of the pesticides listed for certified diets have been banned from use. The Environmental Protection Agency has reported on the 25 most used pesticides in agriculture, and none of these pesticides are on the certified diet pesticide list. Analyses for glyphosate, the most used agricultural pesticide, in 3 lab rodent diets from 3 lab animal diet manufacturers showed the average concentration was 0.94+ 0.178 ppm. During the last 19 y mycotoxins and isoflavones have been included in the NIH lab animal diet QA analyses. Aflatoxin the only mycotoxin on the certified diet contaminant standards list has not been found in any diets. However, vomitoxin, fumonisins, ochratoxin A, and zearalenone are found in the diets. It appears that the contaminant standards for lab animal certified diets need to be reviewed and updated. This will require removing contaminants that are no longer a hazard, determining new contaminants to add to the certified diet standards, and, determining the maximum acceptable levels for the new contaminants.

PS2 Using Clickers to Quiet Monkey Chaos: An Overview of Implementing a Husbandry-based Clicker Training Program

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Nonhuman primate rooms can be a hectic and noisy environment for all involved. Calming and shaping that behavior can be challenging in large nonhuman primate colonies due to limited staffing and time constraints. However, literature review reveals that animals engaged in positive reinforcement training display less stress, aggression, and stereotypical behaviors due to the sense of control, human interaction, and the psychological enrichment the animals recieve from these interactions. Previous studies in canine shelters have shown that clicker training shelter dogs led to decreased barking and an increase in positive behavior changes. Applying the framework from these studies, we modified the daily husbandry routine for our nonhuman primate staff. We incorporated key aspects of using the clicker when providing food enrichment items to animals that displayed positive behavior traits. Animals that demonstrated aggressive behavior were not provided extra food enrichment items and the clicker was not used. Additionally, this interaction helped develop the bridge of the clicker for more advanced behavior training. Implementation of this program was simple and well received by the husbandry staff. Staff members received training from the nonhuman primate enrichment and behavior group on how to use the clicker and recognize positive versus negative behavior traits. Initial subjective feedback from the husbandry, veterinary, and enrichment staff reported favorable results. Data generated from a comparison of the behaviors displayed by these primates before and after the initiation of the clicker training program will be presented. This work tests the value of clicker training for inducing positive behavioral changes in non-human primates and explores methodologies and metrics for implementing a husbandry based nonhuman primate training program.

PS3 Finger Painting: A Novel Intervention for Feces Smearing in Non-Human Primates

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As a component of the environmental enrichment program, behavior management includes observation, quantification, and intervention for abnormal or undesirable behaviors in laboratory nonhuman primates (NHPs). Feces smearing (FS) is a stereotypic behavior observed in approximately 5.88% (6/102 NHPs) of rhesus (Macaca mulatta) and cynomolgus macaques (Macaca fascicularis), and baboons (Papio anubis) at this institution. Currently, standard institutional interventions provided to the NHPs designed to curb FS include destructible enrichment (e.g. box filled with paper and food items), with the addition of therapeutic enrichment (e.g. foraging board), expanded cage space, or a water cup filled with food-grade baking extract as needed. Several studies and case reports on autistic children who exhibit neurological and behavioral limitations have shown the potential use for finger paints as a treatment for these dysfunctions. We hypothesized that the use of finger paints as a novel intervention would lead to a reduction of FS in NHPs. Observations were made 6 h, 24 h, 7 d, and 14 d after cage change for individuals exhibiting FS (n=7). Observations were made for the following experimental periods: baseline, two repeated trials (with paint), and post-trial (without paint). Finger paint was distributed in plastic containers secured to caging daily after husbandry during the 2 repeated trials to evaluate the effectiveness of this intervention. Over the course of this project, a trend towards lower FS scores was observed for day 14 for individuals utilizing finger paints.

AALAS 70th NATIONAL MEETING
PS4 Continued Evaluation of Extended Cage Change Interval for Breeding Mice

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The optimal cage change interval for laboratory mice remains controversial. Our institution previously used a 16-wk cage change interval for mice. This study was undertaken to evaluate whether cage sanitation interval affects breeding performance, cage microenvironment, and animal health and behavior of mice housed in individually ventilated cages (IVC) with pelleted paper bedding. Continuous and noncontinuous breeding trios of C57BL/6 mice were followed longitudinally over 18 wk. Cages were completely changed every 2 wk or had a partial bedding change (75%) with a complete change at 16 wk. A total of 20 cages were followed, 5 per study condition. Breeding success was determined by analyzing litter size, pup weights, and litter mortality. We evaluated cage microenvironment by assessing microbial load and air quality, and we subjectively scored the cage condition, animal appearance, and animal behavior every 2 wk before and after cage change or partial bedding change in both continuous and noncontinuous breeding groups. We found no significant difference in breeding efficiency between groups. Although there were no differences in animal behavior between groups, we did note improved animal appearance in the partial cage change groups that was statistically significant for the continuous breeding trios. Ammonia levels were significantly higher in the partial change cages. Cage wall bacterial levels were higher in the extended sanitation group and taxonomic differences were seen between the groups using microbiome analysis. Extended cage change did not improve breeding outcomes or animal welfare based on behavioral analysis. Cage air quality and microbial growth were worse with extended cage change and the practice may alter the microbiome. Our data do not justify an extended sanitation interval for breeding mice housed on pelleted paper bedding.

PS5 Is a Once per Week Cage Change Possible with Diabetic Mice?

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Studies involving diabetic mice are challenging since they are sensitive to distress that can affect the onset of diabetes. Polyuria causes cages to soil quickly and remain wet, which means near daily cage changes. Such frequent cage changing can induce stress in mice and increase expenses of labor and consumables, such as food and bedding. A novel cage design that uses a perforated false bottom to hold bedding while filtered air is pushed through the bedding was compared with a traditional IVC system housing 5 diabetic (DbDb) mice per cage. The traditional IVC was changed 3 times per week, and the novel IVC system changed once per week. Metrics of ammonia, temperature, relative humidity, and moisture content of the bedding were collected in both caging systems. Ammonia, temperature, and relative humidity were collected using wireless sensors which collect data without opening the cage. Moisture content was measured at 4 corners and the center of the cage. Traditional cages displayed low ammonia throughout the cage-change cycle, whereas the novel cages saw ammonia increase on day 5 but remain under 25 ppm by day 7 of the cage change. We could not allow the traditional cage to go out 7 d due to the wetness, and we had to use a 2-3 days cage change cycle during this comparison study. Both cages stayed within the preferred temperature range. Relative humidity was higher in the novel cage by the end of the 7 days as compared to the traditional cage at 3 days. Moisture content measurements found more dry corners and overall lower total cage average in the novel cage as compared with the traditional cage. Our study indicates that the novel caging system would extend the cage change interval for diabetic mice. Fewer cage changes will reduce labor and supply costs. Reducing the cage changing requirements may also aid in reducing the stress associated with handling. The goal of this study is to safely extend the cage change time period for these diabetic models in the novel cage.

PS6 Temperature and Relative Humidity in Static and Ventilated Cages Housing Mice (Mus musculus)

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The Guide recommends 68-79F temperature (T) and 30-70% relative humidity (RH) for mouse macroenvironment. Intracage T and RH may be affected by factors including macroenvironment, cage type, animal number, activity, bedding, and cage change frequency. Our objective was to measure intracage T and RH and evaluate potential modulating factors. We hypothesized that T and RH are higher in static versus individually ventilated (IVC) cages and vary with macroenvironment, animal number, diurnal cycle, and bedding type. Male or female mice were housed at 1, 3, or 5 per cage in static or IVC cages in 2 rooms maintained at 12h:12h light:dark cycles. Data loggers in cage lids, rooms, and outside the building provided hourly T and RH readings for 28d. Corncob or bedding consisting of specially processed hardwood paper pulpwere each used for 14d, with cage changes at 7d (static) or 14d (IVC). T and RH readings were compared by T-test or ANOVA using significance at P < 0.05. Bedding type and cage change frequency did not affect T or RH in any cages. T was higher in IVC vs static cages housing 1, 3, or 5 mice but did not vary directly with mouse number. T was higher nocturnally in static and IVC cages housing 3 or 5
mice. RH was higher in static vs IVC cages housing 1, 3, or 5 mice and increased with mouse number in static cages. No diurnal RH variation occurred in any cage. Intracage T and RH were both related to room parameters. In summary, our findings included lower T and higher RH in static vs IVC cages, diurnal variation in T but not RH in static and IVC cages, and RH but not T variation with mouse number in static cages. Thus, microenvironmental T and RH are affected by several factors and should be considered for relevance to animal welfare.

**PS8 Mouse Handling with Tunnels**

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Our facility houses 1,000 mice. Approximately 20% of our census are immunocompromised, including NSG™ mice, a severely immunodeficient model generally unable to fight off infections. In 2016, we observed a significant increase in skin lesion reported in this population; 19 cases reported in a 6-mo window versus our typical annual caseload of 1-2 cases. Two factors potentially contributing to the increase included a new staff member and the introduction of *Staphylococcus xylosus*. This challenge provided our team with a new opportunity. Recently, the Unit Manager traveled overseas on an AAALAC International Fellowship Award, where she visited various facilities in and around London. There, she was introduced to an NC3Rs 2010 study discussing moving mice from dirty to clean cages by using tunnels or hands. The study showed that using hands or tunnels was less aversive for mice than using forceps, and resulted in fewer stress behaviors observed after cage change. Following this experience, in May 2017, we obtained both IACUC and principal investigator approval to implement tunnels as part of the cage change process for our immunocompromised mice. We made no other changes to the husbandry of the room, staff, nor to the researchers using the room. We continued making clinical observations looking in particular for additional skin lesions. An analysis of cases from May through November 2017 showed a 100% reduction in skin lesion cases. A more recent check in May 2019 showed that no further skin lesions have been observed in the NSG mice. Due to the success in implementation of tunnels in our immunocompromised room, we’ve expanded to make this our standard model for mouse husbandry across the facility.

**PS9 Going beyond the Isolator: A Novel Process and Facility Design to Acknowledge the Role of the Macroenvironment In Germfree Rodent Derivation Success**

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As the field of germfree and gnotobiotic rodent science grows to support microbiome and gut flora-specific inquiries in basic and advanced research, the need to generate strain-specific animals at germ-ree health status in vivo also arises. This derivation process is technically difficult when limited to working within the microenvironment of the flexible film isolators, as the physical and mechanical constraints of a bioexclusionary containment structure prevent the efficient and successful outcome for the many coordinated parts of the biological derivation process. Constraints include the lengthy contact time of chemical sterilants used in port entry and closure; the use of anesthesia, analgesia, and surgical equipment within an isolator, and limitations of cylinder volume for needed materials. Further, the historical approach to germfree isolator work relies on large quantities of toxic chemicals to clean up the macroenvironment and facilitate the success of isolator port entry of materials and supplies. Many existing facilities are not designed to work with these materials, processes, and equipment successfully, thereby limiting the potential broad-reaching scope of gnotobiotics. As the microbiome field continues to expand, the need to manipulate and handle these germfree animals outside the physical constraints of the isolator grows, as does the need for ventilation support for chemical use within animal facilities. Therefore, an understanding of the baseline and ongoing cleanliness of the macroenvironment must be considered. The process for conducting germfree derivations is reframed around working in a sterile macro and microenvironment. We hypothesize that the clean design of the macroenvironment will improve germfree derivation success rates as well as mitigate and/or dramatically reduce the need for harsh chemical sterilants. The process for conducting a germfree derivation and embryo transfer in a biosafety cabinet is described. In order to maintain acceptable success rates, which is measured by germfree derived pups, we’ve developed a novel decontamination process to mitigate potential contamination scenarios based on identified high-to-low risk points in the derivation process. Also, we’ve developed a novel facility with higher standards of macroenvironmental engineering and equipment controls compared to standard animal and surgical facilities, and we describe the process that drove us toward this concept and outline our parameters for success.

**PS10 The Impact of Visual Cues on Rabbit Behavior**

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Rabbits (*Oryctolagus cuniculus*) are known for being curious and timid animals, are easily frightened, and can be difficult to manage in a laboratory setting. As lab animal technicians we are bound by the Animal Welfare Act, the *Guide*, the IACUC, and our own personal morality to mitigate any unnecessary stress to the animals under our care. In an effort to alleviate stress during acclimation, handling, and husbandry duties, visual cues were initiated in order to indicate to the rabbits what activity was about to occur. After the acclimation period and at the start of the...
protocol, the rabbits were exposed to laminated signage which hung on the wall facing all caging and remained visible to animals at all times. The team used signs consisting of 1 colored shape designated for each procedure type: husbandry/enrichment (blue circle), invasive procedures (red triangle), or status quo (green square). The signs were placed no less than 1 h prior to any activity. This allowed the animals’ time to observe the sign change and prepare for human interaction. Behavioral changes were measured objectively by technicians on a weekly basis. Within 2 wk of the sign placement significant changes in behavior were noted. The rabbits tended to come to the front of the cages and appeared to be more compliant in our presence. There was a marked reduction in foot “stomping” or thumping and hiding, and less resistance when attempting to pick them up. The value of communication is often overlooked when dealing with small laboratory animals but the potential benefits are not to be underestimated. The change in behavior has been paramount and reflects in the success of our study and our relationship with our rabbit population.

PS11 Mass in a Northern Tree Shrew (Tupaia belangeri)

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A 2-year-old, intact female tree shrew (Tupaia belangeri) presented for recurrent mild periocular dermatitis. As an incidental finding during physical examination, a fluctuant, subcutaneous, 1.5cm x 1.0cm mass at her second left abdominal mamma, centered underneath the nipple was identified. Lymph nodes were unremarkable. Very little is published on mammary masses of tree shrews though differentials from other laboratory animal species include intraductal tumor, mammary adenoma/adenocarcinoma, mastitis, myoepithelioma, and lymphoma. A fine needle aspirate was taken of the mass revealing a uniform population of neoplastic epithelial cells. Surgery was scheduled for a planned excisional biopsy. Two weeks later at the time of surgery, the mass had grown another 30% to 1.9cm x 1.2cm x 0.9cm. The tree shrew was induced with isoflurane (2% isoflurane in 100% oxygen). Full body radiographs did not show any evidence of metastasis. Preemptive analgesia of buprenorphine sustained release (0.25mg, SQ) was administered with local analgesia administered via incisional lidocaine before closure. During surgery, anesthesia was maintained with isoflurane (2-4%) as needed and thermal support was provided via a recirculating warm water blanket. The entire mass and overlaying skin was removed to achieve adequate margins and the incision was closed with absorbable subcuticular sutures. Recovery was uneventful, and meloxicam (0.125mg, PO) was administered. On cut surface, the mass was uniformly tan and multilobular. The histomorphology was consistent with a cystic papillary mammary carcinoma. At a 10-wk recheck, radiographs showed no evidence of metastasis and FNA of cranial mammae/lymph nodes showed no evidence of neoplasia. Surgical mastectomy should be considered in cases of mammary carcinoma of tree shrews.

PS12 Dracula the Laboratory Ferret

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A 1-yr-old, castrated, pair-housed male ferret was examined for lethargy, weight loss, and pale mucous membranes. The animal was used solely for annual serology for influenza screening of the naïve ferret colony. On physical examination, the animal had appropriate mentation, ideal body condition (3/5), pale mucous membranes, no murmurs or arrhythmias, normal lung sounds, and no palpable abdominal masses or fluid. The animal was anesthetized with 1-5% isoflurane in an induction chamber before being transferred to the working table on a tightly fitting facemask and maintained on 1-3% isoflurane for a jugular blood collection. A comprehensive analysis including a complete blood count, serum chemistry, packed cell volume, total protein, and blood smear was conducted. The comprehensive analysis revealed an elevated creatinine (0.8mg/dL), severe non-regenerative, normocytic, normochromic anemia (PCV 6%); all other values were within normal limits. Blood smear revealed a lack of reticulocytosis. The differential diagnosis included pure red cell aplasia, chronic kidney disease, immune-mediated anemia, and neoplasia. The animal was prescribed erythropoietin (150 IU/kg IM), iron dextran (10mg/kg IM), ad lib carnivore care (6.4 tbsp dry/kg PO) and daily polyvit (0.5mL PO) for 4 d. The anemia was unresponsive to therapy after three days and, due to animal welfare concerns as well as study-related contraindications of prolonged immunomodulation therapy, euthanasia was elected. Gross necropsy revealed general mucosal pallor and mesenteric lymphadenopathy. Bone marrow histopathology revealed hypercellular marrow with an increased myeloid to erythroid ratio due to selective erythroid hypoplasia. These findings could be compatible with pure red cell aplasia, which is only very rarely described in ferrets. Successful management of pure red blood cell aplasia involves prolonged therapy with multiple immunomodulating drugs (prednisone, cyclosporine, and azathioprine).

PS13 Respiratory Complications in a Rhesus Macaque (Macaca mulatta) with Chronic Indwelling Catheter

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A 70th NATIONAL MEETING
A 10-y-old, singly housed, male Rhesus macaque (Macaca mulatta), involved in a chemical dependency protocol that uses chronic indwelling catheters for self-administration, was examined for acute inappetence and changes in study responses. Cage side assessment found the macaque to be bright, alert, and responsive, and the indwelling right internal jugular catheter was flushing appropriately. Under ketamine (10 mg/kg, IV) sedation the macaque was notably dyspneic with pale blue mucus membranes. Supplemental oxygen was provided via facemask for the remainder of the physical exam, which highlighted crackles auscultated over the chest, predominately on the left side, and a small amount of purulent discharge at the catheter exit site. Complete blood count and biochemistry were unremarkable and culture and sensitivity of the exudate found a moderate growth of Acinetobacter lwoffii. Fluoroscopy evaluation revealed restricted inflation of lungs and a large, round, stationary cardiac silhouette. Thoracic and abdominal ultrasound revealed lobulated pleural effusion, no pericardial effusion, and free fluid in the abdomen. Due to poor prognosis, euthanasia was elected. At necropsy over 700 milliliters of milky, white fluid was removed from the thoracic cavity, and severe bilateral atelectasis and abdominal effusion were grossly appreciated. Fluid analysis of thoracic effusion reported predominately lymphocytes (94%), triglyceride and cholesterol counts of 873 mg/dL and 68 mg/dL, respectively, and no growth on culture, confirming the diagnosis of lymphocytic-rich thoracic effusion, chylothorax. Vascular trauma from repeated attempts of advancing the intravenous catheter to the desired depth has been implicated in adhesion formation which eventually may lead to failure of local lymphatic ducts. As chylothorax is a rare complication of indwelling catheters in rhesus macaques, this case emphasizes the value of radiopaque catheter material and sufficient imaging resources to confirm catheter placement locations and assist in follow up examinations.

PS14 The Tale of a Crooked Tail

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A 30-d-old, male chimera mouse was examined for 2 discrete swellings with dorsal deviations along the tail. The proximal swelling was located at the tail base, with a narrowed, circumferential area of apparent constriction caudally. The area of annular constriction was immediately followed by a second, more distal, fusiform shaped lesion with mild, dark blue to black subcutaneous discoloration, and a lesser degree of swelling and dorsal deviation. The differential diagnoses for these lesions included trauma, handling, nesting material entrapment, ring tail secondary to low humidity, improper tail vein injection(s), a subcutaneous abscess or cyst, a neoplastic process, or a combination of these things. The animal was bright, alert, in good body condition, and was a valuable founder, therefore, the lesions were monitored. After 8 d, both lesions were larger in size, the ventral aspect of the proximal lesion was ulcerated, and the discoloration of the distal lesion was more pronounced. Following euthanasia, radiographs of the animal revealed a significant amount of soft tissue opacity in the areas of the swellings, caudal vertebral malformations, and mild lysis of the dorsal aspect of the malformed vertebrae. Additionally, a firm, raised, pale pink to tan nodule in the right cranial lung lobe was noted. All tissues were placed in buffered formalin, and the tail was subsequently placed in decalcifying solution. Histopathology of both the tail and lung lesions revealed a mixture of tissues from different germ layers, including muscle, bone, fat, squamous epithelium, keratin, and nervous tissue, leading to the ultimate diagnosis of a malignant teratoma with metastasis to the lung. Teratomas are most commonly described in the reproductive organs of animals and are usually benign. The literature describes induced teratomas as a model for assessing pluripotency and tumorigenesis in research; however, this animal was experimentally naive. Although spontaneous neoplasia is less common in young animals, spontaneous teratomas are seen more often in younger animals, and particularly in horses, strain 129, and genetically modified mice. However, the extragonadal location of this teratoma, and the presence of pulmonary metastasis makes this an unusual finding.

PS15 Facial Edema and Mortality in a Cohort of Irradiated B6.SJL-PtprcPepec/BoyCrCrl Mice

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A cohort of B6.SJL-PtprcPepec/BoyCrCrl (B6.SJL) mice presented with facial edema (8/40) and spontaneous mortality (5/40) 1 wk after whole body x-ray irradiation. Mice were irradiated at a dose of 1200 rads and received 2 million bone marrow cells via retro-orbital injection containing either 1 million B6.129S2-Ifnar1tm1Agt/Mmjax or B6.129P2(SJL)-Myd88tm1.1Defr/J cells with 1 million B6.SJL cells. Neomycin was administered prophylactically in the drinking water. This experiment had previously been performed without complication; however a cesium irradiator and C57BL/6J recipient strain were used. The affected mice were euthanized, and 2 were submitted for necropsy. On gross exam, severe facial edema and moderate segmental enteropathies were noted. Splenic cultures grew Enterococcus spp. Histopathology demonstrated multifocal moderate proliferative enteritis and typhlocolitis with villar blunting and fusion and lymph node depletion, characteristic lesions post irradiation. In addition, diffuse edematous facial cellulitis with fibrinoid degeneration, necrosis of blood vessels, and abundant bacterial colonies were present. Differential diagnosis for the facial edema included irradiation damage to facial vessels, infection, or hypersensitivity reaction. In the following weeks, 2 additional mice developed facial edema and were euthanized. Further investigation uncovered that the mice were irradiated in an empty microisolator cage. We hypothesize that due to the ability of the mice to rear, multiple mice received increased doses of irradiation to the
head, causing vascular damage and secondary bacterial infection from translocated gut bacteria. This phenomenon has been described in one case study report, but facial edema is a noted sequela in many irradiation studies. For subsequent studies, the irradiation dose was lowered, enrofloxacin replaced neomycin to provide broader spectrum bacterial coverage, and a restraint device was used to prevent rearing. No further clinical evidence of facial edema or mortality were noted. This case emphasizes the differences between irradiation sources and their effects on dose dependent clinical signs.

**PS16 Abdominal Mass and Thoracic Abnormality in a Rhesus Macaque**

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A 19-y-old, indoor-housed female rhesus macaque (Macaca mulatta), with a previous history of 2 embryo transfers via laparotomy, was examined for an abdominal mass following a routine research MRI procedure. On physical examination, vitals were within normal limits and an immobile firm mass was palpated within the caudal abdomen. Bloodwork was unremarkable, 3+ blood was noted on urinalysis, and urinary retention was reported during the MRI scan. Radiographs and ultrasound revealed a large, thick-walled, fluid-filled mass in the caudal abdomen and increased soft tissue opacity in the left caudal lung fields. The mass was aspirated, and dark brown, hemorrhagic fluid was collected. Differentials for the mass included endometriosis, neoplasia, ovarian cyst, and possible hematoma. Differentials for the abnormality in the left thoracic space included neoplasia and lung lobe atelectasis. Due to poor prognosis, the subject was sent to necropsy. Gross pathology revealed a 12cm-diameter, thick-walled cyst arising from the left ovary, multifocal cysts along the gastrointestinal and diaphragmatic serosa, numerous adhesions, and an anterior uterine/cervical leiomyoma compressing the urinary bladder. Within the thoracic cavity, the left liver lobe and one-third of the greater omentum was herniated through a rent in the diaphragm, leading to atelectasis of the left caudal lung lobe. Histology revealed the large cyst to be an endometrioma, with multiple endometriotic lesions throughout the abdominal cavity. Invasive endometriosis found within the membranous portion of the diaphragm likely led to the weakening of and fenestrations within the tissue, allowing for passage of abdominal viscera into the thoracic cavity. Diaphragmatic hernia associated with endometrial lesions is a rare presentation for a well-known disease. Left undiagnosed, endometriosis in rhesus macaques can lead to various complications, thus routine monitoring and assessments are necessary to improve the prognosis of high risk patients.

**PS17 Anorexia and Weight Loss in a 19-y-old Olive Baboon (Papio anubis)**

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A 19-y-old, 21 kg, individually housed male olive baboon (Papio anubis) previously involved in imaging studies presented with a 2-wk history of hyporexia. Routine physical exam performed 1 mo prior revealed moderate dental disease but was otherwise unremarkable. Examination at the time of presentation showed no change in severity of dental disease and a stable weight and body condition. Lab work revealed marked elevation of ALP (1,621 U/L) and GGT (112 U/L), but all other blood parameters were within normal limits. Differential diagnoses included dental disease, or cholestasis caused by cholelithiasis, cholangitis, amyloidosis, neoplasia, or a gallbladder mucocele. Gastroprotectants, S-adenosylmethionine, and silybin were started along with pain medication and supportive care. Over the next 3 wk, there was no clinical improvement and additional diagnostics were pursued. An 8 percent decrease in body weight was noted. Abdominal ultrasound revealed multifocal dilated cystic structures within the liver parenchyma and a dilated gallbladder containing a moderate amount of sludge. No choleliths were seen and the liver appeared of normal size and echogenicity. Repeat lab work obtained at this time revealed worsening cholestasis (ALP: 2,247 U/L; GGT: 191 U/L; Tbil 1.1mg/dL). Broad-spectrum antibiotics, maropitant, and ursodeoxycholic acid were added to the treatment regimen. However, the baboon presented with jaundice and anorexia 2 wk later and an exploratory laparotomy was performed. During surgery, a thickened, firm, obstructed gallbladder was identified and euthanasia was elected due to poor prognosis. Histopathology revealed a densely cellular, poorly demarcated and infiltrative mass consistent with a gallbladder adenocarcinoma with secondary invasion into liver parenchyma. Hepatobiliary tumors comprise less than 6 percent of all neoplasms in baboons. Only 6 cases of gallbladder adenocarcinoma have been reported, and this is the first reported case in an olive baboon.

**PS18 Episodic Fainting in a Castrated Male Domestic Short Hair Cat**

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A 13-y-old, 4.5 kg male castrated domestic shorthair cat on a vitamin D diet supplementation study presented with episodic syncope and signs consistent with cardiogenic shock. Physical exam revealed pale and tacky mucous membranes, decreased peripheral pulse strength, mild dehydration estimated at 5-7%, rectal temperature of 95°F, a
heart rate of 160 BPM, and episodic fainting during examination. An intravenous catheter was placed and 50 mLs of lactated Ringer’s solution were administered. On auscultation, complete heart sound deficits occurred during the periods of observed syncope. Three lead ECG revealed a left-bundle branch block with intermittent episodes of third degree atrioventricular (AV) block. Hematology, serum biochemistry, and ionized calcium levels did not reveal any significant abnormalities. Thoracic and abdominal radiographs and ultrasound revealed no cardiac abnormalities or signs of metastatic disease. Echocardiogram showed no significant findings outside of complete ventricular standstill during periods of third-degree AV block. Clinical signs of syncope occurred during periods of elongated AV block. The cat was placed in an oxygen cage and observed overnight with continuous cardiovascular monitoring. Clinical resolution of the cardiogenic shock and third-degree AV block occurred 48 h after the initial episode. The cat returned to his normal housing, vitamin D2 supplementation was discontinued, and continuous video monitoring was installed inside of the room. Twenty-four-hour electrocardiographic (Holter) recordings performed 3 times at 4-w intervals revealed a progressive resolution of the third-degree AV block and the cat remained clinically stable. No cardiogenic or systemic cause of the AV block was determined, and clinical resolution occurred over time after termination of the vitamin 25(OH)D2 supplementation. Cardiac pacing was not implemented at this time due to the stabilized condition of the patient. The cat was removed from the study and was placed for adoption. Currently the cat has remained stable with no apparent cardiogenic deficits.

**PS19 Galactorrhea and Hepatomegaly in a New Zealand White Rabbit (Oryctolagus cuniculus)**

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A 3-y-old, multiparous, nongravid, individually housed female New Zealand White rabbit (Oryctolagus cuniculus) presented with multiple swellings along the ventral abdomen. Physical exam revealed uniformly enlarged, soft mammae of normal color and temperature. Milky discharge was easily expressible. Cytology showed prevalent proteinaceous aggregates, as well as scant plasma cells, lymphocytes, monocytes, and neutrophils. Aerobic and anaerobic cultures were negative for bacterial growth. Findings were consistent with normal milk. Abdominal palpation and radiographs were unremarkable. Primary differential diagnoses at this time included mammary gland hyperplasia in response to hyperprolactinemia (prolactinoma vs. hyperestrogenemia [pseudopregnancy vs. uterine adenocarcinoma]), and mammary gland adenocarcinoma. Over 5 wk, mammary enlargement persisted and body condition decreased. Abdominal radiographs and ultrasound exam at this time revealed hepatomegaly with a rounded caudal liver margin. Complete blood count and serum chemistry showed a nonregenerative anemia, increased hepatocellular enzymes, and hyperlipidemia; serum was grossly lipemic. Supportive care was initiated pending protocol-planned euthanasia 1 wk later. Gross necropsy revealed an enlarged, friable liver; histopathology confirmed hepatic lipidosis. The ultimate cause of hepatic lipidosis in this nonanorectic rabbit remains unknown, but prolonged lactation may have been a precipitating factor. Necropsy additionally revealed a normal pituitary gland, a left uterine horn leiomyoma, and diffuse mammary gland hyperplasia. Ovaries were normal with no corpora lutea. Previously reported causes of leporine galactorrhea, including prolactinoma and pseudopregnancy, were ruled out by these findings. Remaining possible causes of galactorrhea in this rabbit include ectopic hyperprolactinemia, pituitary-origin hyperprolactinemia (secondary to dopaminergic dysfunction, stress, or hypothyroidism), idiopathic hyperprolactinemia, or idiopathic galactorrhea. In humans, leiomyomas have been identified as a source of ectopic hyperprolactinemia; uterine leiomyomas occur with some frequency in rabbits, but galactorrhea as a comorbidity has not previously been reported in this species.

**PS20 Head Tilt as a Result of an Unusual Etiology in a C57BL/6J Mouse**

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A 2-mo-old female C57BL/6J mouse presented with a left-sided head tilt. The mouse was otherwise normal. The animal, part of a cohort in a study investigating the influence of inflammation on breast cancer development and progression, had been provided a purified high fat (60%) diet (AIN-93) ad libitum since arrival from the vendor 2 wk earlier. All mice in the cohort were maintained in individually ventilated cages with autoclaved aspen chip bedding, and acidified water. Differential diagnoses included idiopathic necrotizing arteritis, bacterial otitis media/interna (Pasteurella pneumotropica, Pseudomonas aeruginosa, Streptococcus sp. Mycoplasma pulmonis, and Burkholderia gladioli), encephalitis, abscess, neoplasia, congenital malformation, and accidental or iatrogenic head trauma. Magnetic resonance imaging revealed a significant left sided displacement of the olfactory bulb and areas of hyperintense signaling consistent with edema. Following imaging, the animal was euthanized due to poor prognosis. Histopathologic examination revealed a unilateral, full-thickness bone defect at the base of the cribiform plate and a malformation of the nasal conchae, resulting in the herniation of the olfactory bulb into the nasal cavity. There was also a left midline-shift of the frontal cortex and moderate catarrhal sinusitis was present in the left mandibular sinus. The MRI and histopathologic changes are suggestive of a congenital malformation of the nasal cavity and frontal aspect of the skull explaining the clinical presentation. Clinical, imaging, and pathological findings, prevalence in different species and strains of mice, as well as the possible impact on research will be discussed.
PS21 White Growth on the Leg of a Xenopus laevis

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A 9-year-old female Xenopus laevis was reported for a white growth on its left leg. On physical exam, the frog was quiet and had a white, cotton-like mass on the left tarsus. Both eyes had white corneal opacities that were easily irritated and bled freely. Cytology of the growth revealed abundant fungal hyphae, which were identified as Saprolegnia parasitica via fungal culture. Due to the advanced age of the frog and poor condition, euthanasia was elected. On gross examination, there was an approximately 6 mm x 2 mm white skin defect with hyperemia where the growth was previously present on the left tarsus. The spleen was severely enlarged and mottled dark red with a white, fibrous capsule surrounding the serosal surface. The liver and heart were also enlarged and discolored. The spleen and eye were submitted for fungal culture, which yielded no growth. Histology revealed populations of round neoplastic cells, forming sheets and perivascular infiltrates in the spleen, liver, kidney, digestive mucosa, blood vessels, eye, and skin. Immunohistochemistry showed antibodies against CD3 cells colocalizing with neoplastic cells in the spleen and liver, identifying them as T cells. Based on gross and histological findings, the final diagnosis was a multi-organ, T cell lymphoma. Saprolegnia commonly occurs in amphibians secondary to stress or disease. In this case, the frog was likely immunocompromised due to lymphoma, resulting in colonization by Saprolegnia. The presence of Saprolegnia warrants investigation into both environmental and pathological causes of immunocompromise in Xenopus laevis.

PS22 Hypovitaminosis K in Mice: Do Fecal Menaquinones Overcome Dietary Insufficiencies?

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We previously published that antibiotic treatment or increased dietary folate significantly decreases gastric pathology in Helicobacter pylori-infected mice. To determine if combining the treatments would act synergistically, cohorts of male INS-GAS mice infected with H. pylori (SS1) were administered antibiotics and/or high-dietary folate (8 mg/kg vs 2 mg/kg diet) at 18 wk postinfection. Antibiotics, but not high folate, decreased gastric disease severity. However, unexpected morbidity and mortality associated with acute blood loss was observed in 41% of infected mice receiving antibiotics, and in 8% of infected mice not receiving antibiotics. Our presumptive diagnosis was hypovitaminosis K due to dietary insufficiency combined with antibiotic-induced dysbiosis. Mice were fed a chemically defined amino acid diet with menadione (MD) as the form of vitamin K. MD is a synthetic, water-soluble form of vitamin K used in diets for multiple species. Recent recommendations suggest diets should be formulated with the more bioavailable form of vitamin K, phylloquinone (PK). Menaquinones (MKn) are produced by intestinal microbes and provide additional vitamin K through coprophagy. Hypovitaminosis K was confirmed as no additional morbidity was noted following treatment with 100 μg of PK subcutaneously for 3 d and then every 3 d for 2 wk. The diet was also reformulated with 1.2 mg/kg PK. Mice had significantly decreased MK4 in livers of antibiotic-treated mice prior to PK treatment (6+/-0.9 pmol/g) compared to antibiotic-treated mice post PK (13 +/-1.2 pmol/g). Pre-PK fecal samples had significantly lower MK5-MK6 where MK7 and MK11 measured higher in antibiotic-treated mice than in H. pylori-infected antibiotic-free mice. Fecal microbiome analysis in antibiotic-treated mice pre-PK had significant decreases of bacteria in the phylum Bacteroidetes, many of which produce MKs, and an increase within the phylum Firmicutes. Post-PK treatment, the microbiome of antibiotic-treated mice rebounded where the profiles matched H. pylori infected cohorts without antibiotics. Our data demonstrates that marginal vitamin K in the diet when combined with antibiotic-induced intestinal dysbiosis, induced clinical vitamin K deficiency.

PS23 Administration of Oral Diclofenac Results in Signs of NSAID Toxicity in Rats Harboring the Human ATG16L1 Crohn’s Disease Susceptibility Variant

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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. Several environmental factors, either through acute insult or chronic build-up over time, contribute in large part to the multifactorial etiology of this disease. For example, using a knock-in rat model harboring an ATG16L1 gene variant responsible for increased CD predisposition in humans, our laboratory previously found that longterm, low-dose exposure to the nonspecific, nonsteroidal antiinflammatory (NSAID) diclofenac (1.25 mg/kg, 50% of the no-observable-effect level (NOEL)) results in shifts in the gut microbiota composition as well as CD-like histologic signs in ileal and colonic tissues of rats heterozygous for the Atg16l1 CD susceptibility variant (HET). To assess whether acute exposure to the same NSAID could also trigger disease onset, HET rats (4 male, 4 female) from the Atg16l1 strain and their wild type (WT) littermates (4 male, 4 female) were orally gavaged with the NSAID.
PMSG were cultured to 2-cell and surgically transferred into CD-1 surrogate females to compare pregnancy rates. Equal pregnancy rates were obtained for PMSG (3/3) and AIS (3/3) groups, indicating that embryo quality is similar by PMSG or AIS superovulation methods. Further embryo transfer experiments will be performed to determine the numbers of viable pups that were derived from vitrified-warmed PMSG or AIS superovulation methods.

PS25 In Vitro and in Vivo Developmental Comparison of Vitrified-Warmed C57BL/6 Zygotes Derived from Anti-Inhibin Serum or Pregnant Mare Serum Gonadotropin Superoovulation

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Cryopreservation of germplasm such as sperm and embryos is an important and cost effective method to preserve scientifically important rodent models of human diseases and safe guard them against genetic drift, contamination, infectious disease, and natural disasters. Currently, the gold standard consists of superovulation with Pregnant Mare’s Serum Gonadotropin (PMSG). Recent investigation has shown that superovulation of mice by anti-inhibin serum (AIS) yields significantly higher oocytes compared to PMSG method which reduces the number of donor females. However, there is less information available about the cryosurvival of AIS derived embryos. In this study, 10 C57BL/6 female mice were superovulated with either AIS or PMSG. Clutches of metaphase II oocytes were collected from the oviducts following hCG administration, and inseminated via in vitro fertilization. A subset of fresh embryos were immediately cultured to assess their developmental potential to blastocyst stage. Remaining embryos derived from either AIS or PMSG were cryopreserved via vitrification and later warmed to determine both in vitro and in vivo developmental competence. The percentage of fresh embryos that developed to the blastocyst stage from PMSG (85.75±2.09) and AIS (84.30±1.87) mice were not different (p>0.05). The percentage of the vitrified zygotes that were intact after warming for PMSG (93.3±2.06) and AIS (89.3±1.78) mice were not different (P > 0.05). The percentage of vitrified-warmed embryos that developed to the blastocyst stage from PMSG (53.3±1.97) and AIS (56.6±1.71) mice were also not different (P > 0.05). Vitrified-warmed zygotes obtained from either AIS or PMSG were cultured to 2-cell and surgically transferred into CD-1 surrogate females to compare pregnancy rates. Equal pregnancy rates were obtained for PMSG (3/3) and AIS (3/3) groups, indicating that embryo quality is similar by PMSG or AIS superovulation methods. Further embryo transfer experiments will be performed to determine the numbers of viable pups that were derived from vitrified-warmed PMSG or AIS superovulation methods.
PS26 Improvement of Retinal Function in Diabetic Rats After Subconjunctival Injection of Insulin-loaded Nanogels
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Diabetic retinopathy (DR) is the most common neurovascular complication of diabetes, affecting approximately 140 million diabetics worldwide. Current treatment options, including laser vitrectomy and anti-VEGF agents, are only used for advanced DR and have a high non-response rate, many side effects, and do not address the underlying neuroretinal pathology of DR. To target the neuronal degeneration in DR, a sustained release, insulin-loaded, biodegradable nanogel system was developed as a potential long-term therapeutic to rescue retinal neurons from apoptosis and reduce DR onset and progression. The purpose of this project was to determine if a single subconjunctival injection of these nanogels would improve retinal function in diabetic Sprague Dawley rats as measured by electroretinogram (ERG). Insulin was loaded in the nanogels during nanogel synthesis with over 98% loading efficiency. The in vitro release of insulin from the nanogels was studied by dialysis method, and the released insulin was quantified by ultra performance liquid chromatography. The nanogels sustained the release of insulin in vitro for at least 60 days. Male Sprague Dawley rats (n=6) were induced to become diabetic with a single intraperitoneal injection of 50 mg/kg of streptozotocin in citrate buffer. Age-matched control rats (n=6) received citrate buffer alone. One month after diabetes induction, diabetic rats received a single subconjunctival injection of 20 µL of insulin-loaded nanogels in the left eye and 20 µL of phosphate buffered saline in the right eye. Scotopic ERG measurements were performed on both diabetic and non-diabetic control rats after two hour dark adaptation one day before, one week after, and two weeks after treatment. The ERG data showed that a-wave and oscillatory potential amplitudes were significantly increased two weeks after treatment with the nanogels compared with one day before treatment (p<0.05) and were increased to amplitudes similar to those in the naive control rats. These results suggest that the insulin-loaded nanogels improved photoreceptor and amacrine cell function in diabetic rats and can be potentially developed as a long-acting therapy for the treatment and prevention of diabetic retinopathy.

PS27 Effects of Carprofen and Buprenorphine on Tumor Growth in Mouse Models of Prostate Cancer Bone Metastasis
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Murine models of tumor development often require invasive procedures for implantation, potentially causing pain or distress. However, analgesics are often withheld because they may affect tumor development. Previous studies examining the effects of analgesics on the development and metastasis of various tumor lines show that the effect of analgesics on the tumors is dependent on tumor line and analgesic used. A blanket statement that analgesics affect the general growth of tumors is not adequate scientific justification for withholding analgesics, and pilot studies or references are recommended for each specific scenario. In this study, we evaluated the effects of two commonly used analgesics on tumor growth in two models of prostate cancer (PCa) bone metastasis. We hypothesized that a one-time injection of analgesics at the time of intratibial injection of PCa cells would have no effect on tumor growth. C57BL/6 or SCID mice were injected subcutaneously with an analgesic (carprofen 5 mg/kg or buprenorphine 0.1 mg/kg) or vehicle (saline 0.1 ml) at the time of intratibial injection with PCa cell lines (RM1 or PC3, n=10 per group). Tumor progression (bioluminescent imaging, radiographs), behavioral assays (Von Frey), and clinical signs (body weight) were monitored for 2-4 weeks. Neither carprofen or buprenorphine administration affected tumor growth, behavioral or clinical parameters compared to the saline control for either cell line. Overall, this study adds to the growing body of literature demonstrating that animal welfare can be compatible with scientific objectives, and the decision to withhold analgesics must be evaluated on a case-by-case basis.

PS28 A Comparison of Ketamine and Etomidate-based Intraperitoneal Anesthetics in Multiple Mouse Strains
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Intraperitoneal (IP) injection is a common route of anesthetic administration in mice, however few safe and effective protocols have been developed. Ketamine-xylazine IP anesthesia is one of the most widely used IP protocols, but has limitations in efficacy and suitability for some studies. Etomidate is an alternative to ketamine that is used in human and companion animal practice that has not been widely explored in mice. We evaluated etomidate-xylazine (EX) IP anesthesia as an alternative to ketamine-xylazine (KX) anesthesia. We hypothesized that EX anesthesia would be as safe and effective as KX anesthesia, with anticipated sex- and strain-dependent differences. Male and female Crl:CD1(ICR) (n=42), C57BL/6N Crl (n=34), BALB/cf (n=37) and NU/J (n=34) mice were given a single IP dose of ketamine 100 mg/kg and xylazine 10 mg/kg or etomidate 20 mg/kg and xylazine 10 mg/kg. Multiple physiologic parameters, anesthetic time points, and mortality rate were evaluated. Surgical depth was confirmed by a negative pedal withdrawal response to both manual finger pinch and regulated forceps pinch. Sedation time, defined by duration of loss of righting reflex, was similar between KX and EX anesthesia with CD1 mice exhibiting shorter sedation times regardless of protocol. Several mice experienced an adverse hyperexcitement
response during induction, with BALB/cj and NU/J mice administered EX significantly more likely to experience hyperexcitement. Surgical anesthesia was achieved in 43.84% of EX animals compared with 4.17% of KX animals. 93.75% of C57BL/6NCrl mice administered EX lost pedal withdraw reflex and were significantly more likely to achieve surgical anesthesia when compared to other strains. Venous pH was evaluated 40 min post-IP injection for all mice. Male NU/J were significantly more likely to exhibit moderate-severe acidosis as compared to males of other strains. Mortality rates were low for both protocols, with no mortalities noted during KX administration and 1 mortality after EX administration. Overall, these results indicate that EX is a more effective surgical anesthetic than KX. However, due to multiple factors such as adverse reactions, strain- and sex- associated differences, EX IP anesthesia may only be acceptable as a safe and effective alternative to KX anesthesia in C57BL/6NCrl mice. The variability among mouse strains and between sexes requires further investigation to optimize IP EX dosage.

**PS29 Depletion of CD4 T-cells Increased Epstein-Barr Virus Infections in a New Zealand White Rabbit (Oryctolagus cuniculus) Model**

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Epstein-Barr virus (EBV) is a gamma-herpesvirus that infects over 90% of the adult human population, is the main etiologic agent of infectious mononucleosis, and is associated with cancers such as Hodgkin’s or Burkitt lymphomas. EBV has been studied in lab animals using EBV-like viruses, but an adequate animal model has not yet been established. The goal of this project was to compare two immunosuppressive regimens, Cyclosporine A (CsA) and CD4 T-cell antibody depletion to establish a rabbit model of EBV. Six adult NZW rabbits were inoculated with EBV via the marginal ear vein. Two rabbits (female =1, male =1) were treated with 15 mg/kg CsA subcutaneously at the time of EBV inoculation. CsA treatments were continued at 15 mg/kg daily for four days, and then at 20 mg/kg twice weekly for two weeks. Four rabbits (female = 2, male = 2) were treated with 2 mg anti-CD4 T-cell antibody IV concurrent to EBV inoculation and then once a week for two weeks. Peripheral blood lymphocytes (PBL) and body temperatures were monitored until study endpoint. Both rabbits in the CsA group and one rabbit in the anti-CD4 group showed signs of illness such as hyporexia, decreased fecal output, and lethargy; these rabbits reached humane endpoints despite veterinary supportive care. Partial depletion (average 12% decrease from baseline) of CD4 T-cells was demonstrated by flow cytometry. qPCR analysis of spleen cells proved more diagnostic than PBLs, and the anti-CD4 rabbits were strongly positive for EBV within splenic cells whereas the CsA-treated rabbits were weakly positive. Future aims for this model include optimizing the CD4 T-cell antibody depletion regimen with the goal of 100% CD4 depletion to standardize immunosuppression. The rabbit EBV model requires further characterization, but is a promising model to test anti-viral medications and prophylactic vaccines for EBV in patient populations.

**PS30 Placement and Use of an Indwelling Epidural Catheter with Concurrent Behavioral Assessment in Yucatan Minipigs (Sus scrofa)**

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Image-guided percutaneous spine procedures offer an attractive approach to treating spinal targets because minimally invasive techniques are well-tolerated and do not necessitate general anesthesia. A useful preclinical model of this therapeutic modality would provide the means to test tolerability of novel spinal agents delivered to conscious animals in a species that exhibits homology with the structure and scale of adult human spinal anatomy. We set out to establish a model of epidural infusion in 3 conscious Yucatan swine to assess the feasibility of catheter placement in each anesthetized animal with concurrent carprofen analgesia. Behavior was assessed prior to catheter placement and during vehicle infusions (beginning 3 d postcatheter placement) using a modified visual analog scale (VAS) by 2 blinded veterinarians. VAS parameters included restlessness, vocalization, posture, ambulation, injurious behavior, and overall clinical pain. Bolus infusion of epidural lidocaine was available as rescue analgesia. All catheters remained in place throughout the study, demonstrated on CT imaging (at 1- and 2- wk postcatheter placement) by exclusive epidural contrast flow and stability of spinal segments infused. Clinical exams remained within normal limits throughout the study and lidocaine rescue was not required at any timepoint. Necropsy and gross examination at 4 wk did not reveal evidence of infection, inflammation, or fibrosis of neuraxial tissues. Interrater reliability of the modified VAS was strong. No significant difference in animal behavior was observed between vehicle infusion and control timepoints. These results suggest that indwelling epidural catheterization in conscious swine is feasible and safe for a period of at least 2 wk. As a model of adult human spinal intervention, swine offer a robust platform to test tolerability of novel agents intended for use in the awake patient.
**PS31 Comparison of Injectable Anesthetic Management Protocols for Swine (Sus scrofa domestica) Utilized in a Paramedic Emergency Tracheostomy Teaching Laboratory**

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We report comparison of two injectable anesthetic protocols utilized in adult male and female 20-30kg domestic swine for a terminal teaching laboratory in which paramedics conduct an emergency tracheostomy. The laboratory requires the use of injectable anesthetic to prevent exposure of participants to inhaled anesthetics and to maintain a stable plane of anesthesia as the trachea is incised and intermittently obstructed over the course of the lab. An ideal protocol should provide a stable anesthetic plane, cause minimal aberrations in cardiac function, be uncomplicated to administer, cost effective, and utilize readily available drugs. In both protocols, swine were premedicated with intramuscular midazolam 0.5 mg/kg, butorphanol 0.3 mg/kg and xylazine 4 mg/kg followed by isoflurane administration via nose mask, intubation and intravenous catheter placement. All swine received intravenous buprenorphine 0.01 mg/kg prior to surgery. At the start of the lab, isoflurane was discontinued. One group of swine (n=4) received a bolus of alfaxalone 0.47 mg/kg followed by 6 mg/kg/hr continuous rate infusion. A second group of swine (n=4) received 1 ml/kg bolus of 5% guaifenesin 0.1% ketamine and 0.1% xylazine (GKX) followed by a 2.2 mL/kg/hr continuous rate infusion. Vitals including heart rate and non-invasive blood pressure were monitored every 10 minutes. The relative stability of cardiovascular function of the two protocols was determined by comparison of the average slope for linear regression functions for the mean arterial pressure and heart rate. No significant difference was observed between the two protocols in either parameter. However, the GKX group experienced relative higher mean arterial pressures and heart rates trended lower compared to the alfaxalone group. In conclusion, both protocols were comparable in providing a stable surgical anesthetic plane, cost effectiveness, ease of administration and cardiovascular stability. The differences in mean arterial pressure and heart rate between protocols can be attributed to the action of xylazine, an alpha-2 agonist, in the GKX protocol. Ultimately, both protocols present viable options for use in swine and can be used preferentially based on drug availability and targeted length of anesthesia.

**PS32 Pancreatic Cystic Lesions in Laboratory Mice from Predominantly NOD and NOD-derived Backgrounds**

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Reports in the literature of pancreatic cysts in mice are generally associated with polycystic disease (such as polycystic kidney disease), or as a single feature of the overall lesions observed in pancreatic cancer models. However, pancreatic cystic lesions in mice outside those scenarios are exceedingly rare. Here, we report a series of 10 purpose-bred laboratory mice with pancreatic cystic lesions. Generally, animals were submitted to diagnostic necropsy for abdominal distension and/or a palpable abdominal mass. Grossly, cystic lesions were thin walled and often had prominent and tortuous surface vessels. Histologically, lesions were characterized by dilated spaces surrounded by a thin fibrous capsule often accompanied by early fibroplasia and/or edema (7/10). Cystic spaces were lined by cuboidal (10/10) to columnar (7/10) epithelium. In all cases there was lymphocytic infiltration of the fibrous capsule, and most cases also had neutrophilic (8/10) and histiocytic (9/10) infiltrates. There was occasional hemorrhage (5/10) and hemosiderin-laden macrophages. Lesions were also characterized by varying degrees of pancreatic acinar atrophy (6/10) and fibrosis (5/10). Animals were predominantly, but not exclusively, of NOD and NOD-derived backgrounds. Four of 10 animals were NOD.Cg-Prkdc<scid>/J [NOD scid] mice. Four of 10 animals were from the following strains (one of each): NOD/ShiLtJ [NOD], NOD.Cb17-Prkdc<scid>/J [NOD scid], NOD.Cg-Prkdc<scid>/J [aka NOD scid gamma, or NSG] mice. All 10 animals were from the following strains (one of each): NOD/ShiLtJ [NOD], NOD.Cb17-Prkdc<scid>/J [NOD scid], NOD.Cg-Prkdc<scid>/J [aka NOD scid gamma, or NSG], and NOD.B[DbMit93-DbMit124][D4Mit114-D4Mit142]/1112MrkJ [NOD.c3.c4]. Two of 10 animals were not NOD-derived strains: BTBR.V(B6)Lepr<ob>/Wisc [aka BTBR obese] and CBySmn.Cb17-Prkdc<scid>/J [BALB scid]. None of these cases had gross or histologic evidence of luminal obstruction, and the ultimate driver of these cystic dilations could not be definitively determined. Although regarded as rare, pancreatic cystic lesions should be considered as a differential diagnosis for laboratory mice with abdominal distention, and the lesion may be overrepresented in NOD and NOD-derived strains.

**PS33 Development of a Fistula into the Cecum of a Deer Mouse (Peromyscus maniculatus) Post Castration Surgery**

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Male deer mice were castrated to remove the influence of testosterone on the variable of study with testosterone being replaced via administration through an osmotic pump to control dosage and timing. Surgical castration was performed under isoflurane anesthesia using aseptic technique. A 2-incision method was used, incising over each testicle in the scrotum, exteriorizing the testicle, clamping and ligating the vessels and spermatic cord with absorbable suture followed by closure of the skin with tissue glue. Mice were administered meloxicam at 2 mg/kg once every 24 h and enrofloxacin at 10 mg/kg subcutaneously just prior to surgery and for at least 2 more days afterward every 24 h. Treatment with analgesia and/or antibiotic was extended if required based on monitoring observations including but not exclusive to active inflammation, discomfort, or redness at the incision site. One
mouse eventually presented with a complication postsurgery that would not become evident until approximately 2 mo after the initial procedure was performed. The 2-mo-old mouse had no complications noted during surgery. On d 5 post surgery, a small amount of reddish, clear discharge was observed from the right-side scrotal incision. It was treated with topical chlorhexidine ointment and enrofloxacin injectable. Enrofloxacin was continued until d 11 post surgery. The mouse continued eating or drinking; body weight and body condition as well as activity level remained normal. On d 56 post surgery, a brown discharge was observed from the right scrotal incision area. Under isoflurane anesthesia, the area was cleaned with chlorhexidine and flushed with saline. This procedure was repeated for approximately 3 more d until it was observed that the discharge resembled ingesta. The mouse was euthanized and a postmortem exam revealed a fistula formation between a previous abscess and the cecum through the body wall of the mouse. Despite this fistula formation, the mouse had shown no other signs of illness other than the noted brown discharge in the previous few days. This case highlights possible unusual sequelae to a relatively straightforward surgical procedure as well as the time that can elapse before that sequelae becomes evident.

PS34 Unanticipated Disease Severity in the IL10-/- Mouse Model of Inflammatory Bowel Disease

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The IL10-/- mouse is used as a model to study inflammatory bowel disease. An optimal phenotype requires postweaning oral gavage with Helicobacter hepaticus, which serves as a provocateur of an inflammatory response to intestinal microbiota. Mice not inoculated with H. hepaticus develop sporadic and less severe disease. A researcher using this model observed an increase in the severity of disease (rectal prolaphe, weight loss, histologic lesion scores) of IL10-/- saline-gavaged control mice that did not receive H. hepaticus. The concern for increased disease in control experimental mice was expressed after the colony was relocated to a modular unit during vivarium renovation. In order to investigate the cause of the increased disease occurrence in the control mice, metagenomic evaluation of feces, cecal, and mid-jejunal contents was performed to look for a potential dysbiosis, and intestinal tissues were evaluated by histology. Metagenomics data revealed no dysbiosis, histopathology revealed inflammation, and epithelial hyperplasia present in the cecum and colon consistent with mild to moderate disease. Metagenomic analysis unexpectedly identified bacteria in the genus Helicobacter present in the mid-jejunal contents of all control mice analyzed. A PCR to screen for H. hepaticus was then performed in the remaining breeding animals in the colony, and positive results for H. hepaticus were obtained. The increase in disease severity of the control mice was contributed to contamination with H. hepaticus in the colony breeders and stock animals. The timeline of contamination was determined by PCR evaluation of historical paraffin embedded tissues. The colony was reestablished, and routine screening of mice was implemented. This occurrence stresses the importance of pre-inoculation screening to ensure negative status of experimental pathogens.

PS35 Hepatic Lipidosis following Abrupt Diet Change in Wild-caught White-footed Mice (Peromyscus leucopus)

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Twenty adult male and female white-footed mice (Peromyscus leucopus) caught near Cape Cod were socially housed by sex in a barrier room. Quarantine testing by PCR of pooled fecal samples was positive for Cryptosporidium spp., Helicobacter spp., fur mites, and pinworms. After 14 wk, the diet was changed from a standard rodent chow to a medicated rodent chow containing 150 ppm fenbendazole, 25 ppm pyrvinium pamoate, and 1000 ppm doxycycline. Five days later, an adult male was found dead with no premonitory signs. Despite increased monitoring, 2 mice were found dead, and 3 moribund mice were euthanized 3 d later. One moribund mouse exhibited severe neurologic signs, with lateral recumbence, opisthotonus, and paddling. Gross pathologic findings of all mice included thin body condition, empty gastrointestinal tracts, and atrophy of subcutaneous and intracavitary fat. Livers were moderately enlarged, diffusely pale-tan, and friable. On histopathological examination, livers had severe macro- and micro-vesicular hepatic lipidosis. The mouse exhibiting neurologic signs had chronic proliferative gastritis with intralesional eggs and adult nematodes consistent with Capillaria spp. The white matter at the levels of the hippocampus and cerebellum in this mouse exhibited multifocal areas of spongiosis with mild laminar neuronal loss, and astrocytosis with Alzheimer type II cells. These changes are suggestive of hepatic encephalopathy. After the second mortality, the standard chow diet was reinstated. The remaining mice were administered subcutaneous fluids and intraperitoneal dextrose for 2 d, and offered palatable nutritional supplements for 1 wk. Following diet change and supportive care, there were no additional morbidities or mortalities in the colony. Surveillance testing 4 wk later by PCR of pooled fecal samples was positive for Helicobacter spp. and Entamoeba spp. Surviving mice have been clinically normal for 3 mo and breeding has commenced. In conclusion, sudden changes in the diet fed to wild-caught Peromyscus may lead to anorexia and acute onset of hepatic lipidosis. These cases highlight the importance of gradual dietary changes combined with close observation of wild-caught Peromyscus leucopus housed in laboratory settings.
A study was conducted to assess potential therapeutic synergy in treating Helicobacter pylori-infected male INS-GAS mice with increased dietary folate supplementation and antibiotic combination eradication therapy. Mice were infected with H. pylori (SS1) and fed a chemically defined amino acid diet containing 2 mg folate/kg. Eighteen weeks postinfection (WPI), specific cohorts were switched to an 8 mg/kg folate diet and/or received helicobacter eradication therapy consisting of omeprazole (400 µmol/kg/day), metronidazole (14.2 mg/kg/day), and clarithromycin (7.15 mg/kg/day) in a 0.2-mL volume orally twice a day for 7 d. Mice that received the eradication therapy became lethargic and appeared scruffy, hunched, and pale within 4 d after completion of antibiotic treatment. Starting at 19 WPI, 41% of the mice that were given antibiotics were found dead or moribund. Animals that were moribund were euthanized with CO2 and they, along with the mice found dead, were necropsied. On gross examination mice had pale tissues, a pale and friable liver, and a stomach filled with unclotted blood. Complete blood cell counts confirmed severe anemia due to acute whole blood loss. At 24 WPI, 2 mice on 8 mg/kg folate diet that did not receive antibiotics were also found dead with the same findings on necropsy. Our tentative diagnosis was hypovitaminosis K. The source of vitamin K added to both the 2 and 8 mg/kg folate diet was 0.5 mg menadione sodium bisulfite (MD) per kg of feed. Previous studies question the bioavailability and optimal feed concentration of menadione in animal feed and recommends the use of phylloquinone (PK). Fecal and liver samples were collected for vitamin K level analysis. In antibiotic treated mice, liver MK4 was significantly decreased pre-PK treatment (6+/−0.9 pmol/g) compared to post-PK (13+/−1.2 pmol/g). All surviving mice were treated with 100 µg of PK subcutaneously for 3 d and then every 3 days for 2 w. Within 24 h of PK treatment, the mice had improved body condition scores and activity. The diets were reformulated with 1.2 mg PK/kg. No additional morbidity or mortality occurred for the remainder of the study when animals were euthanized with CO2, at the 28 WPI predetermined end point.

**PS37 Urinary Tract Infection Associated with Bladder Augmentation Surgery in Baboons (Papio anubis)**

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Bladder augmentation is a treatment option to increase bladder volume in situations that cause a decreased bladder capacity. Ileocystoplasty is the standard technique for bladder augmentation. However, it has been associated with urinary tract infections (UTI), urolithiasis, intestinal obstruction, and long-term risk of bladder cancer. Various biomaterials have been proposed as an alternative to increase bladder size while minimizing complications. The baboon (Papio anubis) has been developed as a bladder augmentation model, but there is a paucity of information with regards to complications. This case series reports the incidence of UTI following bladder augmentation in baboons. A retrospective review of 24 patients (12 males, 12 females) was performed. There were 4 groups; cystectomy (n=6), ileocystoplasty (n=6), commercial biomaterial (n=6), and experimental biomaterial (n=6). A foley catheter was placed in all animals, while the ileocystoplasty and biomaterial groups also had a suprapubic catheter and penrose drain placed to ensure adequate urine drainage postoperatively. All groups received enrofloxacin postoperatively for the duration that the catheters were in place. The rate of UTI was 16.7% in the cystectomy group, 67% in the ileocystoplasty group, 33.3% in the commercial biomaterial group, and 33.3% in the experimental biomaterial group. The predominant bacteria isolated were Enterococcus faecalis, Staphylococcus aureus, E. coli, Streptococcus dysgalactiae, Corynebacterium renale, and Aerococcus sanguinicola. They were treated with appropriate antibiotics per culture and sensitivity results. No urolithiasis was noted in any group. This case highlights that UTI is the main complication of bladder augmentation, and that postoperative antibiotics and continuous bladder drainage do not prevent infection. Gram + bacteria are the main pathogens, which could reflect elimination of Gram – bacteria by use of postoperative enrofloxacin. We discuss the incidence and significance of the isolated bacteria in human and veterinary urinary tract infection.

**PS38 Thyroid Follicular Carcinoma in an Adult Female Common Marmoset (Callithrix jacchus)**

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A 10-y-old female marmoset (Callithrix jacchus) presented with cervical swelling and bruising during a quarantine examination. Further evaluation revealed poor dentition with significant gingival recession and pytalis. Diagnostics included CBC, serum chemistry, and pharyngeal culture. Ultrasound of the neck revealed bilateral enlargement of suspected cervical lymph nodes with intranodal hemorrhage and subcutaneous edema. No significant findings were noted on bloodwork, with no pathogens identified on pharyngeal culture. The animal was maintained on meloxicam and orbifloxacin to treat suspected reactive lymphadenopathy. Re-evaluation 1 wk later revealed resolution of bruising, no change in ultrasonographic size of presumptive cervical lymph nodes, and bilateral soft tissue opacity enlargement in the neck and tracheal deviation to the right on radiographs. Aspiration
of the node obtained hemorrhagic fluid consistent with a resolving hematoma and biopsy of the cervical skin revealed no evidence of viral inclusions, neoplasia, or infectious organisms. As no clinical signs were present, the animal was monitored for any enlargement of the cervical swelling or development in clinical signs. Approximately 4 mo after initial evaluation, the animal developed dyspnea with stridor and open mouth breathing following sedation for routine bloodwork. The animal was initially managed with antiinflammatories, but was recommended for euthanasia due to poor prognosis for recovery and possible compromise of overall colony health. Upon sedation for terminal perfusion, the animal went into respiratory arrest. Intubation was unsuccessful due to a large mass occluding the dorsal nasopharynx. On necropsy, a large yellow-brown cystic left thyroid mass extending dorsomedial to the larynx with associated lateral deviation of the trachea was noted. Histologic findings were consistent with thyroid follicular carcinoma. Thyroid-related pathology of marmosets is rarely reported in the literature, and to our knowledge this is the first report of thyroid follicular carcinoma in a common marmoset. This case also highlights the need for anatomical description and imaging assessment of normal structures in the common marmoset.

**PS39 Clinical Cytomegalovirus in a Naïve Subadult Rhesus Macaque (Macaca mulatta)**

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An experimentally naïve, 4-y-old male rhesus macaque (Macaca mulatta) presented for weakness. On cage-side exam, the animal was bright and alert but was observed teeth-grinding with generalized hindlimb weakness and ataxia. Sedated physical exam revealed no abnormalities. Whole-body radiographs were unremarkable and point-of-care electrolyte and blood gas values were within normal limits. Cerebrospinal fluid was collected for fluid analysis and blood was collected for blood culture, complete blood count, and chemistry panel. The animal was given buprenorphine (0.005mg/kg) and meloxicam (0.2mg/kg) while diagnostic results were pending. The clinical signs progressed to include a left-sided head tilt, fine whole-body tremor, and hindlimb paresis. Bloodwork was normal, blood culture was negative, and CSF analysis showed increased protein. MRI showed multicentric and poorly demarcated hyperintense lesions within the brain, brainstem, and cranial spinal cord. Given the clinical signs and diagnostic results, top differentials included viral or autoimmune etiologies. The animal was started on antiviral (galvancyclovir 13mg/kg), antinflammatory (prednisone 1mg/kg), antimicrobial (ceftiofur 20mg/kg), and gastroprotectant (famotidine 0.5mg/kg) therapy. Additional diagnostics included standard NHP serology panel (Herpes B, SRV, SIV, STLV), 5V-40 serology, flavivirus serology panel (Dengue, West Nile, and Zika), cryptococcus antigen test, and toxoplasma PCR, all of which were negative. Cytomegalovirus (CMV) serology and PCR were positive. Given the positive CMV results, the lesions seen on imaging were consistent with acute disseminated encephalomyelitis, an autoimmune demyelinating condition that typically follows viral or bacterial infection. CMV is a common beta-herpesvirus in nonhuman primate colonies. The virus undergoes periodic episodes of asymptomatic recrudescence but usually remains latent. Clinical CMV typically requires immunosuppression, and in a research environment is commonly associated with infectious disease or transplant studies. This is a unique presentation of CMV since the animal was experimentally naïve and otherwise clinically healthy until the time of presentation, and highlights the importance of considering this virus as a differential in such cases.

**PS40 Increased Placental Fluid as a Gestational Complication in an African Green Monkey**

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A 7-y-old, 6.6 kg, female, uniparous African green monkey (AGM), housed in an outdoor breeding enclosure, presented for routine obstetric ultrasonographic imaging and was diagnosed with her second pregnancy. Ultrasonography showed a fetus with a heart rate of 160 beats per minute and a fetal crown-rump length of 16mm, therefore estimated fetal age was 44 days. An intact amniotic sac measuring 18mm in diameter was surrounded by echogenic fluid within the placenta, distending the placental lumen to 33 x 46 mm. Other maternal physical examination findings were unremarkable, apart from a distended abdomen disproportionate to gestational duration. Follow-up ultrasound imaging performed 6 wk later revealed a nonviable, partially disintegrated fetus with a crown-rump length of 25 mm, suggesting that the fetus died at approximately 48 d of gestation. The amniotic sac was visible, and the placenta remained distended with fluid. The endometrial lining was thickened and irregularly shaped. The female was subsequently treated with 5 units of IV oxytocin administered over 30 m, which failed to terminate the pregnancy. Seven weeks post fetal death, hysterotomy was performed under general anesthesia to remove the placenta and dead fetus. The placenta was partially detached from the uterine wall and contained brown fluid and an intact amniotic sac. Here we present an unusual gestational complication in nonhuman primates, which may represent a subchorionic hemorrhage or hydroallantois. In the case of gestational complications resulting in incomplete miscarriage in nonhuman primates, medical options include administration of oxytocin, misoprostol, or mifepristone as initial treatment. Due to the severity of the fluid accumulation at the time of detection and the prolonged duration of pregnancy postfetal death, a pharmacological intervention was not further pursued, and surgery was performed to evacuate the nonviable fetus and placenta immediately.
PS41 Making Sense of Workplace Injuries to Help Effect Change in the Workplace

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There are a multitude of ways staff can incur injuries while working in a research vivarium. Many workplace injuries can be prevented by proactively identifying jobs or tasks with a higher injury risk. Work-related musculoskeletal disorders (WMSDs) are reported by the Occupational Health and Safety Administration (OSHA) as the “most widespread occupational health hazard facing our nation today.” WMSDs can occur due to lifting, standing in one position too long, repetitive movements, twisting, poor posture, and more. The creation of a work environment where WMSDs can be avoided is optimal as this category of injury often takes a long time to heal, and in severe cases, may require surgery resulting in lost workdays to turn into lost work months. In line with the aims of our institution, we set out to enhance our method of work injury reporting and tracking. Following modifications to our system of injury reporting we were able to identify the type of injury, the anatomic location of the injury, facility location where the injury occurred, the job task the employee was performing at the time of injury, and the type of equipment the employee was working with at the time of the injury. Based on injury reporting data, we determined 45.8% of the injuries reported from 2018 and 36.0% of injuries reported from the first half of 2019 could be categorized as WMSDs. Periodic workplace injury assessments using data from the modified injury reporting system helped to guide staff ergonomic and safety training, and helped to identify areas for targeted ergonomic improvement. Over a period of 2 y, we have seen a 38% reduction in the total number of injuries reported and a 34.8% reduction in the number of injuries categorized as WMSDs. We highlight changes made to our system of injury reporting while using case-based examples to illustrate how we obtained, analyzed, and used injury reporting data to help enhance workplace safety.

PS42 Benefits of Incorporating an Ergonomic and Injury Prevention Program for Animal Facility Staff

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We implemented a collaborative and progressive ergonomic and injury prevention program June 2017. The program is based on a musculoskeletal wellness and injury prevention blueprint which was customized to the tasks and personnel in the department. This includes ergonomic assessments with corrective actions, a progression of interactive training workshops, individual employee musculoskeletal coaching, and a daily movement maintenance program (MMP). The employees are trained to use specific posture, movement, and exercise tools to prevent musculoskeletal disorders (MSD) associated with manual material handling, awkward work positions, and repetitive manual tasks in AST. Participation rates are high (>90%). All employees who requested individual coaching had excellent outcomes based on operational definitions (average of 94.8 pts out of 100) and the program has achieved longevity. The daily MMP targets improved posture and body mechanics, maintaining flexibility in movements that offset the ergonomic risks in AST and strategic strengthening to increase musculoskeletal resilience. In addition, the program emphasizes training in protective work techniques and use of strategic micro-pausing techniques to combat fatigue. Follow-up surveys demonstrate a universal benefit and improved postural habits and awareness. There have been no musculoskeletal injuries, ROI is estimated to be 10.0 for the whole site with a potential cost avoidance of up to $102,059 for the department.

PS43 Increasing Safety with Universal Chemical Containment Levels

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The administration of chemicals to research animals is continually increasing and evolving. During the IACUC protocol review process, IACUC reviewers, veterinary, and EHS staff are tasked with assessing hazards, including chemicals, in animal models. Assessments are often completed on a case-by-case basis, with inadequate data to reference, or by those with limited knowledge of the daily animal facility operations. In addition, no regulatory guidance is provided for handling chemical hazards in animals. This results in a lengthy and inconsistent review. Consequently, the recommended safety procedures may be unintentionally inaccurate or incomplete, putting both lab and animal care staff at risk. Recognizing a need for an improved review process, EHS and lab animal professionals from multiple research institutions have come together to develop chemical containment levels (CCL). Much like biosafety levels, CCLs will provide the foundation needed to provide an accurate risk assessment. As such, it is important to recognize that the CCL project takes a “cradle to grave” approach, ensuring a review of all activities from administration to waste disposal. A list of the most commonly used chemicals, administered in vivo, at participating institutions was used as a starting point for this initiative. From there, collaborators evaluated the available toxicity and health hazard data for humans and specific animal models (if available). Classes and categories were assigned to the CCLs based on the severity and commonality of the hazards. Overall, it was determined that the majority of chemicals fit within 3 CCLs, with some chemicals needing to be handled at a higher CCL for administration and a lower CCL for animal housing. Minimum housing standards (including engineering controls, PPE, etc.) for each containment level were determined. Information on comparable substitutions for these
controls was included to make CCL universally applicable and allow for flexibility among institutions with different resources. Our hope is that lab animal, IAUCU, veterinary, and EHS professionals can take the information provided back to their institution to help streamline their review process and improve safety for those working with animals exposed to hazardous chemicals.

**PS44 To the Elevator and Beyond: Improving Work-related Conversations with Family and Friends as a Tool to Reduce Compassion Fatigue**

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Our institution is consistently exploring novel methods to identify and improve communication regarding compassion fatigue. We performed a survey over a 2-year period which indicated the majority of respondents (64% and 63%, respectively) experienced symptoms of compassion fatigue. Recognizing the potentially detrimental symptoms related to compassion fatigue, our department created the Compassion Awareness Project, which works internally to prevent, reduce, and mitigate the negative effects of compassion fatigue on laboratory animal personnel. Although the initial programs covered a variety of strategies aimed at reducing compassion fatigue, including seminars on self-care, lectures featuring research performed at the university, and coworker support groups, these programs did not provide resources specifically targeting external social support networks. In addition, focus groups comprised of multiple laboratory animal personnel confirmed the feeling of unpreparedness when talking about their work with people unfamiliar to laboratory animal medicine and indicated an interest in building skills to improve confidence. We felt these were important skills to improve as the lack of understanding from the general public of what our profession entails can hinder the ability to provide proper support to a lab animal care provider who may be experiencing compassion fatigue. Having confirmed the need to develop and implement methods to improve communication confidence, we are designing a novel pilot program to implement or strengthen preexisting communication skills similar to communication styles typically associated with the classic “elevator pitch.” The focus being creating an environment where personnel can seek support from their external support system without concern over judgement or lack of understanding of the field. Judging from initial feedback from laboratory animal personnel, there is an increased sense of pride in their work and improved support from family and friends. As a result, we plan to continue expansion and development of confidence communication skills in our Compassion Awareness Project.

**PS45 Monkey Kitchen: Creative Food Enrichment for Nonhuman Primates**

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Environmental enrichment is a critical part of maintaining animals in a research setting, providing an outlet for species-specific behaviors and enhancing the psychological wellbeing of laboratory animals. Using food as enrichment items for nonhuman primates (NHPs) can be challenging, as these intelligent and curious animals crave novelty and interaction, while also having specific nutritional requirements. We have a standard of many healthy food enrichment offerings as well as nonedible environmental enrichment. However, a group of recently received NHPs did not show much interest in the usual enrichment activities our department has used in the past. This troop appeared indifferent to picture books and television and showed agitation and fear towards bubble and light machines. We decided to get more creative with our edible enrichment leading to many novel ways of presenting food. This has made an imaginative environment for personnel who get to come up with innovative ways of presenting food enrichments, thereby increasing staff morale and job satisfaction, as reported via staff surveys. Video recordings of the NHPs have shown that these complex, fun, and fresh enrichment products have increased NHP inquisitiveness and activity (as measured by percentage of time spent interacting with the enrichment items).

**PS46 Optimizing Husbandry Practices for Armenian Hamsters (Cricetulus migratorius)**

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Hamsters are a preferred species for monoclonal antibody (mAb) generation. The majority of hamsters used in research consist of Syrian hamsters (Mesocricetus auratus) and as a result, most literature and published procedural techniques are based around Syrian hamster anatomy and temperament. This posed a unique challenge when researchers at our facility requested the use of Armenian hamsters (Cricetulus migratorius). Incorporating Armenian hamsters at our facility required our veterinarian, training manager, and technicians to learn the differences between Armenian and Syrian hamsters to provide proper husbandry practices and immunization-related techniques. Armenian hamsters have a more docile temperament compared to their Syrian counterparts. This has allowed our training hamsters, breeding pairs, and weanlings to be regularly pair or group housed. In addition, devices like scoops or bowls were deemed unnecessary for basic cage manipulations. Both of these aspects resulted in a smoother husbandry workflow. They have not shown any signs of aggression towards human handlers and are instead relatively curious. Conversely, they are resistant to manual restraint, and their excess loose skin, similar to other hamster species, further complicates their restraining. Veterinary treatments and evaluations require patience.
and care from the handler due to the challenges associated with restraint. Any type of nontopical veterinary intervention or immunization study work currently requires general anesthesia to reduce as much stress to the animals as possible. Unlike for basic husbandry handling, we found using bowls for topical treatments or tunnels for transferring to anesthesia machines provide the technician with greater control over the hamster while limiting potential escapes. Our veterinarian, training manager, and technicians keep up-to-date on current literature and emerging techniques to provide the proper care to this infrequently used and little known species. This will become even more necessary as we continue to increase our breeding operation to secure long-term continuous access to this important animal model.

**PS47 Novel Spatial and Visual Enrichment for Zebrafish Reduces Aggression**

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The use of zebrafish is research is a growing area, so providing new and different enrichment is important, including social, spatial, visual, olfactory, tactile and other sensory stimulation. Spatial enrichment provides more area for exploration, reduces fighting, allows for escape and decreases stress. Visual enrichment is important because zebrafish have spherical lens, which means they focus by moving the lens closer or further away from thier retinas depending on the amount of light. The light in the tanks is brighter at the surface and gets darker as the fish swims further down. We have created an enrichment that allows the zebrafish to swim up and see out above the tank. Briefly, this new enrichment is a container that is fitted on top of the pre-existing lid by cutting a hole in it. The junction of the existing lid and container are sealed with a safe sealant. Once the sealant is dry, a vacuum is used to suction water up into the container. Once the container is full, then the vacuum is removed. At our facility, this device has been used to reduce fighting by increasing space allowing an area for escape. Additionally, zebrafish have been observed spending a significant more amount od time in the added tank space. In conclusion, this tank modification has decreased figting and aggression issues among the zebrafish as well as increased spatial enrichment.

**PS48 Development of Greek Tortoises and Hyalomma aegyptium Tick Feeding Model at BSL-4 to Study the Transmission of Crimean-Congo Hemorrhagic Fever Virus**

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Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne Nairovirus causing hemorrhagic fever in humans, and is a select agent that must be maintained at the biosafety level-4 (BSL-4). Latest evidence suggests that in addition, mammals and reptiles and their ticks, specifically tortoises and Hyalomma aegyptium, play a role in the maintenance and transmission of the CCHF virus (CCHFV) in nature. Greek tortoises (Testudo graeca) are native to the Mediterranean, and we have shown that they undergo subclinical infection with CCHFV but transmit the virus to their ticks. Researchers at our institution sought to characterize the role of Greek tortoises and tortoise-specific ticks, Hyalomma aegyptium, as reservoirs and vectors, respectively, for CCHFV. Unique housing and husbandry challenges of this model include that tortoises are endothermic with specific temperature, humidity, UV light, and behavioral requirements. For this project, the tortoise housing must contain, and aid with tracking of, free-feeding ticks infected with CCHFV. Finally, the housing and husbandry procedures must satisfy the risk assessments of regulatory bodies such as the Center for Disease Control and Prevention, and be safe for work within the full-suit BSL-4. Despite these challenges, we successfully created appropriate housing by modifying standard guinea pig tubs and bedding, and adding species-specific hides, heat/UV lamps, and shallow water dishes. We adapted current tick-containment protocols for use with our tortoise housing. Here we share the husbandry and housing procedures we developed for the care of Greek tortoises, and their ticks, within high biocontainment.

**PS49 Housing Peafowl for Behavioral Studies of Hearing**

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Comparative studies of animals may involve species not commonly used in laboratories. One such study is determining the ability of birds to hear low-frequency sounds that are inaudible to humans (i.e., infrasound). Although pigeons and chickens hear infrasound, mallard ducks and budgerigars, on the other hand, do not. Thus, the question arises as to which birds hear infrasound and which do not. Because a peacock’s train produces infrasound that is thought to be used in social signaling, we wanted to determine the hearing ability of peafowl. This requires behavioral testing in which food is used to train them to report when they hear a tone, with the results used to construct an audiogram. One male and two female peafowl were obtained from a local farm. They are housed together in a large room within an 11.3 m² area enclosed with snow fencing attached to a sealed wood frame (2.1 m high) with a 2x4 wood perch (0.5 m high). The floor was originally covered with straw bedding which was replaced with aspen bedding (10-15 cm deep) when it appeared that dust from the straw contributed to a sinus impaction which required surgical intervention in one animal. The animals were treated for parasites, and their
nails inspected monthly and trimmed as necessary. They receive ad libitum water and are fed Purina Layena crumbles, which they work for during the week in an auditory test chamber; on weekends, the peafowl are provided free access to the food for 40 min each day. The animals are weighed daily while on test, and this feeding regimen allows them to maintain a healthy body weight. This method of laboratory housing has maintained peafowl health while obtaining useful information about their hearing abilities throughout the testing period.

**PS50 The Role of Emotional Contagion in Distress Exhibited by Grouped Mice Exposed to CO₂**

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The 2013 AVMA Guidelines for the Euthanasia of Animals recommends a chamber volume displacement rate of 10% to 30% per minute (v/min) when euthanizing small laboratory rodents with CO₂. Group euthanasia of mice is a common practice, and grouping strangers is often avoided to minimize distress; however, emotional contagion, which occurs between familiar animals but not strangers, has not been studied in the context of group CO₂ euthanasia. This study examined cagemate- and stranger-grouped mice exposed to 10%, 30%, or 50% v/min CO₂ to determine whether emotional contagion plays a role in this context and whether that role is influenced by CO₂ flow rate. Videos of adult male C57BL/6J mice exposed to different CO₂ flow rates were scored for durations of dyspnea, ataxia, and consciousness as well as the numbers of face pawing (pain response) and jump (escape attempt) behaviors. Blood was collected at time of unconsciousness and assayed for ACTH. Cagemates experienced significantly longer durations of conscious dyspnea and ataxia with 10% v/min CO₂ compared with 30% and 50% v/min. Similarly, strangers experienced significantly longer duration of conscious dyspnea with 10% v/min CO₂ compared with 30% and 50% v/min and significantly longer duration of ataxia with 10% compared with 50% v/min. Cagemates showed significantly more jumps with 10% v/min CO₂ compared with 30% and 50% v/min, whereas jumping was unaffected by CO₂ flow rate in strangers. At 10% flow rate, cagemates showed significantly longer durations of conscious dyspnea and ataxia, and significantly more jumps compared with strangers. There were no significant differences in face pawing or ACTH levels between groups. We conclude that more potential for distress exists when cagemate and stranger mice are exposed to a 10% v/min CO₂ flow rate based on prolonged durations of conscious dyspnea and ataxia. We conclude that emotional contagion may contribute to distress in cagemates at 10% v/min flow rate based on exacerbated jumping behavior. We propose that 30% v/min CO₂ should be used for euthanasia of grouped mice, and that 50% v/min should also be considered humane.

**PS51 Nitric Oxide Inhibition Enhances Immunity of Neonatal Mice To E. Coli-induced Meningitis in an IL-1 Dependent Manner**

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Neonatal meningitis-associated *Escherichia coli* (NMEC) is the leading cause of bacterial meningitis in premature infants. The neonatal immune system has several differences compared to that of adults. It is known that cord blood monocytes secrete decreased levels of mature IL-1 compared to adult monocytes in response to stimulation with bacterial products, and that newborn humans and mice display increased nitric oxide (NO) levels compared to adults. We therefore wanted to determine the role of IL-1 and NO in the outcome of NMEC infection. In vitro, we show here that induction of IL-1 by macrophages and microglial cells infected with NMEC is dependent on NLRP3, a sensor molecule involved in the maturation of IL-1. We also show here that adult IL-1 receptor knockout (IL-1R⁻/⁻) mice have reduced survival and higher bacterial loads in the brain at 18 h following intracranial infection compared to wildtype, C57BL/6J mice (n=15-21/group), indicating a protective role of IL-1 during *E. coli* meningitis. To confirm our results in a neonatal mouse model, we infected 3-d-old C57BL/6J mouse pups intraperitoneally with NMEC and simultaneously treated them with either anti-IL-1R antibody, or an isotype control. Surprisingly, we did not see a significant effect of anti-IL-1R treatment with regards to bacterial loads in either the blood or brains of pups (n=6-8/group). It has been previously shown that nitric oxide can suppress NLRP3 activation and IL-1 production, and that inhibition of nitric oxide enhances resistance to NMEC infection. Confirming previous results, we show here that treatment of pups with an inducible nitric oxide synthase (iNOS) inhibitor at the time of NMEC infection significantly decreased bacterial loads compared to controls (n=5/group). Interestingly, we found that the protective effect of iNOS inhibition was lost when pups were also treated with anti-IL-1R antibody (n=11/group). This suggests that the protective effect of nitric oxide inhibition during NMEC infection is due to improved IL-1 signaling, and may indicate a target for future therapeutics.

**PS52 TKO hu-PBMC Humanized Mouse Model for HIV Research**

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Currently there is no cure or preventative vaccine for HIV infection, thus continued research is needed to end the
HIV pandemic. While there are many animal models used in HIV research, none is used more than the humanized mouse model. Humanized mouse models are all based around the use of immunodeficient mouse strains, most notably the NSG strain. While there are many uses for these current models, they all have a major limitation in common: development of graft-versus-host disease (GVHD). GVHD not only introduces variabilities into the research data, it leads to animal welfare concerns. A new mouse strain, B6.129S-Rag2tm1Fwa Cd47tm1Fpl Il2rgtm1Wjl/J (TKO), has been used to develop a humanized mouse model (TKO hu-BLT) that is resistant to GVHD development. We used TKO mice to develop a new hu-PBMC mouse model. Female TKO mice (n=7) were transplanted with human peripheral blood mononuclear cells (PBMCs) then monitored for engraftment by FACS analysis. A cohort of these mice (n=3) were infected with HIV-1 and monitored for plasma HIV viremia and CD4 T cell depletion. GVHD development was monitored by clinical signs. As a control, NSG mice (n=6) were used to compare the TKO mice because the NSG hu-PBMC model is the most commonly used model. First, TKO mice transplanted human PBMCs supported engraft of human immune cells: CD3, CD4, and CD8 T cells as shown by FACS analysis. Second, the TKO hu-PBMC model supports HIV-1 infection as seen by robust plasma HIV viremia and depletion of CD4 T cells over time as seen in the humans. Lastly, TKO mice showed a delayed onset of GVHD clinical signs (~14 d) compared to NSG mice. Based on these results, the TKO hu-PBMC mouse model not only supports humanization and HIV-1 infection, but is also resistant to GVHD development making this model valuable tool in HIV research.

PS53 Granulocyte Colony Stimulating Factor Plays a Role in the B Cell Depletion of Bone Marrow in MNV-infected Stat1 Knockout Mice

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Murine norovirus (MNV) is highly prevalent in laboratory mice and has been reported to infect macrophages, dendritic cells, T cells, and B cells. We previously showed that MNV infection in Stat1 knockout (KO) mice (129S6/SvEv-Stat1fl/fl) caused a significant depletion of developing B cell populations in the bone marrow (BM). Concurrent with this B cell depletion, MNV infected Stat1 KO mice also had a significant increase in BM granulocytes and serum granulocyte colony stimulating factor (GCSF). Therefore, we hypothesized that the increased GCSF and BM granulopoiesis directly contributed to the BM B cell depletion observed in MNV infected Stat1 KO mice. To test this hypothesis, uninfected female 5-9-wk-old Stat1 KO mice (n=5 per group) were IP administered either isotype IgG (10 µg/mouse) or GCSF (0.5 µg/mouse) daily for 7 days in order to induce granulopoiesis. BM B cells were then evaluated by flow cytometry to determine if B cell losses were observed, similar to that seen in MNV infected mice. We show that daily IP administration of GCSF for 7 days caused a significant depletion (P < 0.05) as expected. To further evaluate the role of GCSF, we administered either anti-GCSF antibody (10 µg/mouse) or isotype IgG (10 µg/mouse) daily for 7 days to MNV infected Stat1 KO mice (n=5 per group) to determine whether preventing granulopoiesis in the BM would rescue the B cell losses observed after infection. We show that daily IP injection of anti-GCSF antibody for 7 days postinfection in Stat1 KO mice resulted in increased (although not statistically significant) pre-B/immature and mature B cell populations (1.5 fold and 1.7 fold, respectively) and decreased granulocyte populations (1.4 fold) in BM compared to MNV infected mice. These results suggest that granulopoiesis induced by GCSF plays a role in the BM B cell losses seen after MNV infection in the absence of Stat1. Further study is warranted to determine why MNV infection induces increased GCSF and granulopoiesis in Stat1 KO mice.

PS54 Conditional Disruption of Hematopoietic Protein-1 In Mice Reveals an Essential Role for Hem-1 In Myeloid Cell Functions

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Hematopoietic protein1 (Hem-1) is a hematopoietic cell-specific subunit of WAVE (WASP-family verprolin homologous protein) complex, which acts downstream immune receptors (including BCR, TCR, TL1, and cytokine receptors) to stimulate filamentous actin (F-actin) polymerization. Inactivating mutations in NCKAP1L, the gene encoding Hem-1, have been recently associated with primary immunodeficiency disease in humans, and high NCKAP1L expression has been associated with a poor prognosis in chronic lymphocytic leukemia. Using constitutive Nckap1l null mice, we previously published that Hem-1 is critical for normal lymphopoiesis and innate immunity. However, the cell autonomous functions of Hem-1 in individual immune cell types remain an enigma. The objective of this study was to test our hypothesis that Hem-1 is critical for migration and phagocytosis by myeloid cells and antiviral immunity, in a cell-autonomous manner. Our approach was to create conditional Nckap1lfl/fl mice, which were bred to LyzMCre mice to delete Hem-1 specifically in myeloid cells. Using flow cytometry, fluorescence microscope, and time-lapse video microscopy, we found that neutrophils from Nckap1lfl/flLyzMCre mice exhibited defective F-actin polymerization and impaired migration in response to the chemoattractant 1 µM fMLP or LTB4, relative to LyzMCre control mice (n=4-5 male and female mice/group). Using 1µm fluorochrome labeled beads and flow cytometry, we found that Nckap1lfl/flLyzMCre macrophages were unable to efficiently phagocytose the beads (n=5 mice/group). Nckap1lfl/flLyzMCre mice were much more susceptible to 10 PFU influenza virus (H1N1/PR8) infection based on significantly increased body weight loss, increased peribronchial inflammation, reduced neutrophil and interstitial macrophage numbers, and increased pro-
inflammatory cytokines in bronchoalveolar lavage fluid, 6 days post-oropharyngeal influenza infection relative to Lyz/MCre control mice (n=5 mice/group). Collectively, our results reveal previously uncharacterized cell autonomous roles for Hem-1 in primary myeloid cells, and suggest that Hem-1 is critically important for effective antiviral immunity. Statistical significance was determined by a two-tailed student’s t-test.

**PS55 Conditional Deletion of Nckap1l Encoding Hematopoietic Protein-1 Alters B Cell Maturation, Antibody Production, and Immune Responses to Influenza Virus**

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Mutations in genes encoding actin-regulatory proteins can lead to primary immunodeficiency diseases (PIDs) characterized by impaired immunity and reoccurring debilitating infections. Utilizing a chemical mutagenesis strategy in mice to discover novel genes involved in immune system function, we previously identified a strain, NTB.1, with reduced B and T cell numbers due to a non-coding mutation in the Nck-associated protein 1-like gene encoding Hematopoietic protein-1 (Hem-1), a hematopoietic cell specific actin regulatory protein. NTB.1 mice are severely immunodeficient, characterized by reduced B and T cells, impaired neutrophil migration and phagocytic abilities. To determine why B cells are reduced in NTB.1 mice, we utilized the Cre-LoxP system to generate mice (Nckap1l<sup>fl/fl</sup>Mb1Cre<sup>+</sup>) that were conditionally deleted for Nckap1l in a B cell specific manner. We hypothesized that Hem-1 is important in B cell development, B cell migration, and antibody production. Flow cytometry revealed that deletion of Hem-1 resulted in impaired follicular and marginal zone B cell development and reduced innate-like B1a cells (4 independent experiments with n ≥4 group). Using flow cytometry and ELISA, we found that Nckap1l<sup>fl/fl</sup>Mb1Cre<sup>+</sup> mice had decreased ability to form germinal centers in mediastinal lymph nodes and impaired antigen-specific antibody production 10 days following oral pharyngeal influenza PR-8 infection, relative to WT littermate mice (n=6 per group). Nckap1l<sup>fl/fl</sup>Mb1Cre<sup>+</sup> had reduced antibody production 5 days following immunization with heat-killed S. pneumoniae (a T-independent antigen), and reduced long-lived antibody production at 3 and 4 wks post-immunization with the T-dependent immunogen Keyhole Limpet Hemocyanin (KLH) (n=6 per group). These results suggest that Hem-1 is essential for normal B cell development and antibody production in a B cell specific manner. We predict that mutations in Nckap1l could be the cause of some undefined PIDs in humans characterized by deficient antibody production and increased susceptibility to bacterial or viral infections.

**PS56 Inhibition of Oxidative Phosphorylation but not Glycolysis Attenuates Lung Injury Caused by H1N1 Influenza A Virus Infection**

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Despite availability of vaccines and antiviral drugs, seasonal influenza A virus (IAV) epidemics cause >300,000 deaths/year worldwide. IAV infection alters lung epithelial cell metabolism. This promotes a shift towards glycolysis and away from oxidative phosphorylation (OXPHOS) for ATP production. We hypothesized that this shift benefits the virus rather than the host and that inhibition of glycolysis would improve infection outcomes. C57BL/6 mice (n=5-6/group) were intranasally inoculated with mouse influenza A/WSN/33 (H1N1). Controls were mock-infected with virus diluent. To inhibit glycolysis, mice were treated daily from 1 day postinfection (dpi) with 1g/kg 2-deoxy-D-glucose (2-DG). To block OXPHOS, mice were injected every other day from 1 dpi with 0.8 mg/kg rotenone (ROT). Treatment controls were treated with saline. Carotid arterial oxygen saturation (S<sub>O2</sub>) was measured using the MouseOx system at 2, 4, and 6 dpi. Open circuit calorimetry and measurement of mouse activity were performed simultaneously using the Oxymax/CLAMS metabolic chambers from 5-6 dpi. On d 6, mice were euthanized and lungs harvested. Viral replication was quantified by serial dilution and plaque assay of lung homogenates on MDCK cells. Whole lung wet: dry weight ratios were calculated as an index of intrapulmonary fluid accumulation. Relative to controls, IAV infection induced severe hypoxemia and pulmonary edema at 6 dpi. There was a significant decline in nocturnal activity and a decrease in the respiratory exchange ratio (RER), indicating a shift towards increased lipid catabolism for ATP generation. Treatment of IAV-infected mice with the glycolysis inhibitor 2-DG and the OXPHOS inhibitor ROT did not alter lung IAV titers; however, 2-DG significantly worsened IAV-induced hypoxemia and further decreased nocturnal activity. In contrast, ROT treatment restored S<sub>O2</sub> to normal levels, normalized RER, and significantly attenuated IAV-induced pulmonary edema. Blockade of OXPHOS with ROT improves outcomes in IAV-infected mice while inhibition of glycolysis exacerbates severity. This indicates that a shift to glycolysis is protective in influenza and suggests that OXPHOS may be a therapeutic target in this disease.

**PS57 Nutritional Gel Supplementation Minimizes Weight Loss in Mice Infected with Influenza A/PR/8/34 Virus**

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Influenza places a large burden on public health, and is therefore a highly researched disease process that uses in vivo studies regularly with mice as a commonly used model. A complication of the clinical course of influenza infection in mice is anorexia and dehydration leading to excessive weight loss often resulting in the early removal of
mice from study based on euthanasia criteria. To reduce the number of mice prematurely removed from an experiment, we assessed the use of a nutritional gel (NG) supplement that is palatable and provides hydration, calories/carbohydrates, and electrolytes. We hypothesized that when compared to the standard of care, supplementation of NG to mice infected with influenza would lead to decreased weight loss and mortality without impacting the immunologic data of a study. Both male and female C57Bl/6J mice were infected with mouse-adapted influenza A/PR/8/34 virus at low (0.2 times the lethal dose 50 (LD50); SOC n=21, NG n=20), medium (0.5 LD50; SOC n=30, NG n=27), or high doses (1.25 LD50; SOC n=23, NG n=22). Mice were provided with either the standard of care (moistened pellets + hydration gel), or moistened pellets + NG supplementation. Weights were monitored daily and mice were removed at 30% loss of initial weight. There was a significant difference (P < 0.05) seen in the maximum percent weight loss of mice given NG in all viral doses used. There was significantly decreased mortality (P < 0.05) seen in the mice given NG at the middle and high viral doses. Flow cytometry on cells from bronchoalveolar lavage fluid, collected at 6 d postinfluenza infection from female mice (n=10) given the middle dose, demonstrated no significant differences (P > 0.05) in percentage and absolute numbers of inflammatory cell populations (specifically resident and interstitial macrophages, neutrophils, eosinophils, T-cells, and B-cells). In summary, the results of this study show that supplementation of NG can be beneficial in reducing weight loss and mortality in mice infected with various doses of mouse-adapted influenza virus.

PS59 Gut Microbiota Alterations in Marmoset Wasting Syndrome: A Cross-Population Study

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In captivity, common marmosets (Callithrix jacchus) are susceptible to marmoset wasting syndrome (MWS), characterized by chronic enteritis and progressive weight loss. MWS, like human inflammatory bowel diseases, may be associated with gut microbial dysbiosis; thus, investigating the gut microbiome of marmosets may inform prevention and management of MWS. Using 16S rRNA sequencing of rectal swab samples, we characterized the gut bacterial microbiota of marmosets in captive colonies with differing MWS prevalence: German research university (MWS 0%; healthy n=15), German primate center (MWS 0%; healthy n=18), and United States (US) research university (MWS 5-10%; healthy n=18; MWS-affected n=6). German-origin (GO) marmoset samples were collected at three time points: 1) upon arrival at the US facility (baseline), 2) following transition to US diet in quarantine (100 d), and 3) following social integration with US colony marmosets (1 y), to dissect the contributions of diet and social integration in shaping the gut microbiota. At baseline, GO samples displayed greater phylogenetic diversity and less species evenness, features of greater microbial resilience, compared to healthy US samples. Evenness increased and richness decreased in GO samples at 100 d, but rebounded to baseline at 1 y. Beta analysis revealed increasing similarity of US and GO gut microbial communities at 1 y compared to baseline; a greater shift in GO samples suggested a role of diet in shaping the gut microbiota. Peptostreptococcusace, implicated in colorectal cancer and colitis in humans, was present in 14/18 (78%) US samples and 4/33 (12%) GO samples at baseline. Both increased occurrence (11/29; 38%) and relative abundance were found in GO samples at 100 d; further enrichment
(17/30; 57%) occurred at 1 y, implicating diet and possibly integration as causative. When comparing healthy and MWS-affected US animals, *Prevotella*, associated with inflammation in mice and humans, was significantly enriched in the MWS-affected group. Significant differences in the microbiota of GO versus US marmosets suggest that the gut microbiome plays a role in development of MWS. Enrichment of *Prevotella* in animals with MWS provides a compelling direction for future studies of disease pathogenesis.

**PS60 Systemic Coccidiosis Causing Fulminant Mortality in a Colony of Wild-caught European Starlings (Sturnus vulgaris)**

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Disseminated disease caused by coccidia (systemic isosporosis, also known as atoxoplasmosis) is a common parasitic disease of passerine birds. Twenty-one European starlings (*Sturnus vulgaris*) were caught in California in 2016. The time between arrival and onset of clinical signs ranged from 2 d to 2 mo, and signs included anorexia, dropping food, neurologic deficits, conjunctivitis, and sudden death. At the time of death, birds ranged from fledglings to adults (n=21), with most of the birds ranging from 3-6 wk of age (n=10). Hematologic revealed peripheral eosinophilia and lymphocytic intracytoplasmic inclusions in most, but not all, birds. Fecal evaluation revealed cysts measuring 39 x 39 to 39 x 44 mm. Necropsy revealed decreased pectoral musculature, blepharedema, prominent keels, enlarged pale livers and spleens, and thickened intestinal walls. Histology revealed florid lymphocytic inflammation with intracytoplasmic parasitic inclusions commonly affecting liver, spleen, conjunctiva, connective tissues surrounding the thyroid glands, and bursa of Fabricius. Samples collected for molecular screening of common avian pathogens were negative. Oral ponazuril treatment was initiated, and this improved the clinical symptoms in some, but not all, birds. Because of the variation in clinical manifestations, circulating parasitic load, intestinal parasitic burden, and parasitic stages present, we query if these differences represent the true spectrum of lesions observed in infection with a single parasite, or if they represent infection with more than one (or different strains of) coccidian parasites.

**PS61 Wound Management of Foreign Body Reactions in Nerve-grafted Dorset Sheep (Ovis aries)**

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Two of seven 2-year-old, female Dorset sheep (*Ovis aries*) on a nerve regeneration study received 7-cm-long bioengineered grafts imbibed with autologous stem cells in their median (left forelimb) and sural (right hindlimb) nerves. Postoperative complications, specifically surgical site edema and dehiscence, occurred on d 6 in the forelimbs of both animals. The edema and inflammation in the first sheep developed to an abscess (cultured as Trueperella pyogenes, Fusobacterium necrophorum, and Bacteroides fragilis) despite medical treatment, and a draining tract eventually resulted. Daily treatments included standard wound management, analgesia, and preemptive antibiotics. Despite multiple attempts to debride and close the wounds, both complications progressed until a discrete, long, thick, white, fibrous tissue was found protruding from the sites. This tissue was sampled for histology, which revealed a granulomatous inflammation consistent with a foreign body reaction to an undetermined, eosinophilic, spheroidal material. Both animals were taken to surgery for removal of the white, fibrous tissue (presumed to be the bioengineered conduit) as well as debridement of necrotic tissue. Both sheep achieved complete resolution of their forelimb complications within 4 weeks post-debridement. Throughout this time, the sheep maintained appropriate mentation, activity, appetite, hydration status, and pain management. Despite the pronounced foreign body reaction witnessed in these two cases, all hindlimbs of the seven sheep healed normally after the engraftment procedure. However, all forelimbs of the post-surgical forelimbs developed some level of edema and inflammation, suggesting that forelimb anatomy (i.e., high tension of skin, increased friction of incision site at axilla) contributes. In these two cases, it is proposed that two separate and unrelated post-surgical events acted as inciting causes for a heightened inflammatory response, ultimately resulting in a foreign body reaction. Foreign body reactions should remain as a differential despite previous successful engraftments.

**PS62 Nursing Care of Rabbits with a Spinal Cord Injury**

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Spinal cord injury (SCI) surgery models and the related postoperative care is well established in rodents, however limited information is available for a rabbit model. Our veterinary staff was approached with clinical management of a surgical SCI protocol in rabbits. All rabbits received a transverse resection of the cord at T8-9; the experimental group received a polymer implant around the spinal cord, providing a cylindrical space for cord repair and regeneration. We present the nursing challenges of the model and animal management. We created a postoperative care plan that addressed major concerns related to SCI, such as ambulation, bladder atony, fecal incontinence,
appetite, and postoperative analgesia. The plan included chewable probiotics and critical care diet gel for gastrointestinal concerns, bandaging feet to prevent autophagia, and bladder expression. Infant pants protected against urine scald and created an additional barrier to prevent autophagia. All rabbits were given a sustained release opioid and nonsteroidal antiinflammatory analgesic. A postoperative schedule was created to perform assessments and care every 8 h, which included manual bladder expression, pain assessment, cleaning of the perineum with pants change, bandage integrity check, and evaluation of food and water consumption. Food and water in shallow bowls, hay, and fresh fruits and vegetables were provided on the cage floor. Hay was also available in stainless steel whisks, and fruit and vegetables were hand fed multiple times a day. All rabbits handled the recovery process differently, behaviorally and physically. Their level of mobility, mentation, and bladder tone varied regardless of their group designation. Fine attention to detail was crucial in keeping the rabbits comfortable for the duration of the study. The success of the experiment, ease of clinical management, and carefully designed nursing care indicates the viability of the rabbit as a spinal cord injury model.

**PS63 Polyglactin 910 Suture-related Pseudoinfection in a Yucatan Pig**

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Cutaneous suture reactions related to inappropriate suture material selection or delay in removal are well documented in both the human and veterinary medical literature. However, foreign body extrusion of an internal absorbable suture has not been well described in the veterinary literature. A female, intact Yucatan pig arrived in apparent good health, and due to closure of study funding, remained naive. As such, a routine ovariectomy was performed for animal health and as required by the vendor to allow for potential adoption. Polydioxanone was used for the entire procedure except for ovarian pedicle ligation in which Polyglactin 910 was elected due to its low memory, ease of handling, and knot security. Approximately 3-4 mo later, recurrent small masses on bilateral flanks of the animal were noted. Diagnostics including cytology, fungal, and bacterial culture, and bloodwork all supported a noninfectious etiology. Biopsy results from the site indicated a suture-related pseudoinfection. Despite treatment including topical, oral, and parenteral antibiotics, surgical debridement, and nonsteroidal antiinflammatory drugs, these lesions did not resolve. Eventually, chronic lameness developed in the worst affected side, and euthanasia was elected. Postmortem necropsy definitively demonstrated a suture-related pseudoinfection with extrusion of suture material from the ovarian pedicle ligatures through the body wall and skin leading to numerous sterile abscesses in the flank areas. Though well documented in the human literature, this is the first report of Polyglactin 910 suture-related pseudoinfection in a veterinary patient. While this may be an isolated incident, it may also indicate an increased sensitivity by Yucatan pigs and support presurgical assessment with vicryl hypersensitivity patch testing similar to what is performed in at risk human patients.

**PS64 Avian Poxvirus Infection in a Colony of Brown-Headed Cowbirds (Molothrus ater)**

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A cohort of 24 adult male and female brown-headed cowbirds (Molothrus ater) were wild-caught using an outdoor funnel trap on an IACUC-approved protocol. The birds were quarantined in an outdoor aviary for 2 mo prior to being brought indoors. Three days following arrival to the indoor facility, 9 of 24 birds were reported for multiple, tan, proliferative, 2.0mm-3.0mm masses on 1 or more digits. Additionally, 2 of the birds had a small nodule located on the dorsal maxilla. All birds otherwise appeared clinically healthy. One wk later, a female bird developed 5 3.0mm-5.0mm masses on multiple digits. This bird was euthanized and submitted for postmortem examination. Microscopically, there was severe multifocal lymphohistiocytic dermatitis with hyperkeratosis and intraepithelial eosinophilic intracytoplasmic inclusion bodies (Bollinger bodies), which are pathognomonic for avian poxvirus infection. Over the next month, masses on other affected birds resolved spontaneously and did not reoccur. However, 1 male bird developed a severe beak malformation resulting in inability to close the beak. This bird was euthanized, and histopathology of the beak showed extensive necrosis and inflammation. Eosinophilic intracytoplasmic inclusion bodies were present in mononuclear cells in the nasal and oral submucosa, which is consistent with poxvirus infection. One mo later, an additional cohort of cowbirds was brought indoors for over-wintering. Several of these birds developed similar small pox lesions on the digits. All birds remained otherwise clinically healthy, and all lesions resolved spontaneously. Other than the 1 bird with beak necrosis, all pox lesions were cutaneous. Since no birds displayed clinical signs until being moved to the indoor housing facility, transport stress likely led to the development of the pox lesions.

**PS65 Feline Atypical Mycobacterial Panniculitis Caused by Mycobacterium porcinum**

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A 12-y-old castrated male DLH cat presented with multiple dry lacerations measuring 3mm to 1cm on the caudal abdomen. The cat failed to respond to systemic amoxicillin/clavulanic acid and topical chlorhexidine soaks. Within 2 wk, additional lesions, some with purulent discharge, were present, and Achromobacter spp., sensitive to...
amoxicillin, was cultured. Lesions did not resolve after 2 mo of antibiotic therapy, and complete surgical excision was attempted. Histologic analysis showed multifocal inflammatory infiltrates associated with granulation tissue and small cavitated spaces with acid-fast-negative, Gram-positive bacilli bacteria, leading to a presumptive diagnosis of Actinomyces spp. New abdominal lesions noted at suture removal continued to wax and wane but never resolve despite varied, intensive systemic and topical treatment. The cat did not exhibit additional clinical signs until 18 mo after the initial presentation when he became lethargic and anorexic. A fast-growing mycobacterium was then cultured from the draining lesions and submitted for identification by whole genome sequencing (WGS). The cat continued to deteriorate and was euthanized prior to final identification of the isolate. The draft genome of the isolate was sequenced followed by assembly into contigs with SPAdes and gene annotation with RAST hosted by PATRIC. The resulting genome was 6,796,350 bp in size with a GC content of 66.8% and contained 6,630 protein-coding genes, 52 tRNA genes, and 5 rRNA genes. Using the tetra correlation search function hosted, the cat mycobacterium genome was most closely related to Mycobacterium porcinum, a fast-growing mycobacterium that belong to the M. fortuitum third biovariant complex. Whole-genome phylogenetic and average nucleotide identity analyses confirmed the cat mycobacterium genome as M. porcinum. M. porcinum has not been reported as a cause of feline atypical mycobacterial panniculitis, nor has it been reported as a zoonotic agent. Historically, inconsistencies regarding published mycobacterium genomes made speciation difficult prior to WGS. Accurate genome sequencing and reporting is essential due to the zoonotic potential of some mycobacterial spp.

### PS66 Observational Learning Facilitates Positive Reinforcement Training in Macaques (Macaca mulatta)

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Studies have pointed to the many benefits of positive reinforcement training (PRT) to animal wellbeing. However, despite the benefits, training can involve a large initial input of time, which may preclude its use in some facilities. Further, not every animal learns at the same rate. Inhibited or shy animals may take longer to train than exploratory animals. Therefore, finding alternate training methods that can make the process less time consuming could be of great value. Because primates are known to learn by observing the actions of others, one potential alternate technique is to have subjects watch conspecifics being trained. In this study, we examined whether observational learning facilitates training for shy female rhesus macaques (Macaca mulatta). All females were housed with their infants. We first trained 6 bold monkeys (“demonstrators”) for 2 tasks using PRT: touch a target and present for menses check. We then trained 12 shy monkeys for the same tasks. Eight of these monkeys (“observers”) watched the demonstrators being trained, while the others (“non-observers”) had not. As expected, bold monkeys were more likely than shy monkeys to learn this task within 12 sessions ($P < 0.05$). Observers were more likely than nonobservers to perform the task as well. While 6 of the observers reliably presented, none of the nonobservers learned this task. To see if observational learning applied to the infants, we trained infants to touch a target. Nine of the infants were with their mothers when they were trained for the 2 tasks, 6 watched a female other than their mother being trained, and 5 did not observe any training. Infants whose mothers were trained learned this task sooner than other infants ($P < 0.01$). These results suggest that observational learning may facilitate PRT for inhibited monkeys. Further, it may be an effective tool for training young monkeys.

### PS67 How Can the IACUC Encourage and Expand Positive Reinforcement Training Programs?

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The process of reviewing and approving research protocols by the IACUC can be a powerful method for enhancing positive reinforcement training of monkeys assigned to research projects. Promoting the increased use of positive reinforcement methods is compliant with many statements in The Guide for the Care and Use of Laboratory Animals (2011). Many behaviors that are commonly part of research procedures can be trained with positive approaches, including shifting to a new cage or location for testing, temporary separation from a partner, receiving an injection, performing a blood withdrawal, or moving into a restraint chair. When reviewing IACUC protocols, IACUC members can ask questions about the planned training processes, the expected duration of training prior to initiating research procedures, criteria for considering a monkey to be fully trained on a particular behavior, what will be done if a monkey does not adequately learn the procedure on time, and how regression will be handled. To accomplish this, at least some IACUC members must be familiar with animal training methods, and be aware of the types of behaviors that are currently being trained across the laboratory animal science field. Some facilities can refer investigators to primate training specialists to help plan for animal training as studies are being designed and this process will be described. Some IACUCs may include assessment of animal training procedures when they conduct postapproval monitoring of research studies. The IACUC can be instrumental in encouraging investigators to consider the role that positive reinforcement training can play in their research, and in improving the welfare of research primates.
Preference assessments systematically and objectively identify items that are preferred and could serve as reinforcers for positive reinforcement training (PRT). This study used a multiple stimulus without replacement (MSWO) preference assessment to determine preference hierarchies of six food items with Cynomolgus macaques (n=14; 11 males and 3 females). Additionally, seven macaques completed concurrent-schedule reinforcer evaluations to confirm the preferred items efficacy as reinforcement. Average food ratings showed the macaques had a preference for yogurt covered treats (74.13 ± 21.55) followed by grapes (58.74 ± 20.01), dried pineapple (33.13 ± 12.96), banana chips (25.73 ± 11.72), dried apricots (20.35 ± 5.08), and peanuts (19.99 ± 10.69). Individual preferences were confirmed by the reinforcer evaluations in which the primates were more likely to perform a task for their most preferred item (average engagement score > 80%) versus the least preferred or control condition. When choice for all food items was examined over a month there was a great deal of instability in preference as shown by less than significant Kendall’s tau correlation coefficients (P > .05). However, when stability of the most preferred item alone was examined, the item chosen first remained consistent for most of the macaques (12 of 14). On average the MSWO required three trials to identify preference and took approximately 4 m to complete. These results suggest the MSWO is a practical and effective method to identify preferred food items and can be used to inform reinforcement choices in PRT programs.

**PS69 Programmed Training-Primate Computer Learning as a Model of Positive Reinforcement Training**

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Positive reinforcement is defined as adding a desired stimulus following a behavior, resulting in the behavior being more likely to occur in the future. Being able to program the exact timing and amount of rewards while maintaining absolute consistency makes computer-based training an ideal model of positive reinforcement as well as a valuable tool for data collection. Automated systems can continually calculate rates of correct trials, giving the trainer immediate feedback on the animal’s learning and the ability to make adjustments that will maximize success. Such computer-based training systems can be set up remotely, thereby allowing 1 person to train multiple animals at a time while also eliminating the potential biases that come with human interaction. One of the major challenges to computer-based training is anticipating the successive approximations needed for learning. Clear and accurate training plans, which break down each task into its smallest components, need to be developed by the trainer and communicated to the task programmers in order to allow the animals a seamless learning process. An accessible user interface which allows the trainer to move the animal through each approximation with minimal interruption can help create that kind of seamless experience. At our facility, computer learning is used in a variety of ways, from environmental enrichment in behavioral management to virtual reality testing in neuroscience. This introduction will show the possibilities for computer learning with examples of tasks that range from the beginning of touch screen interaction to complex memory tasks in virtual reality.

**PS70 The Closed Box Chair as a Refinement in Nonhuman Primate Sperm Collection**

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Collecting semen from macaques for assisted reproductive technology (ART) procedures is critical for the success of those projects. In order to get the samples, monkeys are often restrained in open restraint chairs (ORC) using the pole and collar technique. While commonly used, this restraint is not tolerated by all monkeys; some become anxious or aggressive towards the poles and people. In an effort to refine this procedure and improve the welfare of the monkeys, we recently modified a closed box chair (CBC), a clear, plexiglass box in which the monkey is trained to sit. Unlike the ORC, the CBC does not require pole and collar, and although legs are secured, the monkey’s arms and neck are not restrained, allowing more freedom than with the ORC. Because it is thought to be an improvement in welfare, use of CBCs has increased in recent years; however, there are few studies demonstrating its effects on scientific outcomes. We compared semen quality in samples taken from 5 adult male macaques (2 rhesus Macaca mulatta; 3 cynomolgus, M. fascicularis) trained to participate in semen collection using both methods (8 samples from each method). There was a significant increase in sperm concentration in samples taken in the CBC compared to ORC (536 vs 199 million/ml, respectively; P < 0.05). While there was no change in motility, there was nearly a 3-fold increase in total ejaculatory volume (0.98 mL for CBC vs 0.35 mL for ORC). In addition, it took less time to train monkeys for the CBC compared to ORC, and the monkeys showed fewer behavioral indices of stress. These preliminary data suggest that the closed box chair technique reduces stress on the animals, while enhancing the quality and quantity of sperm samples, supporting the use of the CBC as a refinement.
PS71 The Role of Positive Reinforcement Training in the Restraint of Primates

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Positive reinforcement training (PRT) is applied across the globe with nonhuman primates (NHP) in the laboratory animal science environment. PRT is used to train cooperation with husbandry, clinical, and research behaviors, including teaching NHP to tolerate some restraint behaviors such as the cage squeeze back mechanism, chair restraint, and manual restraint. Benefits of using PRT include increased cooperation with husbandry, clinical and research procedures, increased welfare, decreased stress and fear, decreased time to conduct procedures once training is complete, and increased safety for animals and personnel. There are many factors that contribute to the successful implementation of PRT for restraint behaviors within animal use programs. These factors include a clear understanding of timelines for when the behavior is needed, support from institutional administrators and program managers, PRT knowledge and experience of staff members, strong communication among staff, effective animal training transfer and maintenance plans, and understanding NHP typical and atypical behavior. Successful training for restraint behavior involves the use of problem solving skills and a working knowledge of how to apply PRT with other techniques such as desensitization, acclimation, negative reinforcement, rates of reinforcement, and preference testing. Training for restraint using PRT techniques has been demonstrated to positively impact animal welfare via physiological and behavioral measures and is a powerful tool in the laboratory animal science field.

PS72 Physical Therapy for Captive Chimpanzees Using Positive Reinforcement Training

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Aging captive chimpanzees face age-related health conditions (e.g., arthritis, stroke, and mobility impairment) and using behavioral management programs to establish personalized care routines are a valuable tool for the care and wellbeing of the animals. We are implementing and evaluating the behavioral, welfare, and mobility effects of a physical therapy (PT) program using positive reinforcement training (PRT). Personalized therapy and exercise routines, including squats, standing, finger and toe extensions, weight shifts, and climbing were created for 9 chimpanzees with mobility impairments resulting from arthritis, stroke, or injury. Chimpanzees voluntarily participate in these routines twice per week in an effort to increase strength, dexterity, and flexibility. Veterinary technicians rate each chimpanzee on 6 categories of movement and mobility (on a scale of 1-5) once per month using a subjective scoring system. Lastly, trained caregivers rate affiliative, aggressive, play, fear-related, and anxiety-related behaviors, as well as overall levels of wellbeing, ease of movement, activity levels, and physical health each week. Every month, each chimpanzee’s mobility rating and progress are assessed, and new PT targets are created or increased (e.g., increase squats from 10 to 15). To date, all 9 chimpanzees voluntarily participated in PT without hesitation. After five weeks of participation, paired-samples t-tests showed that caretaker ratings of well-being increased significantly ($P=0.026$), while ratings of ease of movement did not significantly differ ($P=0.098$). These preliminary data suggest that PT using PRT is an effective refinement to captive care that allows chimpanzees to increase their mobility. This may also be an enriching experience for the chimpanzees, as PT increases the level of human interaction and choice within the captive environment.

PS73 The Role of Positive Reinforcement Training in Veterinary Care: Improving Welfare One Grape at a Time

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Positive reinforcement training (PRT) is used to enhance the welfare of NHPs in many ways. Training the animals to participate in their own veterinary care not only decreases the stress often associated with veterinary procedures, it also provides an enriching activity for the animals. Sedations and anesthesia are stressful, even if we train the animals to present for an injection. We can minimize this stress by using PRT to train the animals to voluntarily cooperate with their own care, often eliminating the need for sedation and anesthesia. We have trained NHPs to present wounds for treatment, eyes/ears/throat for diagnostics, and arms/legs for blood sample collection. Nonhuman primates have also been trained to urinate on demand, present for evaluation of blood glucose values and insulin administration, and cooperate with integrative pain-relieving therapeutics such as acupuncture and laser therapy. Not only do these training opportunities improve the welfare of the animals, they are also enriching for the staff. When NHPs are trained to voluntarily provide diagnostic samples, the time required for collection decreases and our programs become more efficient.

PS74 The Interplay between Temperament and Operant Learning in Primates to Cope with Medical Management

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Training primates to cooperate with activities associated with husbandry and medical management is paramount in improving welfare and scientific validity. The laboratory environment presents stressors for primates that have limited experience with close contact with humans and medical equipment. Successful training results in enhanced coping skills and gives animals the opportunity to exercise a degree of control and choice over environment. Previous research has linked temperament in humans and primates to how they respond to stressful situations. We evaluated the relationship between primate temperament, successful behavior acquisition, and coping. We retrospectively reviewed data from 60 primates including rhesus macaques (Macaca mulatta) and cynomolgus macaques (Macaca fascicularis) of different ages and sexes. At entry into the colony, primates were observed by experienced trainers who scored behavioral traits which allowed categorization as either an “exploratory” or “inhibited” temperament. Primates were trained using mixed reinforcement training, primarily positive reinforcement, to perform a complex behavior (present limb to a trainer for medical manipulation in their home enclosure) in 3 phases. We found a negative association between willingness to accept a treat from the hand in primates with an inhibited temperament at colony entry; still, inhibited animals learned at a similar rate as exploratory animals. All animals completed training, learned the task, and demonstrated behaviors consistent with productive coping evidenced by voluntary approach and engagement with trainers. The use of a training paradigm designed to build trust and encourage animals to value rewarding over avoidance, was successful in moderating the role of temperament in performing a complex task as measured by total training time, total training sessions, and task acquisition. After controlling for other variables, we observed rhesus macaques learned faster than cynomolgus macaques, and this difference was significant. Considering animal attributes in relation with training methods allows us to develop efficient behavioral management programs. This improves the wellbeing of our primates and also the rigor of our scientific outcomes by limiting stress-confounding or introduction of enrollment bias.

**PS75 Simplifying Nonhuman Primate Positive Reinforcement Training through Innovative Cage Design**

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We designed and built an indoor group housing area for female macaques (Macaca fascicularis). It was important to design caging that would enhance welfare, facilitate animal training, and improve efficiency by incorporating knowledge of the animals’ behavior into the cage design. There are 27 individual units, each housing up to 11 females that require daily vaginal swabs for menses cycle tracking. To simplify animal training, we designed a tunnel system by which the animals enter via the back of the cage. They are trained to come to a holding cage for swabs before being released into the main unit. Between June 2017 and September 2018, we received 556 females into the group housing area. All of the females were naïve to the caging and training, but by capitalizing on their natural behavior, we successfully trained them how to enter the tunnel within 1–2 training sessions. In September 2018, data collected on 44 naïve females, confirmed 100% compliance with animals coming forward for vaginal swabs before being released into the main unit. Between June 2017 and September 2018, we received 556 females that require daily vaginal swabs for menses cycle tracking. To simplify animal training, we designed a swabs after 21 days of positive reinforcement training. Understanding the animals’ natural behavior and incorporating it into caging design allowed for improved efficiency with regards to animal training, husbandry needs, and research activities.

**PS76 Assessing Stress Levels in Primates Using Real-time Glucose Determinations via Cutaneous Sensors**

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Does acclimatization to physical restraint procedure in an infusion chair over repeated training intervals reduce the stress level in primates? Continuous tissue glucose levels were evaluated and compared with telemetric cardiovascular endpoints (heart rate and blood pressure) taken during repeated physical restraint in infusion chairs. Four cynomolgus macaques where implanted with a telemetry device to obtain BP and ECG data in this IACUC-approved study. Additionally, a flash glucose monitoring system for real-time tissue glucose measurement was attached to the skin on the animals’ back. Animals were dressed with a jacket to avoid interference with the device. Tissue glucose data was continuously measured and remotely collected over 24 h for 8 consecutive d. Telemetric data was recorded for 2 h before, during, and after physical restraint for 6 h in total. To determine the effect of physical restraint, the animals were placed in an infusion chair on 5 consecutive d for 2 h and once again on d 8, following 2 d rest. After animals were hand caught and placed in the infusion chair, glucose levels and heart rate increased in parallel as expected. However, while tissue glucose decreased under physical restraint back to nearly baseline within 1 h, heart rates remained elevated until the animals were released into their home cages. On the following d 2 to 5 tissue glucose peaks were lower each consecutive day whereas the heart rate increase in the infusion chair remained comparable to d 1. On d 8, when animals were replaced in the infusion chair again, after 2 d without restraint, the tissue glucose peak reached comparable d 1 results again. Real-time tissue glucose data capture with the flash glucose monitoring system is feasible in macaques over at least 8 consecutive d. Glucose levels during physical restraint increased in parallel with telemetric heart rate. The consistent increase of the heart rates indicated that at least a period of 5 consecutive d did not elicit any training effects reducing the stress level. The study design did not allow distinguishing clearly whether the increase in glucose levels was clearly attributed to stress or more likely caused by increased muscle activity.
PS77 Light: An Extrinsic Environmental Factor that Influences Animal Health and Wellbeing

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Light is an extrinsic environmental factor in animal facilities that profoundly influences animal circadian, neuroendocrine and neurobehavioral regulation. Previous studies from our laboratory demonstrated that exposure of pigmented and non-pigmented rats to light at night (LAN) of sufficient intensity, wavelength, and duration suppresses nighttime pineal circadian melatonin production and negatively influences metabolism and physiology. In contrast, exposure of rats to blue-enriched (465-485 nm) LED light at daytime (bLAD) revealed a marked positive effect on neuroendocrine and neurobehavioral parameters. Here, in conjunction with our GLAS-supported investigations, we tested the hypothesis in mice that LAN disrupts, while bLAD enhances, circadian rhythms of metabolism and physiology associated with animal health and wellbeing. We examined male and female nude mice (Crl:NU(NCr)Foxn1; n=36 per group), commonly used in metabolism and cancer studies, and exposed them for 12-weeks in an AAALAC-accredited facility to either bLAD or standard broad-spectrum (300-700 nm) cool white fluorescent (CWF) light on a common lighting regimen: 12L(35.9 ± 1.3 lx [within cage];12D, lights on 0600); CWF animals were also exposed to 0.2 lx (0.08 µW/cm²) CWF light during dark phase (LAN). Results showed significantly lower dietary and water intake, and body growth rates in both male and female mice maintained under bLAD versus LAN (P < 0.001). Arterial plasma nighttime circadian melatonin levels were over 400-fold higher in the bLAD- versus LAN-exposed mice, while daily rhythms of arterial plasma total fatty acids, glucose and lactic acid levels, and pO2 and pCO2, were significantly lower (P < 0.001) in bLAD-exposed mice. The present findings suggest that daytime exposure of mice to bLAD, compared to LAN, has a profound positive impact on the circadian regulation of neuroendocrine, metabolic, and physiological parameters that influence laboratory animal health and wellbeing, and ultimately scientific outcomes.

PS78 The Influence of Daytime LED Light Exposure on Circadian Regulatory Dynamics of Mouse Metabolism and Physiology

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Light and lighting protocols, as outlined in the Guide, are important to both biomedical researchers and animal care personnel alike. Light entrains the master biological clock within the suprachiasmatic nucleus (SCN), which regulates the nocturnal pineal melatonin signal that temporally coordinates circadian rhythms of animal metabolism and physiology. Previous studies from our laboratory demonstrated that the wavelength (color) of light impacts these responses in rodents. We tested the hypothesis that daytime exposure to the most commonly used LED lighting, enriched in the blue-appearing portion (460-480 nm) of the visible spectrum (bLAD), compared to standard, broad-spectrum (300-700 nm) cool white fluorescent lighting (CWF), influences circadian regulation of metabolism and physiology in 3 important strains of male and female mice (C3H [melatonin-producing]; C57BL/6 and BALB/c [melatonin-non-producing]; n=120/group). Animals under an IACUC-approved protocol were maintained in an AAALAC-accredited facility for 12 wk on a common lighting regimen 12L (68.8 ± 5.2 lux; 168.6 ± 12.8 µW/cm² [within cage]; lights on 0600 h):12D (0 lux) on either CWF (control) or bLAD (experimental) lighting, and were assessed at 6 circadian time points. Compared mice housed under 12:12-h light:dark cycle in CWF light, C3H mice in bLAD evinced a 6-fold higher peak nighttime plasma melatonin level (P < 0.05). C57BL/6 and BALB/c strains did not produce nighttime pineal melatonin. Body growth rates, dietary and water intake, circadian rhythms in arterial blood corticosterone, insulin, leptin, glucose and lactic acid, pO2 and pCO2, and fatty acids, and metabolic indicators (cAMP, tissue DNA 3H-thymidine incorporation) in major organ systems were significantly lower (P < 0.001) in C3H mice, but not in either C57BL/6 or BALB/c mice, exposed to bLAD, compared to CWF. Phospho-activation of major metabolic signaling pathways (mTOR, GSK3β, and SIRT1) was higher in skeletal muscle, and lower in liver, for C3H mice in bLAD, compared to CWF. These data show that daytime exposure of C3H mice to bLAD has a marked positive effect on the circadian regulation of neuroendocrine, metabolic, and physiological parameters associated with the promotion of animal health and wellbeing.

PS79 Impact of Daytime Blue-Enriched LED Light on Physiological Parameters of Three Common Mouse Strains Maintained on an IVC System

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Many artificial lighting systems around the world, including laboratory animal facilities, are transitioning from conventional cool white fluorescent (CWF) lighting to light emitting diode (LED) lighting technology due to higher efficiency, cost effectiveness, and lower environmental impact. According to The Guide, recommended light illuminance for rodents is about 325 lux at 1 m (3.3 ft) above the floor, however, lighting technologies are not fully addressed. Previous studies from our laboratory demonstrated that the quality of light (wavelength) impacts metabolism and physiology in rodents. Here we tested the hypothesis that daytime exposure to blue-enriched (460-480 nm) LED light (bLAD), the most commonly used LED, compared to broad-spectrum CWF, influences mouse metabolism and physiology. In this ongoing ACLAM-supported investigation, three common strains of age-matched male and female mice (C3H, BALB/c, and C57BL/6) were randomly assigned and maintained in an AAALAC-accredited facility on an IVC system in either CWF or bLAD lighting for a period of 36 days under an IACUC-approved protocol on 12L (300 lx; 123.5 µW/cm²):12D (0 lx) light-dark cycle (lights on 0600). Animals were measured every three days for dietary/water intake, body weights, and feed conversion efficiency. At the conclusion of the 36-day light exposure period blood was collected over one week at 6 circadian time points (0400, 0800, 1200, 1600, 2000, 2400) to obtain blood chemistries and complete blood counts (analysis underway). While there were no differences in light duration or within cage light intensities (illuminance/irradiance) between bLAD (32.16 ± 1.88 ; 78.80 ± 4.80 µW/cm) and CWF (32.25 ± 1.88 ; 78.80 ± 1.88 µW/cm) groups (n = 36 cages/g), respectively, there were significant differences (P<0.05) in dietary intake and body weights (P<0.05) only in C3H male mice maintained in bLAD vs CWF lighting (CWF>bLAD). These data show that daytime exposure of mice to bLAD, compared to CWF lighting, may influence important physiological parameters and assist in our understanding regarding the impact of lighting systems.

PS80 In Vivo Trafficking of Immune Cells by Noninvasive Molecular Fluorescence Tomography Imaging

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Tracking of cells in vivo is an important phenomenon in immunology and immuno oncology research. In vivo tracking of cells after adoptive transfer or engraftment is very challenging. We have developed a method to label cells in vitro using fluorescent probes that integrate into the cell membrane without affecting cell viability or function and the labelled cells can be tracked by fluorescence molecular tomography (FMT) imaging in vivo. Human T cells were labelled with Cellvue815, a near-infrared (NIR) fluorescent dye that intercalates into the cell membrane (excitation ~ 786nm; emission ~814nm). The final concentration of Cellvue815 was at 50µM with 5 million cells in 100ul PBS. Naïve NSG mice were injected with the labelled human T cells. There were 3 mice in each group. Whole body FMT imaging was performed to evaluate the trafficking of human T cells. In vivo (2, 24, 48, 72hr) and ex vivo FMT imaging (24, 72hr) were taken. We showed that T-cells can be labeled by Cellvue815 without affecting the viability in vitro. FMT imaging (in vivo and ex vivo) showed that the trafficking of the labelled T cells in naïve NSG mice. Quantitation showed liver and spleen as major organs for T-cell accumulation.

PS81 Corynebacterium bovis: Antibiotic Susceptibility, Prophylaxis and the Skin Microbiota

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The use of antibiotics to either prevent Corynebacterium bovis infection or treat infected mice has been controversial due to reports of poor efficacy and fears of cultivating antibiotic resistance. However, due to the complexity of eliminating C. bovis from research vivaria, antibiotic therapy could be a valuable tool. Accurate susceptibility testing is needed to dose antibiotics appropriately. An in vitro project was performed to determine the minimum inhibitory concentration (MIC) of 24 antibiotics on 15 novel mouse-C. bovis isolates from across the country. Then, an in vivo experiment was performed to evaluate if oral prophylactic antibiotics can prevent C. bovis infection following acute exposure. An infectious dose of C. bovis was applied to 2 of 3 groups (n = 4-5/group) of singly housed, athymic nude (n = 15) and NOD.Cg-PkdcsdIl2rgtm1Crl/J (NSG, n = 14) mice. Immediately following exposure one group received amoxicillin and clavulanic acid (0.375 mg/mL) in the water for 2 wk. The antibiotic treated, negative and untreated control groups were followed for 8 wk after antibiotic therapy by qPCR. In addition, swabs were collected from nude mice for 16S rRNA sequence analysis to evaluate the impact of C. bovis and antibiotics on the dermal microbiota. Our data demonstrate that the MICs for all mouse-C. bovis isolates obtained do not markedly differ from previous results published for cow-C. bovis isolates. The use of oral prophylactic antibiotics, neither nude nor NSG mice became infected with C. bovis following an acute exposure (P<0.05). Two weeks after infection the dermal microbiota of the positive controls were significantly different from both the negative and antibiotic treated groups and remained that way for the duration of the study (Q<0.05). After 2 wk of antibiotics and 8 wk after antibiotic withdrawal, a significant difference was not observed between the antibiotic treated group and negative control group. Our findings demonstrate that prophylactic antibiotics can be used to prevent C. bovis infections and
has less of an impact on the dermal microbiome than *C. bovis* infection.

**PS82 Whole Genome, Molecular, and Biochemical Characterization of *Klebsiella pneumoniae* Strains Isolated from Immunocompromised NSG Mice**

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*Klebsiella pneumoniae* (*Kp*) is an intestinal bacteria that can cause septicemia, urinary tract infections, and pneumonia particularly in immunocompromised hosts. Some strains of *Kp* exhibit antibiotic resistance and hypermucoviscosity, making infection challenging to effectively treat. NOD-scid gamma (NSG) mice are immunocompromised due to defective immune cell development/function and are used as cancer xenograft, humanized, and infectious disease models. In this study, NSG mice housed at a research institute experienced unexpected diarrhea, morbidity, and mortality. *Kp* was cultured from feces, intestines, liver, lungs, and blood at necropsy from several clinically affected mice. While *Kp* can cause illness in laboratory mice, mouse isolates remain poorly characterized. The objective of this study was to elucidate the pathogenic potential of *Kp* isolated from NSG mice by whole genome sequencing (WGS), molecular, and biochemical assays. WGS was performed on 13 isolates cultured from the feces, cecum, liver, lung, and blood. Pan-genome phylogenetic analysis placed all isolates in a separate clade most similar to human urine, blood, throat, and sputum isolates. All isolates were assigned a multilocus sequence type of ST1165, capsule K antigen of K45, and LPS O antigen of O2:s:2. No genomes exhibited hypermucoviscosity according to the string test and were negative for the hypermucous genes *rpmA* and *magA*. All genomes encoded enterobactin for iron acquisition and type 1 and 3 fimbriae for adhesion and biofilm formation. Biofilm formation was confirmed by crystal violet assay. A plasmid-encoded class 1 integron harboring antibiotic resistance genes for aminoglycosides, chloramphenicol, and trimethoprim/sulfonamide was identified in 12/13 genomes. PCR identified this integron in 20/23 additional isolates. All genomes also contained resistance genes for beta-lactams and fosfomycin. Antibiotic resistance to beta-lactams and trimethoprim/sulfonamide was confirmed by MIC broth assay. In conclusion, *Kp* isolates from NSG mice likely represent opportunistic pathogens. The expression of plasmid-encoded multidrug resistance raises the potential of spreading antibiotic resistance to related *Enterobacteriaceae* colonizing mice housed in the same vivarium.

**PS83 Longitudinal 16S rRNA Gene Sequencing of a Mouse Colony Associated with ‘Wild Mouse’ Gut Microbiota**

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The role of the microbiome in health remains a significant area of research for many investigators with newly discovered links to various disease states. Although many factors can affect the reproducibility and performance of mouse models, it is well established that changes in the gut microbiota can have a major role in driving experimental variability. We hypothesized that germ-free C57BL/6NTac mice associated with gut microbiota collected from wild mice would maintain a consistent microbial profile over time and generations of mice. A group of 12 mice associated at birth were housed and bred for two subsequent generations within an isolator microenvironment under gnotobiotic husbandry practices. Fecal samples were collected for 16S rRNA gene sequencing from the associated founder mice (n=12) and their subsequent F1 (n=40) and F2 (n=19) offspring at multiple timepoints. Analysis of the alpha-diversity of the three generations of mice harboring the ‘Wild Mouse’ gut microbiota revealed no differences (P>0.05) in the species richness of the microbial community with respect to total number of operational taxonomic units (OTUs) detected. Furthermore, species evenness and richness of the microbial community was not significantly altered due to isolator husbandry as measured by Shannon Diversity Index. When examining specific taxonomic levels, no loss of Phyla was detected during the experimental period. In addition, the top 10 Orders accounting for greater than 97% of the total microbial community remained unchanged in terms of relative abundance. However, transient differences in microbial diversity were detected among weanling mice compared to 8-week-old mice in the F1 and F2 generations, with weanlings being enriched with *Lactobacillus spp.* before transitioning to a chow diet. These data support that a defined gut microbiota can be stably transferred across breeding generations using gnotobiotic practices. These findings may improve experimental reproducibility for researchers.

**PS84 Novel Virus Discovery by Next Generation Sequencing**

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Viruses are obligate intracellular parasites and can alter the physiology of infected cells and communication or interaction with other cell types. The existing ongoing threat of rodent viruses and the realization of newly discovered ones present unknown impacts on laboratory animal research. It is imperative to have a rapid and comprehensive screening of virus in research rodent colonies to know the variables that may influence data obtained during biological investigations. PCR detection of viruses is limited by available known virus genome diversity. Therefore genetic variation in undiscovered virus strains can prevent PCR detection of these viruses.
Serology can only determine viral presence based on available virus antigens for the detection of specific antiviral antibody production. Virus isolation and purification is also challenging to establish due to the difficulty of some viruses to be propagated in cell culture. Murine Chapparvovirus (MuCPV), along with other novel rodent viruses, have been rapidly identified by next generation sequencing (NGS) over the last 10 y. In an attempt to better understand the genetic diversity of recently reported viruses, we used NGS to search for additional strains. Two-4 young and adult mice from a pet shop distributor were evaluated for the presence of newly reported viruses to assess strain variation. RNA and DNA was isolated from fecal samples and converted to double stranded DNA by cDNA and dsDNA synthesis. DNA was further fragmented and ligated to primer linkers for sequencing. As a result, compared with GenBank viral genome sequence database, we identified novel viruses not previously reported, such as mouse alpha coronaviruses, Herpes virus 1, Murine Kobuvirus 2, and Murine picornavirus along with novel viruses previously reported. These viruses shared only a 50-74% nucleotide identity to the next closest match in GenBank. TaqMan PCR assays were developed to determine the prevalence in research colonies. NGS is a valuable tool that broadens our understanding of the diversity of viruses in laboratory mice.

PS85 Genomic Characterization of Novel Mouse Kidney Parvovirus strains in Laboratory and Wild Mice

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Chronic nephropathy spontaneously occurs in mice. This renal pathological condition is often associated with intranuclear inclusions predominantly in immune-deficient mice, and to a lesser extent, in aged immunocompetent mice including inbred C56BL/6 and outbred Swiss Webster mice. Recently, chronic tubulointerstitial nephropathy and kidney fibrosis in mice is linked to infection of an atypical parvovirus, termed mouse kidney parvovirus (MKPV) based on its genome sequence and organization being similar to members of Paravovirinae. However, determination of the complete genome sequences of new MKPV strains is essential for revealing phylogenetic relationships and pathogenicity among MKPV strains. In our study, we have identified and determined the complete 4440-nucleotide genome of a new MKPV strain (MKPV MIT-WI-1) isolated from Il2rg<sup>-/-</sup>/Rag2<sup>+/+</sup>/W<sup>W</sup> mice using PCR and genome walking. The genome organization of MKPV MIT-WI-1 is identical to previously known MKPVs. The nucleotide and deduced amino acid sequences of the MIT-WI-1 genome is phylogenetically related to MKPV strains from laboratory mice at the Memorial Kettering Sloan Cancer Center (MKSCC), New York, wild mice (Mus musculus) in the urban areas of New York, and laboratory mice at The Centenary Institute (CI), Sydney, Australia. Notably, there is a dinucleotide deletion at nucleotide 495 compared to the MKPV genome from MKSCC and IC. The deletion is located immediately downstream of the coding region of NS2-P, which does not apparently affect downstream open reading frames. Moreover, PCR and qPCR assays using designed primers conserved among the known MKPV genome sequences were established. Using the newly developed PCR assay, a segment of the MKPV genome, displaying 98-99% nucleotide sequence similarity to the corresponding regions of known MKPV genomes including the MIT-WI strain, was identified in wild mice (Peromyscus leucopus) originating from Massachusetts Taken together, our data indicate that MKPV is prevalent in laboratory and wild mice, and provides new insights into the evolution of the MKPV genomes.

PS86 Development of the Multiplex PCR Assay for Simultaneous Detection of Fur Mites in Laboratory Rodents

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Fur mites, including Myocoptes musculinus, Myobia musculi, Radfordia affinis, and Radfordia ensifera are the most prevalent ectoparasites in contemporary laboratory mice and rats. These parasite infestations are usually subclinical; however, marked lesions, such as pruritus, alopecia, and ulcerative dermatitis have been reported in heavy infestation or in some mouse strains and may also modulate research data in animal studies. Diagnosis of the fur mite infestations is thus required in routine health monitoring of laboratory animals. However, the traditional diagnostic method for these ectoparasites, the subgross examination of pelts, may not efficiently and accurately demonstrate the fur mite infestation status, especially during light infection. Furthermore, M. musculi, R. affinis, and R. ensifera are morphologically resembled and have to be microscopically differentiated by a specialist. Additionally, the subgross examination of pelts will necessitate sacrificing of animals. Besides, recent data reveals that the sentinel health monitoring system may not efficiently and accurately demonstrate the status of ectoparasite infestations in laboratory animal colonies using the IVC system. In this study, we developed a multiplex PCR assay that targets the rRNA genes to simultaneously detect and differentiate these ectoparasites, including Myocoptes musculinus, Myobia musculi, and Radfordia using swab samples. This assay is very sensitive and specific with a detection limit of 10 copies for all target agents. In 15 rodent colonies, the multiplex PCR assay has successfully detected the dual or triple natural infestations of fur mites in 17 of 48 rodents by fur swabs and in 10 out of 25 rodent cages by cage swabs in 5 fur mite-positive colonies. The status of the parasite infestations in these animals was confirmed with pelts examination and specific PCR assays followed by DNA sequence analyses. The rodent fur mites/actin multiplex PCR assay developed in this study could be a useful tool for monitoring rodent health in the future.
PS87 Improving the 3Rs in Preclinical Oncological Research with Multimodal Imaging

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Efficacy of treatment in preclinical oncological research is assessed by measuring tumor volume. This is measured using either medical imaging techniques such as US, MRI, or CT which are expensive and resource intensive; or callipers which are subjective and assume that tumors are spheroidal (measuring only length and width). We have developed a minimally invasive, morphology independent solution for subcutaneous tumor measurement using 3D imaging, thermography, and AI. Our platform captures integrated tumor volume from the 3D surface as well as 3D morphological and thermal features, thereby offering better performance in precision and accuracy than callipers, but also providing extra imaging information. With respect to tumor volume assessment, we will demonstrate the extent of calliper measurement inconsistencies from a dataset of 2,500 tumor volume measurements from 1,600 mice (6 strains), multiple operators and 20 tumor models. Then, a dataset of 2,500 scans captured this year shows 92% and 98% measurement agreement for length and width respectively (within +/-3mm of calliper measurements) and an interoperator variance showing 93% of measurements for length and 96% for width are within 20% of repeated measurements. A further benefit is that users can be trained eight times faster compared to callipers. Further, we use the information contained in the images to estimate tumor symptoms including redness, pallor, necrosis, and ulceration, as well as other biology-relevant features that can be explored as potential pharmacodynamic, toxicity, and animal welfare biomarkers. We demonstrate how these features can be useful to make preclinical oncological studies more statistically powerful. Our solution shows much promise for tackling refinement and reduction and will enable more confident decisions regarding animal welfare to be made in the early stages of drug discovery.

PS88 Examining the Need to Customize Animal Welfare Legislation for Animals used in Xenotransplantation Trials and Production

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Recent advances in overcoming both immunological and pathobiological barriers across species make xenotransplantation a potential solution to ongoing shortage of human organs. Pigs are the best candidates so far for supplying cells, tissues, and organs for treatment of human diseases and traumatic injuries. Genetically modified pigs are being raised in the U.S. to prepare for clinical xenotransplantation trials in humans. The Animal Welfare Act (AWA), enforced by the United States Department of Agriculture (USDA), is the only federal law regulating the treatment of animals used in research in the U.S., while the Center for Biologics Evaluation and Research (CBER), under the Food and Drug Administration (FDA), has regulatory oversight of xenogenic products and xenotransplantation in humans. Animals involved in xenotransplantation are not specifically described under the AWA but are addressed under the FDA’s “Guidance for Industry” documents instead. These guidance documents provide suggestions or recommendations only and do not establish legally enforceable responsibilities. They also focus primarily on public health concerns rather than the welfare of source animals. Current animal protection laws other than the AWA, such as antianimal cruelty laws, vary from state to state, while animal welfare monitoring for livestock production relies heavily on voluntary audits and certification programs. Given the unique function and needs of animals raised for organ or tissue harvest for human use, producing and housing such animals will necessitate separate regulatory oversight different than that for animals in laboratory research or food production. It is critical for U.S. lawmakers to review, revise, and customize current federal and state laws to protect the welfare of this special group of laboratory animals. Communication and collaboration between the USDA and the FDA are crucial, as well as mandatory accreditation and auditing for all facilities housing animals for xenotransplantation. As scientific discoveries lead both humans and animals toward unfamiliar domains, more rigorous and customized legislation is needed to oversee the use and welfare of animals in xenotransplantation programs.

PS89 Behavioral and Reproductive Impacts of Environmental Enrichment and Pseudoloma neurophilia infection on Adult Zebrafish (Danio rerio)

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Coinciding with Danio rerio's expanding popularity in research is the widespread prevalence of zebrafish microsporidiosis, a disease caused by the parasite Pseudoloma neurophilia. Clinical signs of infection include emaciation, spinal deformities, reduced growth, altered behavior, decreased fecundity, and increased mortality. No treatment is currently available for microsporidiosis, making its exclusion from laboratory animal facilities challenging. Environmental enrichment (EE) is another factor which may affect behavior and reproduction in laboratory zebrafish. We investigated the behavioral and reproductive effects that Pseudoloma neurophilia infection and EE have on anxiety-like behaviors and reproductive performance in adult AB strain zebrafish. Specific-pathogen free-P. neurophilia (SPF, n=94) zebrafish and zebrafish experimentally infected with P. neurophilia (n=43) were socially housed in tanks with or without EE. After 5 wk, zebrafish were behaviorally assessed using novel tank test
(NTT) and light-dark test (LDT), 2 established anxiety assays for zebrafish, and then bred once weekly for 6 wk, alternating whether EE was provided in breeding tanks each week. We hypothesized that *P. neurophilia* infection would increase anxiety-like behavior and have a negative impact on fecundity, and that the presence of EE would neither affect anxiety-like behavior nor reproduction. Infected fish had more averaged entries into the bottom of the NTT (16.6) than SPF fish (11.3) \( (P = 0.003) \) and fish housed with EE had more averaged entries (13.97) into the top of the test tank than fish housed without EE (8.04) \( (P = 0.037) \). No statistical differences between groups were found for LDT. SPF fish produced, on average, more eggs (17.49) and more viable embryos at 6 d postfertilization (11.7) than infected fish (4.47 and 1.06, respectively) \( (P = 0.0008) \), with EE in breeding tanks not resulting in a difference in reproductive outcomes \( (P = 0.61) \). *P. neurophilia* infection and EE both affected zebrafish behavior, and disease status had a significant impact on reproduction. Our findings support both the exclusion of *P. neurophilia* from laboratory zebrafish and the use of EE in housing tanks.

**PS90 Cost Modeling: Factors to Consider when Maintaining Inhouse Breeding Colonies of Mice**

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We focus on the practical considerations involved in sizing mouse colonies. We demonstrate how cohort sizes can be produced more effectively with a detailed understanding of productivity factors and animal health requirements. Additionally, we show how genetic drift can affect the reproducibility of experiments using mice bred in small colonies. We introduce principles of cost modeling and demonstrate a tool that was developed to compare purchasing mice from an animal vendor compared with breeding mice inhouse. Data shows that purchasing mice directly from a reputable vendor can have many benefits, including savings for an institution with regards to labor, time, animal space, and animal health. More importantly, use of this tool can also have a positive impact on animal welfare and the 3Rs in certain circumstances. For example, in an experiment that requires 10 B6/J female mice at 3 wk of age for a 10-wk study in an academic lab, we used per diem rates, cage costs, and tech costs provided by a major university to show how to determine the cost variance of ordering the mice directly compared with breeding on site and the results for cost, box space, tech time, and animal welfare. This example resulted in a box savings of over 19 boxes, a decrease in overall costs of over $1,000, and a savings of 138 mice. Genetic drift and the impact and number of potential mutations that occur over multiple rounds of in house breeding without genetic control is also a factor. Purchasing animals directly from a reputable vendor enables principal investigators to obtain animals of the correct age, strain, and sex and reduces the number of discarded animals that do not fit the criteria for the study.

**PS91 Education and Training to Fully Implement Refinement Methods in Practice**

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Retrospective reviews of the application of refinement methods in practice revealed that the knowledge we have gained from refinement research is not fully applied in practice and thus, laboratory animals may endure unnecessary suffering. Experimental refinements, including anesthesia and peri- and postoperative analgesia protocols, humane endpoints, and euthanasia methods were reviewed in over 500 basic and applied animal research proposals. Furthermore, a literature review was conducted to assemble the latest best practice approaches in regards to housing and care. In all areas of refinement, flaws in the application of available approaches which could help to reduce unnecessary pain, distress, and suffering of animals used in science were detected. One potential reason that was identified in the study of experimental refinement use was that researchers might not be sufficiently aware of existing refinement methods. In addition, a recent international survey, conducted by the European Commission’s Joint Research Centre found that very few courses could be identified at university level for the training of career scientists is currently being established to teach best practice approaches and to foster a culture of care for laboratory animals. Several modules cover classical housing and experimental refinement, others comprise refinements of planning, including identifying the most appropriate research model, proper data analysis, and comprehensive reporting. Consequences of poor refinement method use for the animals’ welfare and for the validity of collected data are also discussed. The lectures are complemented by interviews with experts in laboratory science to explore reasons for the currently low refinement implementation rates and to find possible approaches to improve the situation.

**PS92 Caring for Research Animals through Final Transition: What Can We Learn from Veterinary Hospice Practitioners?**

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In laboratory animal medicine, we pride ourselves on the superior care and compassion we provide for the animals
we work with. At the same time, we walk a difficult balance between our attachment to our animals and the need to emotionally detach from our situation. Do we honor that human-animal bond and the grief we might feel when an animal must be euthanized as part of the research study? Animal hospice and in-home euthanasia is one of the fastest growing specialties in veterinary medicine. Hospice veterinarians focus on appropriate pain management, palliation of symptoms at the end of life, humane euthanasia, and grief support for the owners. What can we learn from this specialty in order to better support our staff and the research animals with which we work? We present how the knowledge gained in veterinary hospice can be applied to combat compassion fatigue, assist care staff through the euthanasia process, and honor the human-animal bond in a research setting. Hospice veterinarians assist families through the end of life transition and this can be applied to laboratory animal medicine.

**PS93 ‘WWAG’ing Our Way towards Wellness: Implementation and Assessment of an ASLAP Veterinary Student Canine Enrichment Program**

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The Welfare and Wellness ASLAP Group (WWAG) was created to help maximize the positive human-animal interaction opportunities within our institution’s robust environmental enrichment program. This initiative provided practical hands-on experience for laboratory animal career-focused students. Prior to participation, members of the Ohio State University ASLAP Club completed a written application to assess individual goals for the program. Following the completion of specifically tailored orientation sessions, students completed 20-60 m enrichment sessions weekly throughout each semester. Working in pairs, students engaged their canine companions in leash training, forming playgroups and enhancing canine socialization with one-on-one interactions. Volunteers documented session activities in a dedicated canine enrichment log to track animal-specific behaviors and preferences. A survey was distributed at the completion of the academic year to evaluate individual skills, perceived benefits of the program, and acquire feedback for future improvements. Prior to program initiation, the most frequently reported objectives for program participation included increased experience and comfort working with canines in a laboratory setting, positively impacting dogs through enrichment sessions, and gaining more knowledge about the canine enrichment program. Following participation, 100% of respondents reported their personal wellness was positively impacted by canine interactions and they felt their interactions positively impacted their canine companions. The majority of students indicated they were more knowledgeable about the laboratory enrichment program (91%) and that they had improved specific clinical skills such as low stress handling and restraint (75%) and understanding of canine behavior (66%). From student feedback, we have modified our vivarium tour and adjusted refresher training to streamline the process for returning students. With the overwhelming success of WWAG, we have continued to grow our roster and expand our program to include additional species. Our program exemplifies a unique resource for enriching our animals while fostering valuable experiences for the next generation of laboratory animal veterinarians.

**PS94 Serene Scents: Odors in Nesting Material and Plantar Sweat Influence Male Mouse Social Behavior in the Home Cage**

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Severe wounding, resulting from excessive home cage aggression, is a common reason for premature euthanasia in male laboratory mice. Aggression can be reduced by transferring the old nest during cage cleaning which is thought to contain aggression appeasing odors from the plantar sweat glands. However, neither the deposits on used nesting material nor the contents of plantar sweat have been evaluated. The aims of this study were to identify and quantify compounds deposited in the nest site and to determine if nest and sweat odor profiles correlate with social behavior. Home cage behavior was evaluated in 3 strains: SJL/JOrlCr (high aggression), C57BL/6NCr (moderate aggression), and A/JCr (low aggression; n = 8/strain; 24 cages*5 mice/cage = 120 mice). Aggressive and affiliative behaviors were recorded on d 1, 2, and 7 using 1,0 sampling. Cage hierarchy was assessed on days 5 and 6 via a tube test. On d 7, sweat from the dominant and subordinate in each cage and nest samples were collected for behavior proportions along with nest and tube test. On d 7, sweat from the dominant and subordinate in each cage and nest samples were collected for additional species. Our program exemplifies a unique resource for enriching our animals while fostering valuable experiences for the next generation of laboratory animal veterinarians.
PS95 Increasing Public Engagement in the Evaluation of Animal Research: An Experiment in Transparency

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One potential response to calls for increased openness in animal research is to make protocols publicly accessible, but it is unclear what type of input the public would provide if given this opportunity. We made five different hypothetical research scenarios (describing projects on chronic pain research with mice, organ transplant research with pigs, smoking research with mice, Parkinson’s disease research with monkeys, and cancer research with zebrafish) available to US participants (N = 247) via an online survey. Our objective was to assess participant responses to these animal research protocols, and identify factors that influenced acceptance or rejection of the proposed research. Participants were asked “Do you support the use of these animals in this research?” Response options were yes/neutral/no, and participants were asked to provide open-ended text responses to explain the reasons for their choice. Overall, 54% of the participants expressed support for the research. The scenario describing smoking research with mice garnered the least support from participants (35.6%), while chronic pain research with mice was most supported (63.6%). Many participants provided open-ended comments, showing that an online forum can also provide qualitative feedback on research protocols. Four themes were prevalent in participant reasoning regarding their support for the proposed research: scientific merit, morality, availability of alternatives, and impact on humans. These results illustrate the type of public input that can emerge should research institutions provide opportunities for this participation as part of protocol review. We conclude that this type public input could provide institutions a better understanding of what types of animal research people are willing to accept, and thus reduce the risk that practices fall out of step with community values.

PS96 Use of a Validated Smartphone-based Electrocardiogram in Mice Reveals Severe Handling-induced Bradycardia across Both Sexes and Four Strains

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Noninvasive electrocardiogram (ECG) devices are not often used in mice, as they require restraint or anesthesia, and expensive equipment. As a result, there is little data on the effect of mouse handling on cardiovascular parameters. We validated the use of a smartphone-based, single-lead ECG system in mice by performing simultaneous ECG recordings in conscious, restrained mice and anesthetized mice (n=19) with surgically implanted telemetry ECG systems, the gold standard for ECGs in mice. Heart rates and rhythms collected by smartphone-based ECG and telemetry were equivalent. We observed that mice restrained in a standard immobilizing scruff experienced an up to 80% decrease in heart rate, equivalent to a 600 bpm decrease. We hypothesize that pressure on cervical baroreceptors produced by stretching the neck skin dorsally during immobilizing restraint results in bradycardia, and conversely that restraints that do not result in pressure on the neck would not alter heart rate. Mice of both sexes and four strains (C57Bl/6, FVB/n, DBA/2, and BALB/c, total n=38) were restrained by 3 different restraint methods: a light restraint that allowed substantial head movement; a 3-finger scruffing technique designed to prevent pressure on the neck by creating a transverse fold of skin; and a standard immobilizing scruff designed to draw the forelimbs back, prevent head movement, and create a crease on the ventral neck as a consequence of a longitudinal fold of skin. Three experienced mouse handlers performed the experiments. In C57Bl/6, there was no difference in heart rate between the light restraint and 3-finger restraint method. The median heart rate using the immobilizing scruff was significantly decreased by 50-66% for all 3 handlers (range 143-750 beats per minute). Similar bradycardia was seen in the other strains and both sexes. Moreover, when the 3 restraint methods were compared using telemetry devices, only mice that received an immobilizing scruff produced intermittent delayed beats for as long as 7 m after release from restraint. Due to the profound cardiovascular effects, we recommend use of the light or 3-finger scruffing technique, and avoiding or minimizing the use of the standard immobilizing scruff while handling mice.

PS97 Peer-led Outreach through Biomedical Research Awareness Day

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Accelerating strategies to increase public understanding and appreciation for essential animal research is more important now than ever. Biomedical Research Awareness Day (BRAD) is a day devoted to honoring the role of animals in biomedical research and stimulating conversation about the necessary role of animals in medical advancements. Celebrated annually around the world, BRAD promotes peer education about animal research, offers a platform for those working with or supporting animals in research to discuss their work with animals, and highlights the devoted staff who care for these animals. The event also serves as a pipeline for future careers in biomedical research and laboratory animal care and medicine. The flexible nature of BRAD allows each event to be tailored to reach specific audiences and fit the resources and goals of any organization. We discuss how BRAD has been implemented at an institutional level, within an entire region at multiple organizations, and in new audiences, such as veterinary technician students and educators.
Intervertebral disc disease (IVDD) is strongly associated with spine pain and disability without known regenerative treatments. Etiopathogenesis of IVDD is poorly understood and appears to be multifactorial. Inflammatory and degradative mechanisms are associated with it; however, specific pathways have not been fully elucidated. 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 pathobiology of intervertebral discs (IVD) from dogs and humans would show similarities in metabolite production and responses to cytokine stimulation. With ACUC and IRB approvals, we evaluated IVDs from nonchondrodystrophic dogs (adult mongrel dogs; n=32; female n=28) and human patients (n=85, clinical patients) to elucidate potential similarities and differences between canine and human intervertebral discs. Canine lumbar and cervical IVDs were collected aseptically after euthanasia for reasons unrelated to this study. Human IVD tissues were collected during surgery. Using 6 mm biopsy bunches, IVD explants were created. Explants were separated into 2 groups, control and cytokine stimulated (10 ng/ml IL-1β) in supplemented Dulbecco’s Modified Eagle Medium (DMEM). The explants were cultured for 3 d and media were collected at the end of culture period. 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, IL-8, KC, MCP-1, MMP-1, MMP-2, and MMP-3 levels were noted between canine and human IVD tissues. Human IVD tissues had higher production of inflammatory and degradative biomarkers than canine IVD tissues in both control and cytokine stimulated groups. Both canine and human IVD tissues showed significant increases in biomarker production in response to cytokine stimulation. Collectively, these data suggest that both canine and human IVDs similarly respond to cytokine stimulation by increasing production of inflammatory and degradative metabolites. Ongoing translational studies will be aimed at further elucidation of these important similarities and difference towards understanding and addressing IVDD mechanisms in dogs and humans.

**PS99 Evaluation of Electroacupuncture for Symptom Modification in a Rodent Model of Human Osteoarthritis**

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When faced with the frustration of chronic discomfort and restricted mobility due to osteoarthritis, many individuals have turned to acupuncture as an alternative therapy. Acupuncture is a traditional Chinese practice for pain alleviation that involves insertion of thin needles into the skin and underlying tissue followed by manual manipulation or electrical stimulation. However, the efficacy of acupuncture in managing osteoarthritis is uncertain, as much of the evidence is of questionable quality. The purpose of this study was to evaluate electroacupuncture in a rodent model of osteoarthritis such that unbiased conclusions regarding its efficacy for symptom modification could be drawn. Ten 11-mo-old, male Dunkin Hartley guinea pigs, which characteristically have moderate osteoarthritis at this age, were randomly assigned to receive electroacupuncture (n=5) or anesthesia only (n=5). Acupuncture points were based on the traditional Chinese bi syndrome therapy for knee pain (BL11, BL23, BL40, BL54, GB29, GB30, GB34, GB39, LI11, and ST36). Animals were anesthetized with isoflurane, and treatments were performed 3 times weekly for 3 wk. Using videotracking software, movements in an open field were recorded at baseline (prior to treatment) and then biweekly to measure changes in activity levels as an indicator of knee pain. Animals were harvested 2 wk after the final treatment session. Serum was collected for inflammatory biomarker testing, and whole knee joints were collected for histology and gene expression. Animals receiving electroacupuncture had a significantly greater change in total distance traveled compared to those receiving anesthesia only. There was a trend towards decreased serum complement component 3 and tumor necrosis factor protein concentrations in the electroacupuncture group compared to the anesthesia group. Collagen type 2, fibroblast growth factor 18, and inducible nitric oxide synthase gene transcripts in articular cartilage were significantly increased by electroacupuncture. There was not a significant difference in total joint histology scores between groups. This study provides evidence that a 3-week period of electroacupuncture had a positive effect on symptom, but not disease, modification in a rodent model of osteoarthritis. Further investigations into mechanistic pathways that may explain the efficacy of electroacupuncture in this animal model, as well as longer term studies, are needed.

**PS100 Electro-acupuncture Prevents Spasticity and Orofacial and Somatic Alldynia in a Clinically Relevant Rodent Model of Closed Head Traumatic Brain Injury**

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Traumatic brain injury (TBI) is major health problem in both military and civilian personnel. Approximately 50% of
PS101 A Novel Device to Measure Static Hindlimb Weight Bearing Forces in Pronograde Rodents

PS102 Buprenorphine administration in NZW rabbits: a pharmacokinetic study of intravenous, subcutaneous and intramuscular administration.
the low bioavailability which is not simply compensated for by increasing the dose.

PS103 Comparative Risk of Human Injury/Exposure while Collecting Blood from Sedated and Unsedated Nonhuman Primates

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Collection of blood samples for research or clinical evaluation is one of the most common procedures performed in nonhuman primates. There are several possible methods to obtain samples. In the early days of primate research, manual/physical restraint was used, which was stressful for the animal and risky for the human. As the field developed, chemical immobilization with ketamine or other anesthetics became the most commonly used method. More recently, the use of training using positive reinforcement has allowed collection of blood samples from unsedated primates that are unrestrained or minimally restrained. Elimination of anesthesia reduces risks to the animal. We wanted to know whether there was a difference in risks to humans between the 2 methods of blood collection. We evaluated injury and near-miss reports in conjunction with blood collection data from 2011 to 2019 at a primate center that houses macaques (M. nemestrina, M. mulatta, and M. fasicularis) and squirrel monkeys (S. sciureus). Injuries associated with sedated blood collection included those occurring during the sedation procedure and recovery, as well as those directly associated with blood collection. Injuries associated with unsedated blood collection included those which occurred both during animal training and during blood collection. Overall, there were 16 injury exposures and 3 near misses associated with 62,522 blood collection procedures and 0.022% of sedated blood collections were associated with exposure incidents and 0.088% of unsedated blood collections were associated with exposure incidents. Our data indicate a very low risk of exposure associated with blood collection. The risk was higher for unsedated animals ($P = 0.04$ by chi square analysis), but the low number of incidents makes statistical interpretation questionable.

PS104 A Novel Method of MRI-guided Gene Therapy Agent Delivery to Nonhuman Primate Brain Using a Frameless Stereotactic Device

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The pathogenesis of neurodegenerative diseases, such as spinocerebellar ataxia, involves genetic defects in specific cell populations within the brain. A major challenge for gene therapy research aimed at correcting these defects is developing methods capable of delivering therapeutic genetic material to cells most affected by the disease process being studied. Here, we outline a novel method of delivering adeno-associated viral vectors (AAVs) to Purkinje cells in the cerebellum of rhesus macaques (Macaca mulatta) using a frameless stereotactic device under MRI guidance. Isoflurane-anesthetized animals are placed in the bore of a MRI machine. Percutaneous pins stabilize the skull and a specialized surgical drape that maintains sterility of the prepared surgical site as the animal moves in and out of the bore during the procedure is placed. An MRI-compatible disposable trajectory device, which houses a guide tube containing a liquid contrast agent, is mounted to the skull with percutaneous bone screws. To avoid disturbing vasculature in the brain, liquid contrast is delivered intravenously prior to trajectory planning. Using MRI imaging combined with a specialized trajectory mapping software program, individual nuclei within the brain are targeted for direct AAV delivery. The trajectory is plotted and the device is positioned in line with the target. A small Burr hole is created in the skull, followed by introduction of a cannula to a depth determined by the mapping software. The trajectory of this cannula is confirmed with MRI imaging prior to the commencement of a slow infusion of a phosphate-buffered saline solution containing the therapeutic agent and a contrast agent. Postinfusion imaging confirms that the solution adequately covered the target. The cannula is retracted after a dwell period adequate to minimize reflux up the cannula path, and the skin is closed with suture. Prior to recovery, all hardware is removed. This technique may be used to deliver therapeutic agents to multiple targets within the same animal, and it may be used in other large animal model species, such as pigs and dogs.

PS105 Reducing Variability in Mouse Studies through Continuous Data Collection

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Inconsistencies in the reported behavioral phenotypes of mouse models present challenges for scientific interpretation. Behavioral traits are challenging to measure consistently due to the influence of interacting genetic and environmental factors. As a result, the reported behavioral phenotype of a model can vary widely between laboratories and likely stems from conditions which are difficult to standardize between labs. In an effort to identify approaches to improve the consistency of findings, we hypothesized that continuous collection of data in the home cage would optimize phenotyping by reducing the impact of experimental confounds. The hypothesis was tested in the cuprizone mouse model which is used to study de/remyelination associated with motor deficits. Results from standard behavioral assays are highly variable with reports of open field activity that is increased, decreased, or normal in cuprizone-treated mice. In this study, female C57Bl/6J mice received either control chow (n=6) or 0.2% cuprizone chow (n=6) for 6 wk. Throughout the study, mice were single-housed on racks with cloud-connected
video cameras. In the cloud, computer algorithms processed the video to create a motion biomarker. Analysis of motion revealed variable responses depending on the time of day and occurrence of recent procedures (cage change). Nighttime motion was significantly decreased in cuprizone vs. control mice on nights 5-14, 20-28, and 35-41. Daytime motion was not different with the exception of significantly elevated motion in cuprizone mice following cage change. Continuous monitoring showed that different conditions throughout the study, such as time of day or procedure, interact with the cuprizone treatment to produce different behavioral outcomes. These results reinforce our understanding that traditional behavioral tests, utilizing a limited observation window, are influenced by environmental and procedural factors. In contrast, continuous data collection allows for a more comprehensive characterization of an animal model since transient disturbances are detected as anomalies within a larger data set. The results highlight the utility of continuous collection of undisturbed behavior for better characterization of motor abnormalities in disease models.

**PS106 Exploring Cardiovascular and Metabolic Stress Responses to Carbon Dioxide Euthanasia in Rats**

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Euthanasia is a necessary component in research and must be conducted as humanely as possible. Carefully regulated CO2 exposure in conscious rats is currently recommended but published data are divided on whether this method is more distressing than others, such as adjunct anesthetic use prior to CO2 exposure. Thus, we evaluated cardiovascular and metabolic responses of direct CO2 versus isoflurane exposure prior to CO2 to assess distress in rats during euthanasia. We hypothesized that mean arterial pressure (MAP), heart rate (HR), and plasma glucose (GLU) would initially increase as a stress response to either gas and that these increases would be more marked with CO2 alone. Male Sprague Dawley rats were implanted with telemetry devices to obtain MAP, ECG, and GLU. Animals recovered for two weeks and were exposed to either 5% isoflurane (Iso+CO2, n=6) or 100% CO2 (CO2exp, n=7; 30% chamber volume replacement) in their home cages to induce loss of consciousness (LOC). Once LOC was achieved with isoflurane, Iso+CO2 rats were transferred to a charged CO2 chamber for euthanasia. CO2exp rats continued to receive CO2 in their home cage. MAP, ECG, and GLU were continuously acquired prior to gas exposure through death. MAP increased in both the CO2exp and Iso+CO2 groups when exposed to CO2 or isoflurane, respectively. MAP in the Iso+CO2 group decreased during LOC and further dropped when exposed to CO2 while MAP in the CO2exp group gradually increased until death. HR was elevated during initial isoflurane exposure and returned to baseline before CO2 exposure. When exposed to CO2, HR dropped dramatically in both groups. GLU remained stable throughout LOC with isoflurane and decreased slightly and transiently when placed in CO2. GLU decreased in the first minute after CO2 exposure in the CO2exp group and levels were stable until death. Time to death was shorter in CO2exp. Isoflurane exposure caused increased MAP and HR, whereas direct exposure to CO2 caused an increased in MAP but not in HR. A mild decrease in GLU was detected in CO2exp. These data suggest that LOC with exposure to either gas may be distressful, but euthanasia via CO2 without preanesthetic appears to occur quicker and elicit less of a physiologic stress response.

**PS107 Comparing Male and Female Phenotypes in Common Mouse Models of Cardiovascular and Metabolic Disease**

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National Institutes of Health policy states that animal studies should account for sex as a biological variable by including males and females when possible. We studied key phenotypes in mouse disease models with a large usage bias toward males: cardiovascular disease strains (C57BL/6J mice with Apoe or Ldlr mutations), and diabetes models with inactive leptin (Leprob) or its receptor (Leprdb): two C57BL/6J mutant strains with transient hyperglycemia (B6-db and B6-db) and a C57BLKS/J strain with sustained hyperglycemia (BKS–db). On a standard chow diet, serum cholesterol measured on chemistry analyzer was higher at 8 wk in Apoe-null mice (531 vs 470 mg/dL; P = 0.0036; n=20 per sex) but did not differ significantly by sex in Ldlr-null (P = 0.9673; n=20 per sex); male and female cholesterol was higher than sex-matched controls (P < 0.0001 for both). LDL did not differ by sex in Apoe-null mice while LDL was higher in Ldlr-null females (females: 88 vs 65 mg/dL; P = 0.0006). HDL and ApoA1 were elevated in Apoe- and Ldlr-null and C57BL/6J males (P < 0.001 for each sex-wise comparison). As assessed by histopathology, atherosclerotic plaque formation varied by strain but not sex. In the diabetic strains, homozygous males and females gained weight similarly despite sexual dimorphism in control weights. In homozygous mice at 8 and 16 wk of age, the only significant weight differences between males and females (n=40 of each) were at 8 wk in B6-db (males were 1.7 g more; P = 0.0037) and 16 wk in B6-db (females were 2.5 g more; P = 0.0017). BKS-db/db males had higher blood glucose than females (415 vs 357 mg/dL; P < 0.0001) at 8 wk but no other differences were found at 8 and 16 wk. HbA1c was higher in males for all strains at 16 wk (P < 0.05 for all comparisons). As assessed by DEXA imaging, male and female homozygotes had similar body fat despite differences in control genotypes. BKS-db/db insulin was higher relative to controls at 8 wk, but db/db levels did not differ by sex (n=10 per sex). Leptin was elevated in db/db mice over controls (P < 0.0001 for all comparisons). Leptin did not differ by sex in db/db mice at 8 wk, but was higher in 16-wk females (P = 0.0039). All data are in Mouse Phenome Database as references for using female mice in diabetes and cardiovascular research.
PS108 Subcutaneous Mass in a Naked Mole Rat (*Heterocephalus glaber*)

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A 9-mo-old experimentally naive female naked mole rat (NMR) (*Heterocephalus glaber*) presented with an approximately 2.5 x 1.0 x 0.5 cm soft to firm, dark purple to black subcutaneous mass on the right ventrolateral abdomen. Otherwise the NMR displayed no adverse clinical signs, was in good body condition, and displayed normal behavior. The NMR was from a closed colony that have been bred inhouse for approximately 20 y and have routinely tested negative for common rodent pathogens. The differential diagnosis list included neoplasia, hematoma, or abscess. Naked mole rats are a species known for their longevity and resistance to neoplasia. There are only very rare reports of neoplasia in the literature. Similarly, the young age of the animal also reduced the likelihood of neoplastic disease. Fighting or traumatic injuries are one of the more common health issues reported in this species, which could lead to either a hematoma or abscess. However, no external wounds were seen on physical examination. The NMR was euthanized by anesthetic (ketamine/xylazine) overdose and a necropsy was performed. Gross necropsy revealed that the large, dark subcutaneous mass was the cecum, which had herniated through a defect in the abdominal wall. The abdominal hernia was likely a result of a congenital defect or trauma, though no traumatic events were noted by animal care or investigative staff.

PS109 Generalized Neurologic Disease in an Athymic Nude Mouse

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A 15-month-old, singly housed, female athymic nude mouse was examined for acute hindlimb lameness. The animal was experimentally naive and last observed to be normal 1 wk prior. On physical exam, the mouse was bright, alert, responsive, hunched, and slightly dehydrated and thin. The mild dehydration resolved with moist chow provided on the cage floor, and eating, drinking, urination, and defecation were all observed to be normal. On neurologic exam, there was moderate kyphosis and decreased weight bearing in all limbs. The mouse generally remained in a sitting position with hindlimbs splayed, and nonambulatory tetraparesis was observed. Cranial nerves were within normal limits, and paw placement was intact. Withdrawal reflexes were decreased, and hindlimb splay reflexes were absent. Superficial pain was positive in all limbs. On orthopedic exam, all limbs had decreased range of motion and mildly decreased muscle mass/tone. No overt pain or soft tissue swelling was noted, but the hindlimbs appeared mildly erythemic. At the stifles, 2-3 small, white, firm, round nodules were present. Clinical signs and examination suggested the presence of both a lower motor neuron disease as well as an orthopedic condition. Differential diagnoses for the neurologic disease included neuritis, polyradiculoneuritis, acute inflammatory demyelinating polyneuropathy, peripheral nerve sheath tumor, paraneoplastic polynéuritis, and hypermagnesemia. Differential diagnoses for the orthopedic disease included osteoarthrosis, osteomyelitis, myositis, osteoporosis, muscle atrophy, and osteosarcoma. The animal was euthanized and submitted for histopathology which revealed acute, multifocal, moderate polyradiculoneuritis of the nerve roots and ganglia. It also revealed chronic, moderate osteoarthrosis of the femorotibial joints with cruciate ligament and meniscus involvement. While nearly all strains of mice develop some form of osteoarthrosis with age, acute polyradiculoneuritis is an unusual condition in laboratory mice. This disease shares many clinical similarities with Guillain-Barré syndrome in humans, which has been associated with *Campylobacter jejuni* infection. Clinicians should consider the potential bacterial etiologies of these conditions in mice, such as from *C. jejuni*.

PS110 Cervical Cutaneous Mass in a White Carneau Pigeon (*Columba livia*)

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A 14-y-old male research White Carneau pigeon used in psychology studies was assessed for a soft tissue mass on the right ventral neck near the crop. Two months prior, a mass suspected to be a feather follicle cyst 1cm in diameter was noted at the same location. An open wound over the mass and blood on the bird’s beak indicated self-trauma. The next day, the mass was cavitated, suggesting that a portion of it was pecked and removed by the pigeon. Medical care included oral trimethoprim sulfamethoxazole, meloxicam, and bandage application. Two months later, the mass was 3-4x larger (4.0 x 3.5 x 2.5cm), soft to firm, freely moveable, and extended into the muscularis. Physical examination did not reveal other masses or abnormalities. CBC and biochemistry were unremarkable. Metastases were not seen on whole body radiographs, which were normal aside from the mass. On ultrasound with color flow Doppler imaging, the mass was highly vascular with a multilobular nodular pattern. The mass was excised under general anesthesia, but complete surgical margins were difficult to obtain due to close adherence to the esophageal serosal surface. Recovery was uneventful and the bird resumed experimental operant chamber testing 11 d after surgery. Histologically, the dermis and subcutis were expanded by a well-demarcated, nonencapsulated, densely cellular mass comprised of sheets and haphazardly arranged short-interwoven bundles of neoplastic spindle to elongate cells supported by a thin fibrovascular stroma. Neoplastic cells formed variably sized nodules surrounding dilated veins, arteries, and cystic cavitations containing necrotic debris that extended to
deep and lateral surgical margins. Neoplastic cells exhibited moderate to marked atypia and 91 mitotic figures per 10 HPFs. On immunohistochemistry, neoplastic cells were positive for desmin and ε-smooth muscle actin and negative for pan-cytokeratin and S100. Based on gross, histological, and immunohistochemical features, the cutaneous mass was diagnosed as leiomyosarcoma. Medical oncotherapy was not pursued due to practical limitations. While mass regrowth is expected due to the histopathologic diagnosis, no regrowth was found 4 mo after surgery. Leiomyosarcomas of the skin and subcutis are rarely reported in avian species.

**PS111 Unusual Cause of Hind Limb Paresis in C57BL6 Mice Used for a Behavioral Study**

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An experienced investigator noted that an entire cohort (n=6) of young male C57BL6 male mice exhibited varying degrees of hind limb paresis following short habitation sessions in a restraint device for head-fixed, awake, behavioral experiments. The clinical history revealed that 2 wk prior to the behavior habitation, animals received a craniotomy and head-plate implant surgery. All animals recovered uneventfully and were clinically normal postsurgery until habitation in the restraint device. This investigator had performed both the surgery and behavior habitation procedures in mice previously with no issues. Several animals demonstrated only mild improvement after several days and physical examinations revealed normal mentation with no clinical abnormalities other than hind limb ataxia. Two morbid animals were euthanized for detailed necropsy. Grossly, the skin of the dorsum on both mice was slightly depressed and irregular over an area of 2 cm x 0.5 cm along the spine. On dissection, the underlying subcutaneous tissue and musculature was necrotic and firm. Histologically, in both cases there was regionally extensive acute to subacute necrosis, degeneration, edema, mineralization, and atrophy with associated acute neutrophilic inflammation of the skin, subcubs, and underlying musculature. These findings extended to the distal cervical-thoracic vertebral bones with degenerative myelopathy of the underlying spinal cord consistent with thermal/caustic injury. On follow up queries, the investigator revealed that he had used a halogen lamp to illuminate the head-plate for 5-10 m in close proximity to the animals at the onset of each habituation session. On the basis of the experimental history as well as the clinical and pathological findings, the cause of hind limb paresis was determined to be halogen lamp-induced severe deep thermal injury with myelopathy. This case demonstrates the importance of creating awareness among personnel manipulating laboratory animals regarding types of light sources and their potential to cause thermal injury.

**PS112 Sudden Death after Laceration Repair in a Pair-Housed C1Q Transgenic Rabbit**

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A 13-mo-old, pair-housed, intact male rabbit was found dead in its cage 3 d after treatment of minor skin lacerations suspected to be fight wounds. This rabbit was a C1Q heterozygous knockout. C1Q is a classical complement component and its insufficiency is associated with autoimmune disease including systemic lupus erythematosus (SLE). Skin ulcerations on the ear and dorsum had been clipped and cleaned under inhalant anesthesia, and the animal had been separated from its male cagemate and treated with subcutaneous meloxicam and topical silver sulfadiazine. Differential diagnoses for sudden death included adverse anesthetic/analgesic reactions, hemorrhage or infection of wounds, stress-related intestinal ileus, or glomerulonephritis due to C1Q insufficiency. Necropsy findings included acute necrotizing pancreatitis and focal duodenal perforation with fibrinous, nonsuppurative peritonitis, adrenal gland infarction, and extensive submucosal gastric amyloidosis. Abdominal fluid cytology showed a predominately lymphocytic infiltrate. Taken as a whole, the precipitating cause of death appeared to be acute, severe pancreatitis and duodenal perforation, but the antecedent cause was unclear. Both gastric amyloidosis and pancreatitis are unusual in rabbits. Cutaneous, renal, or other manifestations of SLE were not present. C1Q mutations have been associated with other autoimmune disorders including scleroderma and urtiacial cutaneous vasculitis. Unfortunately, serum samples and skin lesions were not available in this animal. While spontaneous pancreatitis cannot be ruled out, its infrequency in rabbits and the concurrent presence of amyloidosis, adrenal gland infarction, and skin lesions suggest that a C1Q-related autoimmune component may be present. This case illustrates that sudden death in transgenic animals can act as a sentinel event that may guide investigation of potential phenotypes. Biopsy of unusual skin lesions, complete tissue collection from animals found dead, and assessment of serum autoimmune antibodies will be useful in following this colony going forward.

**PS113 Complications following Caesarian Section in a Common Marmoset (Callithrix jacchus)**

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A 2-y-o, pair-housed, primigravid common marmoset (Callithrix jacchus) presented for dystocia. Two stillborn fetuses were delivered following medical intervention. Radiographs and abdominal ultrasound revealed 2 remaining dead fetuses, which were removed through caesarian section. On postoperative d 1, the marmoset developed swelling, erythema, and lameness of the right leg below the stifle. Differential diagnoses at this time included ischemia-reperfusion injury secondary to catheter placement or the leg-tie during surgery, venous
thrombosis, inferior vena cava syndrome, cellulitis, or trauma. Blood work revealed a marked thrombocytopenia (PLT 22K/µL), elevated BUN, and hypoproteinemia. Supportive care, analgesia, and systemic antibiotics were initiated. Right leg swelling and skin necrosis progressed with subsequent loss of motor function, deep pain sensation, and appreciable tarsal pulse. The distal tail and left foot also developed swelling and erythema approximately 48 h after surgery. Due to rapid deterioration and tissue compromise, a mid-femoral amputation of the right leg was performed. The marmoset recovered well following the second surgery, but the distal tail and left foot became necrotic over the next several days. Euthanasia was elected due to poor prognosis. Necropsy and histopathology confirmed ischemic necrosis of the left foot and distal tail, with thrombi near the junction of necrotic and healthy tissue in the tail. Few foci of necrosis and scarring consistent with infarction were present in both kidneys. The cause of morbidity in this patient was likely multifactorial. Hypercoagulability associated with pregnancy and compression of the vena cava while supine during C-section surgery, both of which are common in humans, may have contributed to decreased peripheral perfusion and subsequent injury to the extremities.

**PS114 Icteric Mouse in a Tumor Xenograft Study**

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A 4-mo-old, 20.3g, female athymic nude mouse (Crl:NU(NCr)-Foxn1null) was examined for concerns of yellow discoloration to the skin (icterus). Two mo prior, the lab injected 4.6 x 10^6 MDA-MB cells subcutaneously in the right flank of the mouse. On initial presentation, the physical exam was unremarkable except for the yellow tint to the skin; the animal’s abdomen palpated soft and nonpainful with no evidence of masses or fluid. The lab did not note tumor growth and elected to continue monitoring this mouse. Two w after initial presentation, the lab decided to euthanize the mouse due to weight loss of 2 grams. The list of differential diagnoses included hemolytic disorders, hepatitis, cholangitis, cholelithiasis, cholecystitis, and neoplasia. On necropsy, the gallbladder was severely distended at 7mm in diameter; the gallbladder contained mildly viscous, dark green/black bile with no evidence of choleliths. Clinical chemistry revealed elevation of leakage and induced liver enzymes, hypercholesterolemia, and hyperbilirubinemia. On histopathology, we found evidence of metastatic carcinoma in the gallbladder, lung, pancreas, ovaries, uterus, mesenteric, and peri-pancreatic lymph nodes. The liver also had evidence of cholangiohepatitis and coagulative necrosis. Based on the lack of neoplastic foci on serosal surfaces, inadvertent intraperitoneal injection was unlikely. This case represents an atypical presentation of subcutaneous tumor implantation.

**PS115 Abdominal Mass in a Female Common Marmoset (Callithrix jacchus)**

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An adult, experimentally naïve, multiparous female common marmoset (Callithrix jacchus) presented with a large, approximately 3 x 4 x 2 cm, firm, immobile mass in the right cranial abdomen during quarantine. Diagnostic test results and the clinical evaluation were otherwise unremarkable. The animal was cohoused with a male. Ultrasound revealed the presence of a hyperechoic mass with solid and fluid-filled compartments in the right cranial quadrant of the abdomen. MicroCT revealed an intraabdominal hyperdense fetiform structure in the region of the palpated mass. Differential diagnoses included teratoma, ectopic pregnancy, or fetus in fetu. The animal was euthanized after an acute terminal procedure and submitted for complete necropsy. On gross examination, a large, oval heterogenous mass was found adhered to the margins of the left liver and abdominal wall. Macroscopically, the mass’s consistency ranged from soft to hard depending on location. On cut section, it appeared to contain different tissue types with a variable level of organization. Microscopically, the mass had a thick fibrous capsule and contained bony structures surrounding 2 skull-like structures, as well as segments of axial and appendicular skeleton, soft tissues and organs. MicroCT, anatomic, and histologic findings, differential diagnoses, and prevalence in nonhuman primates will be discussed.

**PS116 Dyspnea in a Long-Evans Rat (Rattus norvegicus)**

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A 3-mo-old male Long-Evans rat (Rattus norvegicus) presented acutely with open-mouthed breathing. The only experimental manipulation was an intravitreal injection of an adenovirus vector one month prior. On physical examination, the rat was bright, active, and responsive, and in addition to the dyspnea was sneezing with production of white foamy nasal discharge. Differential diagnoses included upper respiratory infection, pneumonia, or congestive heart failure. Euthanasia was elected because of poor prognosis. Gross necropsy findings were pulmonary hyperinflation, esophageal and intestinal dilation suggestive of aerophagia, and a markedly thickened left cardiac ventricle. Histologically, there was extensive cardiac myocyte hypertrophy but without significant degeneration, necrosis, inflammation, or fibrosis. Neither pulmonary edema nor hepatic congestion were
A cohort of 20 adult male and female STAT2 knockout Syrian hamsters (Mesocricetus auratus) was imported from another academic institution. Approximately 8 wk after arrival, and prior to the start of an experimental infectious disease study, 6 hamsters presented with multiple swellings on the face and/or legs or with poor body condition and an enlarged abdomen with a firm, palpable mass. The animals were euthanized and necropsies were performed. Gross necropsy findings varied among the hamsters and included exophytic growths on oral mucosa and within cheek pouches, multifocal grey raised nodules in the skin, and mottled mesenteric and retroperitoneal masses that adhered to or displaced other viscera. Livers were enlarged and pale or mottled. Histopathology was completed on 3 hamsters. Findings in the skin were multiple epithelial neoplasms showing areas of differentiation to all segments of the hair follicle including hair bulb, inner and outer root sheath, and accumulation of keratin and matrical shadow cells within cystic centers. Where rupture of the neoplasm exposed keratin, a secondary granulomatous dermatitis was present. In abdominal tissue sections, sheets of moderately pleomorphic neoplastic round cells formed mesenteric masses and infiltrated the liver, kidney, GALT, and mesenteric shadow cells within cystic centers. Where rupture of the neoplasm exposed keratin, a secondary granulomatous dermatitis was present. Gross and histologic lesions were characteristic of trichoepitheliomas and lymphoma. A diagnosis of transmissible lymphoma induced by hamster polyomavirus (HaPyV) was made based on prior pathogen surveillance testing at the sending institution, clinical presentation, and gross and histologic evaluation.

PS117 Cutaneous, Oral, and Abdominal Masses in a Cohort of Immunomodulated Syrian Hamsters (Mesocricetus auratus)

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A cohort of 20 adult male and female STAT2 knockout Syrian hamsters (Mesocricetus auratus) was imported from another academic institution. Approximately 8 wk after arrival, and prior to the start of an experimental infectious disease study, 6 hamsters presented with multiple swellings on the face and/or legs or with poor body condition and an enlarged abdomen with a firm, palpable mass. The animals were euthanized and necropsies were performed. Gross necropsy findings varied among the hamsters and included exophytic growths on oral mucosa and within cheek pouches, multifocal grey raised nodules in the skin, and mottled mesenteric and retroperitoneal masses that adhered to or displaced other viscera. Livers were enlarged and pale or mottled. Histopathology was completed on 3 hamsters. Findings in the skin were multiple epithelial neoplasms showing areas of differentiation to all segments of the hair follicle including hair bulb, inner and outer root sheath, and accumulation of keratin and matrical shadow cells within cystic centers. Where rupture of the neoplasm exposed keratin, a secondary granulomatous dermatitis was present. In abdominal tissue sections, sheets of moderately pleomorphic neoplastic round cells formed mesenteric masses and infiltrated the liver, kidney, GALT, and mesenteric shadow cells within cystic centers. Where rupture of the neoplasm exposed keratin, a secondary granulomatous dermatitis was present. Gross and histologic lesions were characteristic of trichoepitheliomas and lymphoma. A diagnosis of transmissible lymphoma induced by hamster polyomavirus (HaPyV) was made based on prior pathogen surveillance testing at the sending institution, clinical presentation, and gross and histologic evaluation.

PS118 Changing Human Behavior Towards Animals to Improve Laboratory Animal Welfare

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Visits to research facilities in Canada and the U.S. by various regulatory and accreditation organizations commonly demonstrate gaps in routine implementation of tools, practices, and techniques to enhance the environment and care of research rodents. While part of the answer for this may lie in a need for further animal welfare science and 3Rs research, some of this is also related to resistance in changing behaviors and practices in those working with research rodents. This study focused on the need for understanding principles of human behavior change to improve animal welfare. The specific objective was to assess the current attitudes of laboratory animal professionals, including veterinarians, veterinary technicians, and animal care personnel towards research animal welfare to determine major animal welfare concerns for rodents. Study participants were recruited from amongst the memberships of CALAS and CALAM. Methods consisted of 1:1 structured 30-60-m interviews with 17 veterinarians and 18 veterinary technicians/animal caregivers from a range of research facility types from across Canada. Volunteers were asked between 13-17 questions, depending on their responses, and they were additionally asked to complete a 5-question written demographic survey after the oral interview. Interviews were recorded and transcribed verbatim and transcripts were subsequently coded using a thematic analysis. Our results identified no discernible differences in response comments between gender, age, geographic distribution or institutional type, although responses did differ in weighting between veterinarians and veterinary technicians/animal caregivers. Both groups consistently identified current rodent housing standards and pain management as their biggest welfare concerns. Historical standards and speciesism were identified as the primary reasons for current welfare issues, but most interviewees remain optimistic that it is possible to move beyond these limitations. We hope to use these findings to develop a general action plan that may be used to address research rodent welfare issues.
PS119 Cognitive Dissonance in Laboratory Animal Medicine and Implications for Animal Welfare

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People experience cognitive dissonance when they entertain 2 conflicting ideas at the same time. Cognitive dissonance may cause a negative emotional state, which can lead to engagement of compensation mechanisms to resolve the dissonance. This report describes a survey which explores cognitive dissonance in laboratory animal veterinarians and veterinary technicians and some ways in which veterinary staff manage dissonance associated with research animal use. Respondents, 164 veterinarians and 145 veterinary technicians, were asked to rate their opinions of various statements on a scale of 1-10, where the numbers represented “strongly disagree to strongly agree” or “never to always.” Statements assessed negative emotions (shame, powerlessness, and frustration) and compensation mechanisms (devaluing, emotional distancing, and shifting responsibility). Responses were evaluated overall and compared by level of training (veterinarian versus veterinary technician), years of work experience (0-5, 6-10, >10), and species tended (large, mixed, small species). Respondents strongly agreed that animal wellbeing and animal use in research were important. Respondents reported feelings of shame, powerlessness, and frustration associated with work, but did not consistently agree to feeling powerless to initiate changes affecting animal welfare. The most frequent compensation mechanism noted was shifting responsibility onto the IACUC and institutional rules. Devaluing the animals was also reported as a compensation mechanism. Responses to emotional distancing statements were divided. The survey supports the existence of cognitive dissonance associated with laboratory animal medicine. Potential negative and positive impacts on animal welfare are discussed.

PS120 Conflict of Interest in Research: What Is It and Why Should You Care?

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Conflict of interest (COI) is receiving increasing scrutiny in biomedical research. Discussions about COI impact all research due to requirements found in federal regulations, scientific journals, research contracts, grant agency guidelines, and institutional policies, many of which originate out of concerns about industry influence on academic research from over 20 years ago. Recently, the failure to disclose industry relationships in scientific manuscripts has resulted in increased media and public attention to academic research programs along with faculty consulting arrangements. Concurrently, academic research programs have heightened institutional policies regarding COI disclosures and management as well. Since several studies indicate that even small financial relationships may influence research results, public concern about COI will only continue to intensify. We review the foundations of the current concerns with academic-industry research affiliations, which directly resulted in the current federal regulations on financial COI (FCOI). The most common types of COIs will be discussed and made relevant to animal research oversight. Differences between FCOIs and COI for IACUC review, as per Animal Welfare Act Regulations and the Public Health Service Policy requirements, will be critically examined. Additionally, the impact of rigorous COI review, management, and enforcement on animal research and researchers at various institution types (academic, industry, government) will be evaluated.

PS121 Development of a Laboratory Animal Care Training Program

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Laboratory animal technicians are tasked with immense responsibility in caring for laboratory animals that support life-saving research. Attention to proper husbandry and animal care are imperative for upholding animal welfare. Many academic institutions now offer training programs for animal technicians, and companies exist to provide training for animal care technicians. There are also several other resources available for training content, such as the AALAS Learning Library. It is also helpful for individual institutions to develop their own training programs based on their own standards of care and vivariums. We share the development and implementation of a training program for a large commercial institution where lessons learned may be applied to any institution. We discuss how the training program was developed, including use of best practice sharing amongst facilities, creation of a training guidebook for 2 types of housing environments, as well as program implementation and assessment.

PS122 Focus On Severe Suffering: How a Scientific Animal Welfare Organization Can Promote Change

E Lilley

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All laboratory animal suffering is a concern, but the Royal Society for the Prevention of Cruelty to Animals (RSPCA) believes that ending ‘severe’ suffering (equivalent to some procedures within category D/E of USDA Pain
and Distress Categories) should be a top priority. There are a number of reasons to do this: the ethical obligation to avoid or reduce suffering, the requirement to adhere to the 3Rs principles, and the scientific benefits. It is widely acknowledged that good quality science goes hand in hand with good welfare, and that unalleviated suffering can introduce avoidable variation and reduce the power of experiments. As a scientific animal welfare organization with a high level of national and international liaison with scientific and regulatory communities, we have been able to establish an integrated program of work aimed at reducing and ultimately ending severe suffering. We promote constructive dialogue between those who are involved in the use, care, and regulation of research and testing to identify practical strategies to avoid, or reduce the impact of, severe models and procedures. Our approach is well supported by the scientific community and the UK Government. Our pioneering initiative has so far included the organization of 2 major international conferences, the convening of several expert working groups, and the production, publication, and dissemination of a range of resources, including a dedicated web resource to help reduce and ultimately end severe suffering. We highlight how the RSPCA, as a scientific animal welfare organization, working with, rather than against, the scientific community has managed to improve implementation of the 3Rs. Practical examples of approaches to reduce severe suffering and a range of resources that we have produced will be presented.

**PS123 Increasing Benefit in Harm/Benefit Analysis by Robust Study Design**

J Rigney

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IACUCs work to minimize harms and ensure judicious use of animals. Harm-benefit analysis (HBA) of the experiment is usually discussed during committee review. The HBA assesses whether the inflicted harm on the animals is outweighed by the potential benefit of the experiment. Committees generally accept the science justification for the procedure is valid, based on the scientist’s citing of published data or previous work. Data generated from preclinical studies should be reproducible and translatable to the human condition, pathway, or target. If not, the benefit of the study may not outweigh the harm to the animals. What are the tools to select the best model and design a study that is reproducible, translatable, and robust? Are there ways to avoid publication bias, that is, studies with “positive” outcomes validating a hypothesis, or a novel scientific discovery get published rather than those with uncertain or negative outcomes. Knowing this, how can we be assured that we are minimizing harm and maximizing benefit? Tools developed in house facilitate determination of quality study design driven by preclinical-clinical continuity and translational relevance and pathobiology of clinical disease. The tools are a prestudy review by a multidisciplinary Animal Model Strategy Team (AMST), and employment of an Animal Model Quality Assessment (AMQA), followed by retrospective After Action Review (AAR) of animal models. Additionally, applying robust study design principles, such as randomization, blinding, and power analysis of experiments uses animals judiciously and avoids progression of projects with misleading data, and nonreproducibility of experiments. Application of these tools, in conjunction with robust study design, increase benefit and minimize harm. Rapid advances in data analytics and data sharing initiatives could further decrease the effects of publication bias generating advances in 3Rs principles.

**PS124 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 resource 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 group has made significant progress over the past year, and the site is currently in development and testing. We provide an overview of the CUSP project, as well as an update on the current status and what attendees can expect moving forward.

**PS125 Reaching Research Goals through Animal Behavioral Training**

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Animal research models are necessary to adequately study new medical technologies and are required to bring new products to market. To obtain viable data, animals should be physically healthy and housed in an environment with minimal external stressors. Behavioral acclimation and training can decrease stress and allow for easier data collection. Dogs, sheep, and pigs here walk on treadmills, spend time in slings, carry external equipment in saddlebags, and are acclimated to a variety of conditions. Recently we introduced clicker and target training to provide better animal welfare and easier animal handling for animal care staff. Clicker training is a type of operant conditioning which uses a distinct click to mark desired behaviors. Animals are taught to associate the click with a food treat. After learning the association, the sound is used to train animals to other behaviors, such as touching their nose to a target. Target training encourages animals to step onto a scale to be weighed, stand still while
being examined or data is being collected, return to their kennel, and walk on a treadmill. These refinements have
decreased behavioral signs of stress in the animals when they’re being handled and provide additional cognitive
enrichment. The care staff can perform their duties in a safer and more efficient manner since the animals are
better trained. Overall these behavioral refinements have improved study data collection, animal welfare, and staff
morale.

PS126 Setting New Principal Investigators up for Success by Streamlining the On-boarding Process
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New principal investigators (PIs) require guidance in both establishing their first animal protocol and
understanding their responsibilities as PI. Our IACUC identified a need to inform relevant parties when new
PIs are being on-boarded and to establish a process to provide new PIs with targeted resources to assist with
their transition. In this large research program, there are many channels through which animal research service
departments may learn of the arrival of a new PI. Once a new PI is identified, the IACUC office is alerted and then
acts as the centralized communication source to notify key personnel across departments. Standardized messaging
allows on-boarding tasks between departments (e.g., granting system access, coordinating animal shipments) to
occur in tandem, reducing burden on administrators as well as the new PI. The IACUC office also sends each new
PI a welcome message guiding them to a New PI Resources webpage. This webpage was created by the IACUC
office in an effort to establish a “one-stop-shop” for new PIs, providing relevant information on topics such as
accessing the electronic protocol management system, writing an animal protocol, completing required training,
and obtaining animal facility access. Additionally, a link to OLAW’s What Investigators Need to Know About
the Use of Animals is provided as an introductory resource. Complimenting this webpage, the IACUC office also
arranges a meet and greet between each new PI, the IACUC chair/vice chair, and an IACUC administrator. Meeting
the new PI in person allows for customized discussion and the ability to answer questions about their specific
research needs. It is also an opportunity to convey the IACUC’s expectations for taking ownership of an animal
protocol. Each month, the IACUC is provided with metrics communicating both the volume and type of new PIs
joining the program. By streamlining the PI on-boarding process, relevant information is disseminated efficiently
and a positive relationship is fostered with the new investigator and the IACUC.

PS127 Understanding Compassion Fatigue: Associations between Euthanasia, Social Support, and Other Factors
with Personnel Quality of Life
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Laboratory animal personnel may be subject to significant workplace stress because of constantly caring for animals
that may experience pain or distress and are typically euthanized at the end of the project. Workplace stress is often
described as compassion fatigue, which is comprised of burnout and secondary traumatic stress (a result of viewing
stress in others). We explored the associations between professional quality of life (compassion fatigue as well as
the opposite, compassion satisfaction) and potential risk or protective factors in laboratory animal personnel. A
total of 802 laboratory animal personnel, from the United States and Canada, completed an online survey regarding
professional quality of life, social support, euthanasia, stress/pain levels, and general interactions with laboratory
animals. Personnel worked in a wide range of settings (e.g., industry, universities), research types (e.g., basic,
applied), species (e.g., nonhuman primates, mice), and roles (e.g., animal care technicians, veterinarians). Data were
analyzed using general linear models. Our results indicate that higher self-reported levels of burnout and secondary
traumatic stress were associated with less social support, enrichment provision, and control over euthanasia ($P <
0.05$). Additionally, lower levels of burnout and secondary traumatic stress were associated with lower levels of
stress or pain in the animals being cared for, the personnel’s desire for more enrichment provision, and general
positive behaviors towards laboratory animals ($P < 0.05$). Surprisingly, neither primary species the personnel
worked with nor frequency of euthanasia performed by the survey participant was associated with workplace
stress levels ($P > 0.05$). In summary, higher reported levels of social support, enrichment provision, and control over
euthanasia, as well as lower levels of animal stress/pain, were associated with less compassion fatigue. Developing
interventions targeting these areas could improve the professional quality of life of laboratory animal personnel.

PS128 Impact of Euthanasia on Compassion Fatigue in Personnel Working in Animal Research
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A cross-sectional study design was used to investigate the association between euthanasia and compassion fatigue
among employees working in animal research at a large academic medical center. The Professional Quality of
Life Compassion Satisfaction and Fatigue questionnaire was modified to refer to animal research, and scores from 3 domains (compassion satisfaction scale, burnout scale, and secondary traumatic stress scale) were compared between individuals who euthanized animals and those who did not using a 2-sample t-test. The overall web survey response rate was 30.8%, with a total of 414 responses out of 1,341 surveys. A total of 273 self-reported in the survey that they euthanized animals and 73 self-reported that they did not. There was no difference \( (P = 0.10) \) in the compassion satisfaction T score between those who euthanized animals (mean 49.6, SD 10.0) and those who did not (mean 51.7, SD 10.1). There was a significant difference \( (P < 0.0001) \) in the burnout T score with greater burnout reported by those who euthanized animals (mean 51.1, SD 9.8) compared to those who did not (mean 45.2, SD 9.2). Those who euthanized animals also reported significantly higher \( (P = 0.04) \) traumatic T scores (mean 50.5, SD 9.96) than those who did not (mean 47.8, SD 10.0). The effect sizes for score differences were further examined using Cohen’s D to quantify the strength of the association between compassion satisfaction, burnout, and traumatic Stress scores and animal euthanasia. Effect sizes were small for compassion satisfaction and traumatic stress scores, -0.21 and 0.27, respectively, and medium for the burnout score, 0.61. Animal research workers who euthanize animals reported significantly more burnout and traumatic stress than workers who do not, and the magnitude of the differences was greater for burnout than for traumatic stress. Going forward, we will analyze our survey data further to determine whether factors such as years of employment, animal species, or method of euthanasia are associated with feelings of burnout and traumatic stress among individuals involved in euthanasia. This information may be used to inform targeted interventions to mitigate these aspects of compassion fatigue.

**PS129 Murine Norovirus Mediated B Cell Depletion in Stat1 Knockout Mice Does Not Impair Effective Antibody Production**

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Noroviruses have a profound global epidemiologic impact on human health, and it was recently shown that noroviruses can infect B cells. We previously demonstrated that murine norovirus (MNV) infection significantly reduces all stages of B cell populations in the bone marrow in a signal transducer and activator of transcription 1 (Stat1) dependent manner as early as 7 d postinfection. Because B cells are the source of antibody production, we hypothesized that depletion of B cells in the bone marrow may cause an impaired ability to effectively produce antibodies when exposed to novel antigens. To test this hypothesis, uninfected Stat1 knockout mice (129S6/ SvEv-Stat1tm1Rds) were immunized with the T-dependent antigen keyhole limpet hemocyanin (KLH) and then compared to KLH immunized mice infected with MNV-4 (n=5 mice/group), or uninfected Stat1 knockout mice were immunized with the T-independent antigen NP-Ficoll, and then compared to NP-Ficoll immunized mice infected with MNV-4 (n=5 mice/group). Serum levels of antigen-specific IgG were measured by ELISA 4 wk after immunization. After immunization with NP-Ficoll, MNV-4 infected Stat1 knockout mice had an average OD value of 1.04 compared to uninfected Stat1 knockout mice with an average OD value of 0.36. After immunization with KLH, MNV-4 infected Stat1 knockout mice had an average OD value of 1.58 compared to an average OD value of 1.11 in uninfected Stat1 knockout mice. We demonstrate here that the significant depletion of B cells in the bone marrow of Stat1 knockout mice infected with MNV-4 does not result in a significant difference \( (P > 0.05) \) in serum IgG levels compared to uninfected mice immunized to either KLH or NP-Ficoll. These results suggest that B cells in MNV-4 infected mice can still mount an effective adaptive immune response to novel antigens, even in the face of diminished populations of developing B cells in the bone marrow. This effect may be due to B-cell responses outside of the bone marrow that are still able to effectively respond to antigens. Further investigation is warranted to determine the mechanisms of this effective response.