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Abstracts of Scientific Presentations

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Poster Sessions

P1 Bridging The Gap Between Surgical Training and Best Practices: A Team Approach

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Our campus is home to a diverse animal research program that encompasses over 700 animal use protocols and 200 principle investigators. The majority of animal research conducted is performed in mouse and rat models, and a substantial proportion of this research requires rodent survival surgery. Historically, 100% of rodent survival surgery is conducted by research faculty or staff, with operators trained by more experienced research personnel. While researcher-to-researcher training is ideal for teaching protocol-specific surgical procedures, this paradigm may be less effective for teaching foundational principles of aseptic technique, intraoperative care, and postoperative analgesia. The Department of Veterinary Medicine and Surgery (DVMS) identified the gap between laboratory-based surgical training and accepted best practices for rodent survival surgery as a potential OLAW compliance and animal welfare concern. In response, DMVS developed a 3-pronged approach for identifying and addressing investigator knowledge gaps in basic principles of surgery and patient care. This approach entails semiannual rodent survival surgery record reviews by the IACUC veterinarian, observation of surgery and completion of surgery checklists by DVMS personnel, and free hands-on training through nonsurvival surgery wet labs organized and taught by DVMS veterinarians and technologists. Unlike some research programs of comparable size, this facility does not have a formal postapproval monitoring program, nor is there an institutional requirement for veterinary supervised training programs or competency assessment in rodent survival surgery. Our team approach, with its emphasis on voluntary participation, collegiality, and client support, helps fill this gap while building positive working relationships between the veterinary and research staffs.

P2 Refinement of IV Tail Catheterization in Support of Imaging Procedures

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We were conducting imaging studies that required us to give multiple IV tail injections of contrast agent to the same groups of mice per week. Proper and successful administration of the contrast agent was necessary for high-quality imaging results. The traditional IV catheter that was being used gave us excellent results at the first and second time points, but created vasculitis, contusions, and swelling in the tail, rendering it unusable for the third and subsequent time points. This led us to the development of a new type of tail vein catheter. First, we found and purchased a different type of catheter which was a smaller gauge and had a needle at the end for easy insertion. The new catheter was then modified to fit our needs by connecting it to extension tubing using a modified 27G needle as a connector. Animals were implanted with this cannula catheter for a 90-minute time point, and for each time point a new cannula catheter was placed. We also created a tail splint and modified induction box for this new type of catheter. This prevented the mice from dislodging the new catheter during the anesthetic induction period. This novel method of catheterization led to an improved outcome for both the animals and the science by virtually eliminating damage to the tail and producing more consistent and reliable administration of contrast agent.

P3 Vitamin D Deficiency in Broiler Chicks (*Gallus gallus domesticus*)

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Fourteen-day-old broiler chicks presented with retarded growth rates, lethargy, and leg deformities. Broiler chicks were purchased from a commercial vendor at 2 days of age and then housed in battery cages in a poultry facility at an AAALAC-accredited institution. They were enrolled in a nutrition study assessing novel protein sources to optimize the physiologic performance of broiler chickens. On physical exam, chicks showed varying severity of clinical signs including retarded growth, lethargy, stilted gait, reluctance to stand or walk, splay legs, and increased respiratory rate with open mouth breathing. The affected chicks were euthanized and necropsies were performed. The beaks and long bones were soft and pliable. The keel was deviated from midline. There was also "beading" of the ribs at the juncture with the spinal column. Histopathology of long bones showed expansion of the zone of proliferation in the physeal cartilage, retention of cartilage in the medullary spicules, widened osteoid seams lined by osteoblasts, thickened periosteum, and fibrosis in the medullary cavity. Vitamin D deficiency, or rickets, was diagnosed. This was due to expired Vitamin D in the lab-mixed diet. The remaining chicks on study were placed on a commercial grower diet and clinical signs of rickets resolved within 2 days.

P4 Spontaneous Nonneoplastic Bone Proliferation in a Brown Norway Rat

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A 24-month-old, 650 gram, single-housed, male Brown Norway (BN/Crl) rat presented with history of swelling of the right forelimb. Physical examination was unremarkable with the exception of a markedly expanded, solid, hard, boney mass in the right antebrachium. The principal investigator was contacted and opted to end the study at an earlier time point but allowed dissection of the affected limb for further analysis. The animal had no previous medical or treatment history and was part of an aging study that did not involve the use of any tumor cells or carcinogenic substances. Radiographic and 3-dimensional reconstruction of micro-computed tomography (3D-µCT) images were used to reveal a radiopaque lobulated and trabecular mass encompassing the radius and ulna in the dissected limb. Further 3D-µCT analyses also revealed that the mass had a total volume of 3,074 cm^3 and density of 698.82 mg/cc, which corresponded to approximately 59% of the density of healthy cortical bone. Histopathological analyses were conducted to attempt diagnosis of one of the proposed differentials (osteoma, osteosarcoma, nonneoplastic bone proliferation, and osteomyelitis). Microscopically, the mass was primarily composed of large, interconnected trabeculae of

well-differentiated, lamellar bone. Osteocytes of the trabeculae were small and numbered 1 per lacuna. Few osteoblasts and osteoclasts were observed. The trabeculae were surrounded by fibrous tissue, often with central, large, blood-filled spaces. Occasional areas of hemorrhage were noted. The lesion was considered a nonneoplastic bone proliferation possibly related to a chronic nonunion fracture of the radius and/or ulna but further evaluation (immunohistochemistry using bone-specific markers) may be necessary to achieve the final diagnosis of this spontaneous condition.

P5 Retrospective Analysis of Trichobezoar and Phytobezoar Cases in Rhesus Macaques (*Macaca mulatta*)

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Trichobezoars and phytobezoars are stomach masses composed of compact hair or plant material, respectively, and mucus. Both can present asymptomatically in rhesus macaques (*Macaca mulatta*); however they may be associated with vomiting, anorexia, and weight loss. The incidences of these gastric masses are unknown in rhesus macaques, and contributing factors are not yet completely understood. Surgery and necropsy records between 2001 and 2014 from a single institution with a large breeding population of rhesus macaques were used to calculate bezoar annual incidences, overall incidence, and relative risk of sex. Animal ages and locations at presentation, recurrences, and minimum time to recurrence were also described to summarize cases. Retrospective analysis revealed 33 phytobezoars and 106 trichobezoars. Trichobezoar incidence (1.48 cases per 1,000 animals per year) was significantly higher than phytobezoar incidence (0.43 cases per 1,000 animals per year). Females were 1.8 times more likely to develop trichobezoars and 3.0 times more likely to develop phytobezoars than males. The mean age of animals with trichobezoars and phytobezoars was not statistically different. Recurrence events were not significantly different between trichobezoars (10% of animals) and phytobezoars (11.4% of animals). The mean time of bezoar recurrences were not significantly different. Fifteen trichobezoar cases were from a single outdoor field pen that houses multiple families of macaques. Overall, with up to 19 cases a year, trichobezoars and phytobezoars are relatively common in a large population of rhesus macaques. These masses are more often seen in females than males and appear to be uncommon in rhesus macaques less than 2.7 years old. Bezoar recurrences accounted for a minority of cases, but related animals from a single field pen accounted for a large proportion of trichobezoar cases. Further investigations of field pen variables may be needed to clarify risk factors associated with this cluster.

P6 Unilateral Hydronephrosis Secondary to Endometriosis in a Rhesus Macaque (*Macaca mulatta*)

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A 12-year-old, female, rhesus macaque (*Macaca mulatta*) presented with poor appetite and 11% weight loss over 2 months. Attempts to breed this animal were unsuccessful in the 2 years prior to presentation. A physical exam and results of routine hematologic and serum biochemical analyses revealed a body condition score of 2.5 and a mild azotemia with creatinine 1.6 mg/dL (range: 0.8-1.2 mg/dL). Abdominal ultrasound revealed a severely dilated left renal pelvis and severe dilation of the left ureter until the insertion on the bladder. Additionally, 3 round, hypoechoic structures were identified on the serosal surface of the uterus near the trigone and the cervix. A presumptive diagnosis of ureteral obstruction secondary to endometriosis was made. Due to the poor long-term prognosis, the animal was euthanized. Necropsy revealed entrapment of the left ureter by endometrial cysts and unilateral hydroureter and hydronephrosis. The residual left kidney parenchyma was pale. Two 3 mm pale tan masses were clustered in the bladder epithelium at the trigone in possible obstruction of the left ureter. The right kidney was unremarkable on gross examination. A left ureter urine biochemistry analysis showed a mildly elevated protein/creatinine (UPC) ratio of 0.6. Representing right kidney function, urine from the bladder had a moderately elevated UPC of 1.9. The combination of mild azotemia and protein loss confirmed renal disease in the right kidney. Ureteral obstruction by endometriosis has been reported in a Guinea baboon (*Papio papio*) and humans, but this is the first report of such a case in a rhesus macaque.

P7 Alleviating Pressure Sores in Swine (*Sus scrofa domesticus*) during Prolonged Anesthetic Events

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Our facility was requested to provide veterinary support for an IACUC-approved procedure in swine (55-65kg) involving 24+ hours of anesthesia. Since this timeframe is considerably longer than our routine procedures, refinements to our surgical positioning were vital to prevent hypoperfusion and neuropathy of compressed structures. During pilot studies, animals were placed supine in a thickly padded v-trough (35″L x 14″W x 8″H), as is common in large animal anesthesia. Intraoperatively, compression of the ear pinna affected patency of peripheral catheters. During postoperative care, discolored necrotic areas of skin were noted on high pressure areas of the body, such as the shoulder blades and dorsal aspect of the hips. For these reasons, the use of padded troughs was deemed unacceptable due to necrosis of the skin and underlying musculature, as well as potential for development of neuropathies. Study objectives such as evaluation of end organ histopathology, hematologic, and serum chemistry values may also be confounded compromising this model's utility. To solve our problem, a radiolucent cost-effective air mattress (110.2″ x 35.4″ x 2.5″) was used to alleviate the constant and concentrated pressure in the affected areas. In addition, the animal was rotated into a lateral position. Throughout the course of the procedure, the 130 bubble-cell mattress would inflate and deflate select areas to facilitate pressure redistribution and minimize negative skin effects. A technician periodically manipulated the animal's extremities to help aid circulation. This refined technique ensured catheter patency and greatly reduced the size and severity of the pressure marks noted on the body of the animal. Additionally, the increased frequency of contact with the patient granted more opportunities to detect abnormalities. As a result, our facility has adopted these practices for prolonged procedures. When planning studies that require prolonged anesthesia, strategies that prevent dermal necrosis, myopathies, and neuropathies should be weighed, in addition to monitoring standard vital parameters. Thus, alternating pressure pads should be considered in all large animal species undergoing prolonged procedures.

P8 Idiopathic Myofasciitis in a Domestic Ferret

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A 9-month-old, castrated male ferret presented for an acute onset of lethargy. On physical examination the animal was laterally recumbent with minimal response to manipulation and handling and was dehydrated. Due to rapid clinical decline and poor prognosis, euthanasia was elected, and the animal was submitted for a postmortem examination. The animal was found to be in fair to

adequate body condition score, but had generalized muscular atrophy with multifocal regions of pale tan discoloration within the skeletal muscle of the limbs and cervical region. Diffusely, the esophagus was mottled red to tan and thickened up to 3 mm, and the spleen was massively enlarged, soft and light red. Histologic evaluation of the esophagus revealed marked circumferential and mural expansion by numerous neutrophils and macrophages with fewer admixed lymphocytes and plasma cells. The inflammatory infiltrate was primarily centered on the muscularis externa and serosa with extension into the periesophageal connective tissue and adjacent adipose, and sparing of the mucosal epithelium and lamina propria. A similar inflammatory infiltrate was within the peripheral skeletal muscle and also within the heart surrounding vessels and within the endocardium. The splenic architecture was disrupted by florid myeloid hyperplasia and numerous megakaryocytes. These findings are consistent with idiopathic myofasciitis of ferrets, a disease of unknown etiology and pathogenesis that was first recognized in 2003. The disease typically affects young ferrets of both sexes and presents as muscle atrophy, weakness, lethargy, pyrexia, and leukocytosis of neutrophilic predominance. Extensive evaluation for an infectious etiology has proven unsuccessful. A vaccine-related immune mediated mechanism or genetic susceptibility has also been suggested, but neither have been substantiated so the cause remains unknown. Ultimately animals affected by this disease have been unresponsive to medical interventions, and the disease has been uniformly fatal.

P9 A Mixed Mammary Tumor in a Breeding Galago (*Otolemur garnettii*)

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An 11-year-old, multiparous, pregnant galago (*Otolemur garnettii*) in an established breeding colony presented with an alopecic, semimovable, firm, multilobulated subcutaneous mass of the right middle mammary gland. Fine needle aspirate was consistent with a neoplasia of epithelial origin, consisting of sheets of densely packed epithelial cells with anisokaryosis, anisocytosis, and an increased nuclear:cytoplasmic ratio. Individual cells show abundant cytoplasm, stippled chromatin, and multiple nucleoli. Since the animal was pregnant and there were no signs of ulceration, pain, or distress, the animal was allowed to deliver and nurse the infant while being closely monitored. Following weaning of the infant 20 weeks after initial presentation, the mother was euthanized and presented for necropsy. On gross exam, the mammary mass measured 3.5 x 2.5 x 1.5 cm, a 13-fold increase in size since initial presentation. Microscopically, the mass was well circumscribed with characteristics of malignancy in both stromal and glandular components. The stroma was composed of densely cellular streams and whorls of spindloid cells, with anisokaryosis, anisocytosis, and a high mitotic rate (2-3 per 40X field). Glandular structures within the mass were lined by multilayered neoplastic cells displaying multiple nucleoli, anisokaryosis, and a moderate mitotic rate. Adipose tissue infiltration was noted throughout the mass. There was no evidence of metastasis in draining lymph nodes. In humans, biphasic malignant proliferation of cellular stromal and glandular components in the breast is uncommon and known as a phyllodes tumor. While mixed mammary tumors have been reported previously in *Otolemur crassicaudatus*, to the authors' knowledge this is the first reported case of a mammary tumor with malignant glandular and stromal components in *Otolemur garnettii*.

P10 A Method to Produce Contamination-Free Bronchioalveolar Lavage in Nonhuman Primates

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Bronchioalveolar lavage (BAL) is an important diagnostic procedure widely used to characterize a number of pulmonary disorders, particularly those due to an opportunistic infection in an immunocompromised host. In the research setting, BAL is used to access the pulmonary mucosal surface for detection of specific cells generated by the vaccines introduced to the nonhuman primate (NHP). A BAL collection procedure consists of two parts: intubation of the lung and washing of the pulmonary mucosal surface with sterile saline. Typically a bronchoscope is used for intubation which allows for the technician to visually navigate past the larynx, the trachea, the bronchi, and into the lung. Once the bronchoscope intubation has been completed, the lavage of pulmonary mucosal surface is done through the bronchoscope. When using the bronchoscope, the principle investigators too often would receive blood contaminated samples. The entire BAL procedure was reviewed after reports of the blood contaminated samples came in from the investigators. It was determined that the use of the bronchoscope, in addition to performing the procedure on the nonhuman primate in sternal recumbency, could have likely been the cause of the blood contaminated samples due to accidental injury to the larynx, the trachea, and/or the bronchi during intubation. The review of the BAL procedure led to several changes to the procedure. The modified procedure consists of the following: 1) use of Laryngoscope and a size 10 French feeding tube to intubate the animal, 2) placing the nonhuman primate in Fowler's position (45-60º semiupright sitting position) instead of sternal recumbency to assist with intubation and reduce the chances accidental injury to the larynx, the trachea, and or the bronchi, 3) no pooling of the sample aliquots, and 4) no longer adding R10 media to the collected samples in order to visually confirm that the samples are not contaminated with blood. Here, we present and illustrate the recent changes in methods to the BAL collection procedure which has effectively reduced the instances of blood contamination in the bronchioalveolar lavage samples.

P11 Treatment Matrix for Murine Dystocia of Primiparous Versus Multiparous in a Breeding Facility

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Efficacy of treatment options for mice in dystocia has been limited historically. The default prescription was oxytocin. By completing a study on the efficacy of treatments for murine dystocia study, we found that there are physical and historical situations that affect the dam's onset and response. After treating and tracking this condition for 2 years, we can now recommend 1 of 2 treatments, depending on breeding history. Our study included 192 females of all backgrounds. Five treatment groups received 1 of 5 options: dextrose calcium cocktail (0.15ml), oxytocin(0.2ml), oxytocin cocktail(0.35ml), PGF2a(0.1ml), and PGF2a cocktail(0.25ml). The best treatment of the 5 groups was found to be PGF2a cocktail. But when the mice were grouped by breeding history, the results varied. Out of 192 females, 96 were primiparous or first time dams. A total of 40 females survived, with a survival rate of 41.6%. Additionally, 96 were multiparous, or dams that had previous litters. A total of 47 survived, with a survival rate of 48.9%. The primiparous females responded significantly ($P = 0.042$) to the PGF2α cocktail (8/14) when compared to the oxytocin cocktail (4/18). On the contrary, oxytocin cocktail was found to be a significant treatment ($P = 0.0059$) in multiparous females (11/16) when compared to primiparous females (4/18). PGF2a cocktail had no significant response between both primiparous and multiparous females, with survival rates of 57% (8/14) and 60% (12/20). But it was a viable treatment option when compared to the control group, where the survival rate was 20% (3/15). PGF2a cocktail was the most beneficial in the primiparous females. Oxytocin cocktail yielded the highest resolution in multiparous females. Regardless of the onset of dystocia, specific treatments are indicated if you have historical breeding information of your colony.

P12 Spontaneous Polycystic Liver in Cd-1 Mice

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Polycystic disease is a rare inherited disorder in humans and is characterized by the development of multiple cysts in the liver and kidney. While several genetically modified rodent models are available to study polycystic diseases, we report here the spontaneous occurrence of polycystic liver in CD-1 mice. Several of our 13 to 14-month-old female sentinel mice (CD-1) were found with distended abdomens, but otherwise appeared healthy. When the mice were euthanized and necropsied, the liver was markedly enlarged with multiple fluid-filled cysts of various diameters throughout the liver. These cysts occupied 50% to 80% of the liver mass. The gross examination of the other major organs was unremarkable. Histopathology of the liver showed multiloculated cysts with complete displacement of the hepatic parenchyma. The cystic structures were lined by cuboidal epithelium with distinct cell border and were consistent with bile duct origin. There were rare foci of minimal epithelial dysplasia with piling of epithelial cells and loss of cellular orientation, but there was no evidence of neoplastic transformation and no evidence of either cholestasis or cholangitis. The number of hepatocytes was markedly decreased with intact portal structure. There was multifocal and mild lymphoplasmacytic, eosinophilic, neutrophilic periportal inflammation that rarely extends into the cystic spaces. The histopathological findings were consistent with primary biliary hyperplasia and polycystic liver with secondary inflammation. While single to few biliary cysts are sporadically encountered in aged mice, the spontaneous occurrence of multiple biliary cysts throughout the liver in our mice are characteristics of polycystic liver and is comparable to the polycystic liver lesions in humans. Hence, further characterization and generating a new line of these mice with spontaneous development of polycystic liver would be a useful model to study pathogenesis and molecular mechanisms of polycystic liver.

P13 Multiple Venous and Arterial Access Ports in a Model of Extracorporeal Circulation in Conscious Yorkshire Swine

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A clinically relevant model of extracorporeal circulation (ECC) with multiple venous and arterial access ports in conscious large animals enables monitoring and sampling of clinical physiologic parameters during normal activity, eliminating the cardiodepressive effects of anesthesia. Yorkshire swine (20-45kg) were chosen for anatomic and physiologic similarities to humans, allowing extracorporeal flow rates of 150–300ml/min. The external jugular veins, the inferior vena cava (IVC), and the right carotid artery were cannulated to provide sufficient ports for central line monitoring, blood draws, and administration of medication during ECC. Surgery consisted of bilateral jugular cutdowns to isolate the right and left external jugular vessels and the right carotid artery. The jugular vessels were cannulated with standard venous ECMO cannulae, secured and connected to extension tubing ensuring enough length to safely reach the extracorporeal circuit while limiting the blood volume outside of the body to a minimum (max 10% total blood volume). The right carotid artery was cannulated with a sheath catheter and extension line. All tubing was subcutaneously tunneled using a sharp tip chest tube trocar and exteriorized between the shoulders. A midline laparotomy was performed to access and cannulate the IVC with a sheath catheter. All incisions were closed once catheter was secured and the extension exteriorized. The pigs were recovered from anesthesia and individually housed before ECC. The pig was heparinized to an ACT >450 seconds prior to ECC to eliminate clotting of the circuit. ECC was executed by connecting circuit tubing to both jugular lines via a swivel. Flow was initiated by pumping blood from the left jugular into the circuit then returning via the right jugular at a rate of 200ml/min. This surgical approach allowed successful conscious ECC of 4 pigs without complication for up to 9 hours and allows custom monitoring, sampling and intervention for dialysis, ECMO and other extracorporeal therapies.

P14 Psychometric Assessment of a Composite Measures Sedation Scale for Dog

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Sedation of dogs is a common procedure in laboratory and clinical environments. Available scales for the assessment of sedation level have not been tested for validity or reliability. Testing is essential to ensure acceptable agreement between raters, appropriate scale sensitivity when evaluating new sedatives and sedation protocols, and in comparing results between studies. The purpose of this study was to evaluate a sedation scale (range 0-21) for inter-rater reliability, construct validity (discrimination between sedation levels) and internal consistency (relationship between scale items). Our null hypothesis was that the chosen sedation scale would be unreliable when used by different raters and show poor discrimination between sedation protocols. Sixty-two dogs scheduled to receive sedation at 2 veterinary clinics were filmed, both before and 15 minutes after receiving sedation drugs (study ID: 13-103). The sedation scale was composed of 7 items: posture and general appearance, palpebral reflex, jaw relaxation, eye position, noise response, and resistance to placing in lateral recumbency. Five experienced animal health technicians, untrained in use of the scale, independently scored 15 of the videos. Reliability between raters was very good (intra-class correlation coefficient$_{single}$ = 0.95). Construct validity was shown with a significant difference ($p = 0.002$) between the 2 most commonly used protocols: dexmedetomidine-hydromorphone (10.9 ± 5.9, mean ± SD, n = 20) and acepromazine-hydromorphone (5.7 ± 4.2, n = 36). Scale items showed excellent internal consistency (Cronbach's alpha, 0.89). The null hypothesis was rejected as these data show that the sedation scale can be used reliably by untrained raters and is therefore a useful tool to discriminate between different levels of sedation in dogs. This facilitates comparison between studies employing the same sedation scale.

P15 Abortion and Abdominal Distension in a Guinea Pig

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A 1.07 kg pigmented pregnant (estimated day 20-25 gestation by the vendor) guinea pig of unknown age had a normal physical exam upon arrival. Three days later, she had a decrease in appetite and fecal output with a 0.14 kg (13.1%) weight loss. Physical exam at that time was otherwise normal and 8-10 ml of a commercially available recovery food was provided daily as a nutritional supplement. Sixteen days later, she had regained the 0.14 kg lost since arrival, but began to have mild loose stool. A fluid-filled structure was palpated in the abdomen, but no fetuses were palpable, suggesting fetal demise due to presumptive pregnancy toxemia. Her weight had stabilized and nutritional supplementation was stopped. Over the next 5 months, mild recurrent loose stool with a few episodes of mild hematochezia were observed and treated with daily hay supplementation. During this time, she also exhibited progressive abdominal distension. Abdominal palpation revealed a fluid consistency with no discrete masses. To determine the cause of abdominal distention, an exploratory surgery was performed, revealing numerous clear and red fluid-filled cysts (0.5 to 5 cm in diameter) arising from the

omentum and approximately 20 cm of the serosal surface of the distal colon. At one point the omentum was adhered to the colon and appeared to infiltrate the lumen. One uterine horn was thin with a 0.1 x 0.5 cm necrotic focus. Based on the extent of tissue involvement and poor prognosis, the guinea pig was euthanized. Histopathology indicated a multicystic peritoneal mesothelioma, but immunohistochemistry was required for a definitive diagnosis. Immunohistochemistry ruled out hemangiosarcoma and lymphangioma, but could not distinguish between mesothelioma and adenocarcinoma due to technical issues with staining. However, based on current findings, mesothelioma is considered the far more likely diagnosis than adenocarcinoma.

P16 An Assessment of the Safety of Recuvyra following Topical Administration in Mice

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Mice are commonly used in surgical procedures requiring the use of analgesic medications. Most analgesics require repeat administration, leading to suspected animal stress secondary to handling and additional staff time requirements. Recuvyra, a topically-applied fentanyl solution, is currently approved for use in dogs. It is effective for providing up to 4 days of analgesia with one topical dose. In this study, multiple doses of Recuvyra (5mg/kg,12.5mg/kg, or 20mg/kg) were topically administered to 5 mice per treatment group to evaluate the safety and efficacy. We hypothesized that Recuvyra would be safe for use in mice, and provided multiple days of analgesia with one application to the dorsal tail base. Mice were assessed for weight loss, behavior, and nociception for 4 days following application. Behavioral tests (nest complexity, time-to-integrate-to-nest test, and open field testing) were performed to assess individual mouse behavior. Nociception following application was assessed with the tail flick test. All mice survived dosing with Recuvyra; however 1 mouse that was dosed at 20mg/kg was euthanized due to lethargy and dehydration. All dosed mice initially lost between 1g and 1.5g of body weight, but regained it prior to the study end. Nest complexity scores decreased with dosing for all groups, returning to normal by day 4. Time-to-integrate-to-nest testing showed a decrease in nesting behavior initially. Tail-flick latencies were increased significantly for all groups at 24 hours, and gradually decreased to normal latency time by day 4 for the 12.5mg/kg and 20mg/kg groups. During open field testing, dosed mice exhibited less exploratory behavior, with lower center duration times and less rearing than control mice initially. Defecation during open field testing was decreased on day 1 for all groups, returning to normal day 2. Dosing at 12.5mg/kg was determined to be a safe analgesic for use in mice that remains effective for approximately 3 days.

P17 Thermal Imaging as an Alternative to PIT Tagging for Monitoring Body Temperature and Clinical Disease Progression in Mice

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Body temperature is thought to be an important parameter to track when monitoring clinical disease progression in conjunction with clinical signs, such as sick rodent posture and other animal behaviors. Passive Integrated Transponder (PIT) tags have been used to monitor mouse body temperature with relatively little stress on the animal. We hypothesized that a thermal imaging camera may be used to obtain body temperatures from mice in manner that is less obtrusive than PIT tags with comparable results. PIT tags were implanted subcutaneously into 2 groups of mice. One group was then infected with *Burkholderia* spp, and one group remained uninfected. Mice were assessed for clinical behavior and body temperature the day prior to infection and daily for 7 days postinfection. Clinical behavior was scored on a scale of 0-4 (0- normal; 1- questionable illness; 2- mild but definitive illness; 3- moderate illness; 4- severe illness). Body temperatures were taken with both the PIT tag, as well as thermal images of the eye, base of the ear, and flank. Although thermal imaging temperatures were lower than PIT tag temperature, they trended similarly with eye temperature being the closest to PIT temperatures followed by ear and flank. Increased clinical behavior scores correlated well with lower body temperatures from the PIT tag and thermal imaging. This indicates that a thorough clinical observation scoring system may be a more efficient and effective to track disease progression in mice than monitoring body temperature.

P18 Pharmacokinetic Profiles of a Sustained-Release Formulation of Buprenorphine in Mice

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Buprenorphine is one of the most widely used analgesics in research mice. A new reformulated biopolymer-based sustained-release formulation of buprenorphine (Bup-SR), was recently developed with the goal of deriving a single dose, 3-day sustained analgesic effect, which would not only offer stable pain management but also decrease handling-associated stress and result in more cost-efficient pain management. Here, the new formulation administered at 0.5 mg/ml was studied at 3 different subcutaneous doses (1, 1.5, and 2 mg/kg) in 7-week-old, male Swiss-Webster mice to determine the last time point (endpoint) when all mice retained blood plasma levels above 1 ng/ml. This plasma concentration is assumed as the minimum concentration that may result in adequate analgesia based on previous reports. Intracardiac blood samples were collected at 0, 0.5, 2, 4, 8, 12, 24, 48, 72, and 96 hours after dosing in triplicate, and blood plasma were evaluated using liquid chromatography-mass spectrometry. Results suggested that at 1.0 mg/kg the desired endpoint was ≥ 24 hours, at 1.5 mg/kg it was ≥ 24 hours, and at 2.0 mg/kg it was ≥ 72 hours. These results indicate that a 3-day analgesic effect after subcutaneous administration of this reformulated Bup-SR is practical. These results are promising, and suggest this Bup-SR formulation when dosed properly at 2.0 mg/kg concentration is easy to use, saves labor, and provides reliable levels for at least 3 days.

P19 Marked Trematodiasis in a Population of Wild-Caught Trinidadian Guppies

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Wild-caught Trinidadian guppies (*Poecilia reticulata*) in a research laboratory were found to have an unusually high rate of morbidity and mortality for a month following capture. Reported clinical signs included emaciation, erratic swimming, external sores, and death in greater than 50% of the guppies. Ten affected guppies from 3 river drainages (Caroni, Marianne, and Oropuche) were sacrificed for histologic assessment. Fish from all drainages sampled were affected by multifocal trematodiasis which was often associated with chronic-active inflammatory changes. Trematodes were identified in the coelom, pericardium, liver, and peri-renal tissues of many guppies. In 1 fish, multiple embedded trematodes were associated with development of pancreatic sarcoma. One fish from the Oropuche drainage had marked granulomatous meningitis of unknown etiology. Numerous fish also had microsporidia xenomas in skeletal muscle. Cumulatively, the findings suggest that infection with multiple infectious agents, especially visceral trematodes, was the most likely cause of morbidity and mortality in most fish. Fish from the Marianne and Oropuche drainages were treated once with 10 mg/L praziquantel in tank water for 5 hours, and fish from the Caroni and Oropuche drainages received salt treatment with a commercially available sea salt (3 tbsp/5 L tank water) for 3 weeks and 1 week, respectively. Clinical signs and mortalities were

reportedly reduced in all populations following treatment. The location of these trematodes in viscera suggests that they are of the platyhelminth group Digenea. Importantly, no digenean parasites have been previously described in a wild population of Trinidadian guppies. Parasite distribution in the fish populations suggest that there could be variations in parasite-host ecology across the sampled drainages. In addition, investigators should be aware that wild-caught specimens used for experimental purposes may have multiple comorbid conditions that could compromise research outcomes.

P20 Disinfection Quality Control in an ABSL-3 Environment Housing *Burkholderia pseudomallei*-infected Mice

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Environmental sanitation is one of the most important concepts in laboratory animal facility management because it provides protection against the transmission of disease-causing microorganisms to both animals and humans, in addition to ensuring the integrity of the research being performed. Disinfection of equipment and surfaces is even more crucial in specialized biocontainment laboratories in which biologic agents regulated by the CDC and/or USDA are used, such as the ABSL-3 facility at our university's Infectious Disease Research Complex. Although strict measures are taken to mitigate environmental contamination by select agents in the ABSL-3 facility, it is important to perform environmental testing in these areas periodically to ensure that the procedure and disinfection controls are working correctly. The objective of this study was to collect and test samples in the areas housing *Burkholderia pseudomallei*-infected mice to determine if laboratory surfaces were being kept free of contamination. To accomplish this, a systematic grid, mixed with judgmental sampling, was used to include floor and vertical surface swabbing in the animal room, anteroom, main hallway, laboratory spaces, and exit vestibules. The ~160 samples were processed using an inhouse system compliant with handling suspect select agent pathogens, and processing was optimized to permit rapid genomic extraction providing low, but sufficient, yields of genomic DNA. After genomic DNA extraction, PCR was performed to amplify a fragment of the ORF-13 gene segment. ORF-13 is unique to *Burkholderia* species and is located in the type III secretion system gene cluster. For this reason, it can be used as an identifying factor for the detection of *Burkholderia*-specific DNA. The results from this study show that the local ABSL-3 surface environment is negative for *Burkholderia* contamination. This internal quality control project confirms that the procedures and engineering standards currently in place, when properly used, control bacterial trafficking within the ABSL-3 environment.

P21 Assessment of Pain Associated with The Injection Of Sodium Pentobarbital For Euthanasia In Mice

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The *AVMA Guidelines for the Euthanasia of Animals: 2013 Edition* includes injection of sodium pentobarbital as one of the few methods of euthanasia that is acceptable without conditions in laboratory rodents, but states that pain may be associated with intraperitoneal (IP) administration. Our goal in 2 separate studies was: 1) to assess mice for signs of visceral pain during IP euthanasia using sodium pentobarbital; and 2) to objectively measure the onset of acute somatic pain in mice using the paw lick test. We administered a euthanizing dose of sodium pentobarbital (250mg/kg) to male and female C57BL/6J and CD-1® mice (n = 77; 9-10 mice/group) by IP injection using one of two concentrations (pentobarbital at 50mg/mL and a 5mg/mL dilution) and observed the mice for a range of behaviors including writhing and loss of righting reflex (LORR). We did not observe writhing in any mouse euthanized by IP injection of sodium pentobarbital. The observed time to LORR was more rapid in mice euthanized with the 50mg/mL compared to the 5mg/mL solution (75.3+/-3.3s vs 98.0+/-5.1s, respectively, $P < 0.001$), and there were differences between sex and strain. In the second set of experiments, we injected 15uL of the same solutions subcutaneously in the plantar hind paw of male and female C57BL/6J and CD-1® mice (n = 44; 5-7 mice/group) and recorded each time the mouse licked the site within a 15-minute period to assess acute pain. The time to the first paw lick tended to be earlier in mice that received the 50mg/mL compared to the 5mg/mL solution (255+/-117s vs 293+/-178s, respectively, $P = 0.50$), but this was not statistically significant due to a high degree of interindividual variation. From these observations, we conclude that sodium pentobarbital induces a rapid onset of sedation based on LORR, and mice show no sign of pain following IP injection for euthanasia. Furthermore, the time to loss of righting following IP injection occurs well in advance of the time to the initial perception of pain as assessed by the paw lick test. Therefore, IP administration of sodium pentobarbital provides for humane euthanasia in mice, and acute pain due to chemical irritancy occurs after the time to loss of righting.

P22 Use of Enrofloxacin to Eliminate *Pasteurella pneumotropica* Infection in *SFTPA1* and *SFTPA2* Transgenic Mice Used in Respiratory Studies

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Surfactant protein A (SP-A) plays a vital role in lung innate immunity. SP-A binds to alveolar macrophages, enhancing their ability to affix to debris and pathogens. Humans and animals deficient in SP-A are more likely to have recurrent and more severe respiratory infections. Mice differ from humans in that they have only 1 surfactant protein, while humans have 2. Recently, humanized *SFTPA1* and *SFTPA2* transgenic mice used in respiratory studies, as well as knock-outs deficient in SP-A, rederived via cesarean section at our institution cultured positive for *Pasteurella pneumotropica* upon post-rederivation testing using contact sentinels. *P. pneumotropica*, a gram-negative opportunistic pathogen, is known to exacerbate multifactorial pneumonias and is the leading cause of failure of C-section rederivation, as pups can be infected *in utero*. Elimination of this pathogen was imperative to the outcome of the study since SP-A deficient mice would be at greater risk for respiratory infection. Previous reports show that enrofloxacin in drinking water at a daily dose of 25.5 mg/kg for 2 weeks resulted in consistent negative results, but there is no information illustrating complete elimination of the pathogen or carrier state in mice deficient in SP-A. In our study, oropharyngeal cultures of 228 colony mice revealed 118 positive animals, and testing revealed sensitivity to enrofloxacin. All animals were treated with 0.16 mg/mL enrofloxacin in autoclaved water for 2 weeks. Eight consecutive monthly follow-up oropharyngeal cultures of treated mice and their offspring showed 100% efficacy in eliminating *P. pneumotropica*. This is the first report of successful elimination of *P. pneumotropica*, including prevention of vertical transmission, in humanized *SFTPA1* and *SFTPA2* transgenic mice, including knock-out animals deficient in surfactant protein A.

P23 Malaria in a Simian/Human Immunodeficiency Virus-Infected Pigtail Macaque

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A 4.7-yr-old, 7.4-kg intact, male pig tail macaque (*Macaca nemestrina*) on tether presented for severe anemia (Hct decreasing to 11.1% from 38% 3-days prior). This animal was infected with chimeric simian/human immunodeficiency virus (SHIV1157ipd) 424 days prior to presentation and received total body irradiation followed by an autologous bone marrow transplant and daily treatment with 700 µg granulocyte colony stimulating factor (GCSF) for 26 days prior to presentation. He received 10 irradiated whole blood transfusions beginning 3 days after the bone marrow transplant, 4 of which occurred in the week prior to presentation. At presentation the monkey was in thrifty body condition (BCS 2/5), markedly obtunded, and was pale and jaundiced with multiple small petechiae present on the mucous membranes. A CBC revealed marked anemia, leukopenia, and thrombocytopenia. The monkey was diagnosed with *Plasmodium* species infection via examination of thick and thin blood films. Antimalarial therapy (6.5 mg primaquine phosphate PO q24 hrs) was begun the following day. Two transfusions comprising a total of 163 mL irradiated whole blood were performed, which improved the monkey's mentation, skin, and mucous membrane color. Four days after diagnosis the monkey was found dead in its cage. Necropsy revealed severe hemolytic anemia with marked pallor, petechial hemorrhages, and scant adipose tissue stores. Significant histologic findings included erythrocytic protozoal organisms and hemozoin and hemosiderin pigments both intravascular and in multiple organs, as well as erythroid hypoplasia with myeloid hyperplasia in the bone marrow. The *Plasmodium* infection was later determined to have originated from transfusions given to the monkey by two separate Indonesian-origin blood donors that tested positive for malaria by PCR. This case highlights the utility of routine screening of all blood donors for erythrocyte parasites such as *Plasmodium* species and *Trypanosoma cruzi* even when blood products are irradiated.

P24 Modifying Rabbit Anesthesia for Increased Surgical Duration

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Isoflurane is commonly used for general anesthesia in laboratory animals and is associated with dose-dependent cardiorespiratory depression. During extended studies with intubated New Zealand White rabbits and spontaneous ventilation, higher levels of isoflurane (> 2%) were required to maintain anesthesia. Cardiac arrhythmias, inconsistent end-tidal CO_2 levels, and morbidity were noted, especially when isoflurane via face mask was used for induction and intubation. We did not notice significant differences due to weight (1.6–3.2 kg), age (2–6 months), or sex. The use of a flared Cole intubation tube to improve the tracheal seal and a positive pressure ventilator with peak inspiratory pressure (18–25 cm H2O) increased the efficacy of isoflurane delivery, oxygenation, and ventilation as indicated by lower inspired isoflurane concentrations, oxygenation saturation >96%, and end-tidal CO2 levels within or slightly above normal limits. To improve intubation efficiency and provide multimodal balanced anesthesia with lower isoflurane levels (average 2%), we premedicated with dexmedetomidine and ketamine and maintained as separate intravenous infusions at rates of 0.2–1 mcg/kg/h and 6–20 mg/kg/h respectively. Vitals signs including heart rate, respiratory rate, blood pressure, end-tidal CO_2, and temperature were continuously recorded during surgery to monitor trends. We had a decrease in procedures with surgical complications (6/7 to 1/6), an increase in control over surgical endpoint (3/7 to 5/6), and success in survival procedures (0/1 to 2/2) in moving from isoflurane alone to this combination anesthesia regimen. We plan to continue investigating modifications in the injectable infusion rates and isoflurane levels, which we expect will further decrease the need for inhalant anesthesia, provide additional cardiorespiratory stability, and eliminate surgical morbidity for future animals.

P25 Peracute Otitis Media and Interna in a Farm Pig

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A 4-month-old, 36.5 kg neutered male Yorkshire/Landrace/Spot-cross pig was examined for sudden inability to rise. The pig arrived 5 days previously in a group of 5. At the farm, the pig was vaccinated against parvovirus and *Mycoplasma hyopneumoniae*, and the pig's dam had been dewormed and vaccinated against Porcine circovirus and *Clostridium perfringens*. Physical exam on arrival was normal. The pig appeared to be healthy, until found at the morning health check recumbent on his left side with his neck extended, and vertical nystagmus. He could not rise or stand. TPR was within normal limits. He would eat if hand fed, but could not raise his head. He could move his limbs and had a strong toe pinch withdrawal reflex. He had no external signs of trauma. When lifted to sternal recumbency, his head moved side to side, and had a pronounced left tilt. He was separated and given 5mg/kg of ceftiofur antibiotic IM, and supportive care. The results from a CBC and chemistry screen were unremarkable. The next morning, the pig's condition had not improved. Due to poor prognosis, we elected euthanasia. On gross necropsy, the left lung had focal atelectasis and the liver had mild regional scarring. The brain appeared grossly normal. The left medial and inner ear had copious exudate. The cultured exudate grew *Arcanobacterium pyogenes* and *Bacteriodes*, both sensitive to ceftiofur. The final diagnosis was peracute otitis media and interna. Otitis is common in swine. In one report, 69% of pigs slaughtered due to clinical signs of illness had otitis, although few showed clinical signs characteristic of otitis. The peracute onset of severe clinical signs of otitis interna is an unusual presentation of this disease.

P26 Extra-Prostatic Transgene-Associated Neoplasms in a Female TRAMP Mouse

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A 6-month-old female of the transgenic adenocarcinoma of the mouse prostate strain (C57BL/6-Tg(rPb-SV40Tag)8247Ng/J) was reported for a large mass impeding its ability to ambulate. Physical examination revealed a large firm mass extending from the ventral neck, with respiratory distress upon restraint. The mouse was euthanized and submitted for complete necropsy. Gross examination revealed a large, firm, lobulated, light tan mass effacing the sublingual, submandibular, and parotid salivary glands and lymph nodes. There were numerous white, soft to firm, nodules visible from the surface of the lungs. Histologic examination of salivary gland revealed an invasive, densely cellular, expansile, and infiltrative mass consisting of solid nests and acini of epithelial cells with large basophilic nuclei. The lung parenchyma was effaced by multiple similar masses (pulmonary metastasis). Separately, there was a large, focally extensive, invasive, and expansile mass composed of sheets and cords of anaplastic cells present in the midbrain. Based on these findings, final diagnoses of 1) salivary gland adenocarcinoma with pulmonary metastasis and 2) malignant anaplastic tumor of the midbrain were made. Several extra-prostatic transgene-associated neoplastic lesions have been described in TRAMP mice, including both salivary adenocarcinomas and anaplastic midbrain tumors. The rat probasin promoter is an androgen-dependent promoter initially thought to restrict transgene expression to the prostate; however, androgen receptor expression has been described in other tissues. This is the first report of 2 different extra-prostatic transgene-associated neoplasms occurring within a single TRAMP mouse.

P27 Hookworm Infection and Treatment in Newly Imported Rhesus Macaque (*Macaca mulatta*)

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Hookworm (*Ancylostoma ceylanicum*) infections can cause impaired host cellular immune responses and possible cross-transmission between humans and animals. The purpose of this report is to introduce the procedure and treatment against the infection of hookworm that occurred in our animal facility. Twelve rhesus macaques (9~10-year-old male) were imported from China. During the quarantine and acclimatization periods, 8 out of 12 animals showed intermittent diarrhea and adult larvae in feces. Adult parasites and fecal samples were analyzed by expert parasitologist. A direct smear was first performed followed by a zinc sulfate flotation. As a result, these were diagnosed with hookworm (*Ancylostoma ceylanicum*) which can cause diarrhea, anemia, weight loss, and hypoalbuminemia. For treatment, Ivermectin was subcutaneously injected at a dose of 0.3mg/kg BW, and we found that there were no more findings of adult parasites and eggs of hookworm. To confirm treatment effectiveness, microscopic examination was performed for fecal sample, which was collected 11 days after treatment. The infection of hookworm can cause to affect the experiment results, especially for our rhesus macaques under immunosuppressive condition for xenotransplantation. For these reason, our report suggests that improved hygienic practices, regular examination, and appropriate immediate treatment against parasitic infection are required when rhesus macaques are newly acquired.

P28 Ultrasound Guided Blood Vessel Access in Large Laboratory Animals

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Blood vessels accessed for venipuncture and intravenous catheter placement in large laboratory animals are often not visible, and deep vessels are difficult to palpate. Repeated attempts to puncture a vessel may cause trauma and hematoma formation. In addition, selective catheterization of arteries and veins with accuracy is also difficult. To increase the success rate for venipuncture and catheterization of blood vessels, the use of ultrasound was employed. Studies have proven that ultrasound guidance improves the success rate and safety of invasive procedures. In addition, color Doppler may be used to visualize flow and distinguish arteries from veins. Laboratory animals such as rhesus monkeys, sheep, and swine are routinely anesthetized prior to venipuncture and arterial catheterization. Following aseptic preparation of the skin, ultrasound guided venipuncture and catheterization was performed, mainly on femoral arteries and veins. Although a second person was sometimes necessary to hold the ultrasound probe while the initial person performed the blood collection or catheterization, fewer attempts were required to accurately access blood vessels. Overall, visualization of the blood vessels using ultrasound guidance resulted in successful venipuncture and catheterization.

P29 Exudative Epidermitis in a Group of Duroc Pigs (*Sus scrofa domestica*)

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Six, approximately 3-month-old, female Duroc pigs presented with multifocal exudative lesions on the cervical and scapular regions. No other significant findings were present on physical examination. Dermatophyte Test Medium (DTM) and dermal skin scrapes of the affected regions were negative. While awaiting final bacterial culture results of the lesions, daily treatment was initiated with an oral broad spectrum antibiotic and topical cleaning with 2% chlorhexidine gluconate medical scrub. The culture results were positive for *Staphylococcus hyicus*. Clinical diagnosis of exudative epidermitis was supported by gram stain and histopathology at the conclusion of the study. The lesions improved and decreased in size during treatment but did not completely resolve prior to reaching the experimental end point. *Staphylococcus hyicus* is a gram-positive coccus that is commensal flora of various animals and can cause skin disease when subjected to stressors such as trauma or environmental irritation. This organism also causes exudative epidermitis, otherwise known as "greasy pig disease," in piglets. Early clinical signs of exudative epidermitis include listlessness and anorexia. The skin generally appears reddened but not pruritic and initial lesions are generally found in the groin region. Focal erosions develop in the stratum granulosum and extend into the hair follicles causing a suppurative folliculitis. Excessive sebaceous gland secretion leads to an accumulation of greasy exudate over the lesions. Dehydration along with serum protein and electrolyte losses contributes to death in piglets less than 8 weeks of age. Lesions are uncommon in adult swine but may be observed on the dorsum and flank regions. To our knowledge, this report is the first description of exudative epidermitis in Duroc pigs.

P30 False Positive Plasma Relaxin Results in a Canine Research Colony: A Collaborative Response Between Facility, IACUC, Commercial Laboratory, and Manufacturer

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Initial necropsy confirmation of 2 pregnant dogs in our AAALAC-accredited, nonbreeding research facility led to screening of the entire female colony for pregnancy. Screening consisted of plasma determination of the placentally derived hormone relaxin. We selected plasma relaxin rather than abdominal palpation, radiographs, or ultrasound to determine pregnancy because phlebotomies are easy to perform and theriogenologists consider positive test results to be reliable. Ten of 66 (15%) dogs had positive plasma relaxin test results, prompting retraining of animal caretakers, gender-separating of dogs by room, and reviewing of animal and housing records. Review of facility surveillance tapes did not identify a free-roaming male or purposeful breeding. Additionally, through different combinations of abdominal palpation, radiographs, ultrasound, and end-of-study necropsy, we did not confirm pregnancy. The 10 plasma relaxin test results were falsely positive. We worked with personnel at the commercial reference laboratory who performed relaxin testing and who subsequently worked with the manufacturer of the test kit to identify a reason for these false positive results. Samples continued to yield positive results at both commercial and manufacturer laboratories. Although the test has reported high specificity (low false positive rate), false positive results may develop because of low prevalence of pregnancy in the dog population tested, incorrect sample, mishandling of the sample, technical error with performing the test, misinterpreting the test results, and inherent problems with a specific test lot. The manufacturer confirmed a problem with the specific lot used. The positive results led to considerable energy expended in confirmation and communication activities. We have learned a measured response including follow up testing is needed, especially with unexpected or incongruent results.

P31 Validation and Refinement of a New Blood Pressure and Electrocardiogram Telemetry System in the Canine for Cardiovascular Safety Studies

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Cardiovascular safety assessment in conscious unrestrained animals is a regulatory requirement for new chemical entities prior to testing in humans. Canine jacketed external telemetry (JET) has been our primary method used to collect high-quality cardiovascular data such as blood pressure and heart rate. Because of some disadvantages of JET, which include jacketing of the animals, inconsistent electrocardiogram (ECG) quality, and the necessity for single-housed animals, we validated a new fully internal telemetry system as an alternative to JET to address these limitations. A minimally invasive intramuscular implantation surgical approach was developed. Eight animals were successfully instrumented with an internal blood pressure/ECG telemetry device without surgical complications. Following full recovery, animals were administered with 2 validation drugs known to modify cardiovascular parameters. Cardiovascular data from the new internal devise were collected for 24 hours and were similar to previously acquired data collected from JET. While the implantation surgery is more involved, the new devise is a completely internal system and does not require an external jacket. Therefore, acclimatization and jacket management are eliminated which allows animals to be socially housed. In addition, the digital signal reduces ECG dropout compared to JET technology. All data that was acquired and validated is supportive of this change in methodology.

P32 Postoperative Lung Atelectasis in a Common Marmoset (*Callithrix jacchus*)

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A 3-year-old marmoset (*Callithrix jacchus*) underwent cranial implant surgery. The marmoset was premedicated with Ketamine (15 mg/kg)-Atropine (0.02 mg/kg) IM, induced with Isoflurane, intubated with a 2 mm endotracheal tube (ET), and maintained with Isoflurane (2-3% and O_2 at 1L/min) on spontaneous respiration using a non-rebreathing circuit. The length of the tube was controlled to prevent endobronchial intubation. The animal received IV fluids (LRS 10 ml/kg/hr), antibiotics (Enrofloxacin 10 mg/kg IM) and analgesia (Carprofen 2 mg/kg SQ and Lidocaine-Bupivicaine block) in pre-op. The animal was placed in sternal recumbency in a stereotactic apparatus. The surgery was uneventful and lasted 4 hours. Except for slightly low $EtCO_2$, anesthetic parameters remained normal. The animal recovered uneventfully but developed acute respiratory distress 8 hours after surgery. Physical examination revealed severe expiratory dyspnea, tachypnea, as well as slight crackles on right lung field auscultation with respiratory acidosis on blood gas analysis. Thoracic x-rays demonstrated an atelectatic right cranial lung lobe with no other significant abnormalities. Ultrasound did not reveal pleural effusion, cranial mediastinal lesion, lung masses, or lobe torsion. Differential diagnoses included mechanical compression due to prolonged recumbency or trauma, as well as airway obstruction from the ET tube, a mucus plug, or hemorrhage. Despite the absence of x-ray lesions, we could not rule out aspiration pneumonia. We created a O_2 chamber using a rat microisolation hooked to an O_2 supply humidified through a sterile saline bottle. The animal improved immediately in the chamber. The animal also received Albuterol (90 mcg in chamber TID), antibiotics (Orbafloxacin 7.5 mg/kg PO SID), SQ fluids (LRS + 5% dextrose BID), and daily gavage with yogurt and a commercially available nutritional supplement. Follow-up blood gas analysis 2 days post-op revealed mixed respiratory and metabolic acidosis. CBC-Chem showed moderate dehydration but renal function was normal. The animal received calcium carbonate as an adjunctive treatment for the metabolic acidosis. The animal improved on treatment, was gradually weaned from the chamber over 5 days and recovered after a week. Controlled chest X-rays showed complete resolution of the atelectasis.

P33 Use of an Animal-Specific Glucose Meter for Mice and Rats and Its Potential Impact with Future Research

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Purpose bred mice and rats are critical for metabolic disease research. Blood collection from these rodents challenges research scientists with difficult collection methods and low sample volumes compared to larger research animals. Studies of glucose homeostasis typically require repetitive sampling, further complicating the blood collection process and stressing the animals. Use of an accurate, portable, animal-specific glucose meter requiring a small volume (0.3 µl) of blood using nontraditional collection methods, such as lancet tail prick, may mitigate scientist challenges and animal stress. Three groups of mice C57 (normal; n = 22 samples), BKS (n = 21 samples), and pound mice (n = 20 samples) received insulin or dextrose injections to obtain blood glucose within the desired range of 20-750 mg/dl. Three groups of rats CD (n = 50 samples), ZDF Lean (n = 50 samples), and ZDF Obese (n = 49 samples) were similarly subjected. Measurements obtained with the animal-specific glucose meter and with 2 commercially available human glucose meters were compared to plasma glucose results. Average percent bias calculated from the differences of the results obtained with the animal-specific glucose meter or the human glucose meters and at the laboratory across all mice strains were -8.4%, -22.0%, and -12.9%, respectively. Results for rats were 7.9%, -16.7%, and -14.2%, respectively. Average bias <+/-10% is considered acceptable. For both species, the animal-specific glucose meter yielded results that were <+/-10% average bias compared to the human glucose meters and therefore more closely approximates plasma glucose results obtained through a laboratory. Because the animal-specific glucose meter only requires a small volume of blood, its use may allow scientists to refine research protocols and reduce the number of animals needed for study while simultaneously improving the accuracy of results and reducing animal stress.

P34 Endometrial Carcinoma in a 16-year-old Rhesus Macaque (*Macaca mulatta*)

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A 16-year-old female rhesus macaque (*Macaca mulatta*) with a history of painful cycling is presented. She was treated with 40mg Depomedroxyprogesterate acetate (DMPA) intramuscularly monthly in addition to 0.1mg/kg meloxicam (an NSAID) for analgesia as needed. Long-term history noted gradual increase of the uterine diameter and presence of a palpable uterine mass. During an acute episode, the macaque was noted to be lying on the cage floor bottom with bloody vaginal discharge. Physical examination revealed that the monkey was hypothermic, pale, and bradycardic. A moderate amount of dark red bloody vaginal discharge was present. On abdominal palpation a large, firm, 10cm diameter mass was located in the caudal abdomen. Vaginal palpation revealed a hemorrhagic polyp protruding into the vagina. On ultrasound, a 10cm diameter heterogeneously echogenic mass located within a fluid filled structure, presumably the uterus was noted. Differential diagnosis included endometrial polyp, benign neoplasia, or malignant neoplasia. Despite overnight treatment with analgesics (buprenorphine and meloxicam), intravenous fluid support and thermal support, the animal remained recumbent and in obvious pain. A decision was made to euthanize the primate. Necropsy revealed a distended uterus with hemorrhagic necrotic material terminated by a large blood clot that extended through a dilated cervix into her

vagina. The clinical presentation of abnormal vaginal discharge and pelvic pain were consistent with the histopathological findings of intraluminal endometrial carcinoma, in addition stromal hyperplasia with decidualization was also found, expected in an aged female given DMPA.

P35 Seizures and Subdural Hemorrhage in a Rhesus Macaque (*Macaca mulatta*)

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A 17-yr-old, male, singly housed rhesus macaque was noted to have arm tremors and difficulty grasping with his right hand. This macaque was used in neuroscience research and had two left cephalic recording chambers in place, although the macaque was not actively being recorded. The tremors were first observed by the research staff during routine cephalic implant maintenance and increased in frequency over the following 3 weeks. Physical examination, complete blood count, and serum chemistry panel were unremarkable during quarter annual examination a few weeks prior to the onset of the tremors. Differentials included focal seizures, cerebellar disease, acquired myotonia, or myelin disorders. Two weeks after the first onset of tremors, a grand mal seizure lasting approximately 5 minutes was observed. The macaque was started on oral diazepam at a dose of 0.85 mg/kg twice daily. Due to the progression in frequency and severity of the seizures, the investigators elected to pursue a terminal magnetic resonance imaging study. Although ketamine is commonly used for sedation of nonhuman primates, its use in neurologic cases is controversial due to concerns for increasing intracranial pressure and lowering seizure threshold. In this case, the macaque was first sedated with midazolam (0.45 mg/kg, IM) followed by a low dose of ketamine (3 mg/kg, IM) 10 minutes later. This sedation protocol permitted handling and intravenous catheter placement. General anesthesia was induced with propofol (3 mg/kg IV), followed by endotracheal intubation and maintenance on inhalant isoflurane (2%). Cerebrospinal fluid was collected by atlanto-occipital tap following MRI. Necropsy revealed marked, subdural hemorrhage over the majority of the left cerebral hemisphere. CSF culture was negative, and protein and specific gravity were within normal limits. Interference from the cranial implant complicated interpretation of MRI images. The etiology of the subdural hemorrhage is unknown but may be secondary to the cranial implant, or trauma secondary to unobserved seizure activity. Subdural hemorrhage is uncommonly seen, but should be included in differential diagnoses for seizures in cranially implanted macaques.

P36 Nonhuman Primate Body Temperature Method Comparison

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We compared nonhuman primate (NHP) temperature data to evaluate the differences between rectal, infrared (inguinal and chest), and telemetry temperature readings in sedated NHPs. We sought to replace the standard rectal temperature with the infrared device and not compromise our evaluation of body temperature for diagnostic or clinical examination purposes. The rectal thermometer requires physical restraint or sedation, has a slight risk of rectal trauma, and takes 30 seconds to 1 minute to record the temperature. The infrared thermometer readings are instantaneous and require no animal contact. We measured temperature data on 206 (138 male and 68 female) cynomolgus macaques under ketamine (10mg/kg) IM sedation over 3 months as part of scheduled physical examinations. Digital rectal and TempIR infrared temps were taken on all animals. The infrared device measurements were taken 5cm from the chest and inguinal areas (least hair). We used 10 ketamine (10mg/kg) IM sedated cynomolgus macaques (5 male and 5 female) instrumented with a commercially available telemetry signal simulator in a muscular pouch between the internal and external abdominal oblique muscles on the flank to compare the telemetry temperature to rectal and infrared inguinal and chest measurements. We determined the body temperature mean, median, and standard deviation for the telemeterized and nontelemeterized animals. Nontelemeterized animals showed a mean value of 102.30 F for inguinal, 101.41 F for chest, and 101.33 F for rectal. The data set for these animals showed a slight but statistically significant difference between inguinal and rectal measurements and no significant difference between chest and rectal measurements. The telemeterized group showed a mean of 100.6 F for rectal, 99.5 F for telemeterized animals, 99.5 F for inguinal, and 99.6 F for chest measurements. The trend for this group was excellent consistency between infrared methods and telemetry (core body temp) readings and a consistent deviation of approximately 1 degree F between rectal and telemetry readings.The results confirmed our hypothesis that the infrared thermometer could be used to replace our standard rectal thermometer due to all readings being within the macaques normal temperature range of 99.5-102.5 F.

P37 A Pruritic Primate: Atopic Dermatitis in an Adult Female Rhesus Macaque

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A 7-year-old, 6.0 kg female rhesus macaque (*Macaca mulatta*) presented with diffusely erythematous skin, moderate pruritis, regional alopecia, and excoriations prior to transfer to our facility. Monthly baths with ketoconazole/chlorhexidine shampoo and application of zinc oxide ointment improved the symptoms, but her records indicate that all recurred upon discontinuation of treatment. Therapy with diphenhydramine (12.5 mg IM BID) and omega-3 fatty acids (140 mg PO SID) did not result in improvement. Upon arrival at our facility, physical exam revealed diffusely erythematous skin, moderate pruritis, and regional alopecia. Complete blood count and serum chemistry values were within normal limits. No ectoparasites or other abnormalities were noted on skin scrape. Differential diagnoses included allergic dermatitis (parasite hypersensitivity versus food hypersensitivity versus environmental), chemical irritation, keratinization disorder, sebhorreic disorder, nutritional deficiency, endocrine disorder, infectious disease, and neoplastia. Histopathologic analysis of punch biopsies revealed acanthosis with perivascular mononuclear cell infiltration in the superficial dermis, with variable numbers of eosinophils and mast cells. Dermal edema was present, as well as thickening of the walls of superficial blood vessels and dermal fibrosis. Histopathologic lesions were consistent with atopic dermatitis (AD). AD is a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features associated with IgE, most commonly directed against environmental allergens. Treatment was initiated with 30 mg/day (5 mg/kg) of cyclosporine PO (Atopica oral solution). There are two previously published case reports describing AD in rhesus macaques; one in a juvenile female and one in an adult male. Oral cyclosporine at 5mg/kg/day, and daily application of 0.1% topical tacrolimus have been used effectively to treat macaques with AD. Oral cyclosporine solution at 6.6 mg/kg SID (40 mg/day) has resulted in clinical improvement of the affected animal in our colony. Atopic dermatitis should be a differential diagnosis in macaques with erythematous, pruritic skin lesions.

P38 Pneumothorax from Upper GI Endoscopy in a Yorkshire Swine

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A 2.5-month-old, 25kg female Yorkshire swine was anesthetized with Tiletamine–zolazepam (5mg/kg IM) and xylazine (2mg/kg IM) for

upper GI endoscopy and device delivery as part of an experimental manipulation. The device was constructed out of elastic enteric polymer gels fitted into a ring shaped polydimethylsiloxane mold folded to fit into a 000 gelatin capsule for endoscopic placement. The device was placed via standard gastroscopy with a 12.8mm endoscope and overtube. Esophageal mucosal inflammation was noted during endoscopy, as was bloody, mucoid fluid in the oral cavity upon removal of the scope. The gilt was placed on oxygen 2 L/min via face mask and yohimbine (0.05mg/kg IM) was given to reverse xylazine. Upon recovery from anesthesia, sucralfate (1 gram) was dissolved in 8o ounces of a nutrition shake and provided in feeding bowl for treatment of both stomach and esophageal inflammation. A physical exam of the gilt 3 hours after recovery from anesthesia revealed tachypnea (80bpm), nasal discharge, lethargy, and pale mucus membranes. Pulse oximetry showed an SpO2 reading of 78-84%, and absent breath sounds over the left lung fields. For further diagnostic workup, anesthesia was induced with isoflurane provided via facemask. Thoracic radiographs revealed evidence of a left-sided pneumothorax with collapse of the left lung lobes as well as a mediastinal shift. To confirm the suspicion of an esophageal tear, endoscopy was repeated with evidence of a full thickness esophageal tear distal to the thoracic inlet. Given the grave prognosis, the gilt was euthanized with a barbiturate overdose. At necropsy, an 8cm longitudinal full thickness esophageal tear was confirmed distal to the thoracic inlet with placement of the ring shaped device within the mediastinum. The esophageal tear may have occurred during introduction of the over tube. Risk of esophageal tear should be considered prior to upper GI endoscopy procedures in animals smaller than 30kg.

P39 Comparative Performance of Two Bench-Top Hematology Instruments for Macaques and Mice

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Automated hematology analyzers are used in diagnostic and research settings to obtain quantitative information on cells in blood. The complete blood count (CBC) quantifies up to 20 blood cell parameters, in less than 3 minutes for modest cost. CBC data are relevant to diverse degenerative, developmental, genetic, infectious, neoplastic, toxic, and experimental conditions. Automated analyzers use different technologies, and settings are adjusted for different species. This study compared the results from 2 two commercially available benchtop hematology analyzers on blood from macaques and mice. Potassium EDTA anticoagulated whole blood samples from 87 macaques (49 *M. nemestrina* and 38 *M. mulatta*), and 68 mice, in their original collection tubes, were tested in parallel on both instruments. The coefficient of determination (r^2) and slope were reviewed for each parameter. Correlation was defined as excellent (r^2 > 0.9), good (0.8 - 0.9), fair (0.7 - 0.8), poor(0.5 - 0.7), or no correlation (< 0.5). For these 3 species, correlation between analyzers was good to excellent (>0.8) for quantitation of white blood cells, neutrophils and lymphocytes, and correlation between analyzers was poor or worse (<0.7) for monocytes and platelets. Correlation between analyzers was good to excellent for several important measurements. Although many differences between results from 2 instruments may not have great diagnostic impact, they may confound statistical analyses and interpretations in research settings. When the same instruments and technologies cannot be used for the duration of a study, comparative analyses should be done to define these differences.

P40 Enhanced Magnetic Resonance Imaging of Hepatocellular Carcinoma in a Cynomolgus Monkey (*Macaca Fascicularis*)

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The number of cases of hepatocellular neoplasia has increased with advancing age in humans. However, very rarely has spontaneous hepatocellular neoplasia been reported in nonhuman primates. In humans, radiography or ultrasonography cannot be used to assess and clearly identify differentiation of the organs or abnormal structures that include masses. Superparamagnetic iron oxide enhanced (SPIO)-magnetic resonance imaging (MRI) tends to be used practically to noninvasively identify tumors and determine their size, location, and the border between neoplasia and normal tissues in the liver. A 13-year-old female cynomolgus monkey exhibited signs of clinical illness, such as anorexia, with no serological abnormality of liver enzymes such as alanine aminotransferase and aspartate aminotransferase. After fluid therapy, the condition remained in remission for a few months under observation. Laboratory analysis of serum parameters after 2 weeks of recovery showed high levels of creatinine, alkaline phosphatase, and lactate dehydrogenase. In addition, we confirmed the presence of a firm, palpable mass and performed ultrasonography, radiology, and SPIO-MRI using a 3-T scanner. After MRI, we administered Ferucarbotran as a superparamagnetic iron oxide contrast agent. We then performed imaging to assess the liver mass noninvasively. The mass was difficult to surgically remove because of their location and infiltration despite the laparotomy after imaging. Hence, the MRI and laparotomy revealed massive neoplasia in the liver, and euthanasia was elected due to the poor prognosis. Histopathologic analysis showed the hepatocellular carcinoma with large, extremely irregularly shaped nuclei. Hepatocellular neoplasia can be reliably detected by SPIO-MRI. In particular, T2-weighted imaging clearly depicted the border between hepatocellular carcinoma and normal liver, yielding images similar to necropsy findings. The SPIO-MRI technique is therefore practicable and useful for the diagnosis of hepatocellular neoplasia in nonhuman primates. This is the first report to demonstrate SPIO-MRI for hepatocellular carcinoma in nonhuman primates.

P41 Comparison of PCR to Microbiologic Culture for Detecting *Klebsiella oxytoca* Colonization of Mice

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Klebsiella oxytoca is an opportunistic bacterial pathogen of mice and rats reported to cause otitis media, urogenital tract infections, or pneumonia in substrains of C3H/HeJ mice, inflammatory lesions in NMRI-*Foxn1*^{nu} mice, and urogenital tract infections in LEW.1AR1-*iddm* rats. In addition to being an opportunistic pathogen of mice, *K. oxytoca* is also a ubiquitous contaminant of plants and soil, and as such may be a contaminant of rodent feed and bedding. While microbiologic culture has historically been used to detect bacteria, PCR tests are increasingly being used to detect bacteria, including *K. oxytoca.* However, a *K. oxytoca* positive PCR result does not determine whether the source of *K. oxytoca* DNA is from viable bacteria or environmental contamination with DNA. In some cases our laboratory has detected *K. oxytoca* in rodents by PCR, but these results were inconsistently confirmed by microbiologic culture. The goal of this study was to determine the most accurate method for detection of *K. oxytoca* colonization in mice. Fecal samples from cages of athymic nude mice suspected to be colonized with *K. oxytoca* were collected and held at 4° C overnight prior to culture and PCR analysis. Identification of cultured bacteria was performed by MALDI-TOF mass spectroscopy and *K. oxytoca* DNA was detected using real-time PCR. *K. oxytoca* was detected in 0/45 (0%) feces by direct culture, 4/45 (9%) feces by broth culture followed by plate culture, 18/45 (40%) feces by *K. oxytoca* PCR testing of broth-cultured samples, and 29/45 (64%) feces tested by *K. oxytoca* PCR. The average *K. oxytoca* DNA template copy number per PCR reaction was 850,000 in broth-cultured feces and 40 in DNA extracted directly from feces.

In conclusion, PCR testing of feces provided the highest sensitivity of *K. oxytoca* detection while broth culture of feces followed by PCR allowed demonstration of the presence of viable *K. oxytoca*.

P42 Effects of Long-Term Intraperitoneal Injections of Corn Oil and Testosterone in Mice

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Intraperitoneal injection is a common route of injection in laboratory mice in which obtaining intravenous access is difficult. Intraperitoneal injection is easily performed and substances given by this route are quickly absorbed. Possible complications from the intraperitoneal route of administration include peritonitis from puncture of the intestinal tract, chemical peritonitis, or creation of fibrous tissue adhesions. This study looks at a group of 14 castrated male SCID mice that were implanted with subcutaneous prostatic tumors and administered supra-physiologic doses of testosterone in corn oil by intraperitoneal injection once daily, 5 days a week for between 2.5 and 8.5 weeks. All injections were performed by experienced personnel. The testosterone and corn oil used in the injections were both pharmaceutical grade. The solution was prepared in a biosafety cabinet using sterile instruments and sterile vials. In mice receiving less than 14 injections, no gross abnormalities were present although there was histologic evidence of mild inflammation associated with the mesentery, kidneys, and pancreas. In mice receiving >30 injections, 0.5 to 2.5 mls of oily fluid was present in the abdomen and there was gross and histologic evidence of regionally moderate to severe peritonitis involving the pancreas, mesentery, left kidney, and accessory reproductive organs in all animals. Fibrous adhesions were also present. There was light growth of *Burkholderia cepacia* on peritoneal cultures from 6 mice that received between 30 and 42 injections, although no bacteria were seen histologically. This study shows that serial intraperitoneal injections of corn oil is associated with peritonitis, presumably secondary to irritation from unabsorbed oil accumulating in the abdomen.

P43 Hind Limb Paralysis and White Matter Degeneration in a NOD.cg-*Prkdc*^{scid}*Il2rg*^{tm1wjl}/szj (nsg) Mouse with a Patient Derived Xenograft Mammary Tumor

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A female NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ (NSG) with a first passage, patient-derived xenograft (PDX) mammary tumor implanted into the axillary mammary fat pad presented with hind limb paralysis. Physical examination confirmed the mouse had bilateral hind limb paralysis with moderate to marked muscle atrophy, absent withdrawal reflex in both hind legs, and urine staining around the perineum. There were 2 masses between the scapulae. Both were firm and each measured approximately 0.25 cm in diameter. A second mouse in the cage had right hind limb paresis with a positive withdrawal reflex. There was concern that the tumor cells may have invaded the spinal cord, leading to the observed neurologic deficits. Gross necropsy did not reveal abnormalities aside from the hind limb muscle atrophy and the masses that had been palpated during physical exam. The masses, spinal column, and hind legs were submitted for histopathology. The spinal cord demonstrated bilateral white matter degeneration with vacuolation and axon swelling of the lateral funiculi, ventral funiculi, and nerve roots. Both hind limbs had myofiber degeneration and necrosis and the masses were confirmed to be carcinomas. There was no evidence of carcinoma metastasis into the spinal cord, and the lesions observed raised suspicion of viral contamination of the PDX. The PDX tumor was tested by PCR for 13 murine viruses and was positive for lactate dehydrogenase elevating virus (LDEV). LDEV is one of the most common viral contaminants of murine biologics and has been reported to cause poliomyelitis in AKR, C58, and immunocompromised mice, including one report in an ICR-SCID mouse. This is the first report of LDEV with white matter degeneration in an NSG mouse with a PDX. This case demonstrates the importance of testing PDXs after being passaged or combined with murine biologic material to protect the immunocompromised host and avoid spread to the colony.

P44 External Jugular Vein Stenosis in a Dog (*Canis familiaris*)

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An 11-month-old, 17.8 kg, female, purpose-bred hound was enrolled into a 4-hour in vivo thrombogenicity test. A facility surgeon noted a markedly small right EJV with a diameter of 2 mm compared to the left EJV which was 8 mm. When the animal was euthanized, a gross necropsy was performed and stenosis of the right EJV was noted. The right internal jugular vein and the hyoid venous arch were enlarged (9 mm and 3.5 mm, respectively) to compensate for the stenosis of the right EJV. Venous anomalies in dogs are not commonly reported in the literature and knowledge of intra-species variation is important for interpreting results from a canine *in vivo* thrombogenicity test.

P45 A Case of *Chilomastix* in a Common Marmoset Successfully Treated with Metronidazole

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Recently, the use of the common marmoset (*Callithrix jacchus*) has increased in biomedical research as animal models. Common marmosets were imported from a specific pathogen-free colony in Japan. To monitor bacteria and parasites in the marmosets, we tested fecal samples quarterly. A total of 118 fecal samples of 35 common marmosets were examined for infection with *Salmonella, Shigella*, *Helicobacter,* intestinal helminths, and protozoa. *Salmonella, Shigella, and Helicobacter* were detected by culture and PCR methods. Intestinal helminths and protozoa were detected by fecal centrifugation concentration (FCC). We identified nonpathogenic bacteria, such as *Proteus mirabilis, Enterococcus faecalis, Staphylococcus saprophyticus, Staphylococcus intermedius*, and *Escherichia coli* in feces of normal common marmosets. Interestingly, *Chilomastix mesnili* was isolated from a common marmoset with diarrhea by FCC technique. Also, we identified the *C. mesnili* using real-time PCR observations. *C. mesnili* is a non-parasitic member of primate gastrointestinal microflora. Although *C. mesnili* is considered nonpathogenic, it often occurs with other parasite infections. And, *C. mesnili* may be confused with other pathogenic species during diagnosis. This is the first reported case of *C. mesnili* infection in a specific pathogen-free common marmoset. The monkey infected with *C. mesnili* was treated orally with metronidazole (30 mg/kg body weight) to protect laboratory animal technicians. After treatment, *C. mesnili* was not found in the feces using FCC and real-time PCR methods.

P46 Increased Mortality in a Zebrafish (*Danio rerio*) Colony Infected with *Pseudocapillaria tomentosa*

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An investigator observed an increase in mortality, from 2-3% up to 7%, within the zebrafish colony over a month. The majority of the mortality was observed in a single mutant line that had not been thoroughly phenotyped by the group. However, mortality was also noted in other strains, including well-established lines that had no history of increased mortality. The group reported that several months prior to noticing the increased mortality, feeding frequency and water temperature had been increased in an attempt to promote fish growth. On examination, fish from the affected tanks exhibited normal behaviors and were normal on external examination, but did appear moderately small in size. Differential diagnoses included water quality abnormalities, toxin exposure, and infectious disease. A sample of the system water was tested for standard water quality parameters and found to contain elevated nitrite and nitrate levels. The investigator increased the water exchange rate in the recirculating system and reduced feeding frequency of the adult fish to decrease nitrogenous wastes. Concurrently, 5 fish from the most affected tank were submitted for necropsy. Gill biopsy of 1 of 3 fish submitted revealed a single bi-operculated, barrel-shaped egg. Histopathology performed on 2 fish revealed numerous intestinal nematodes in both fish, consistent with an infection of *Pseudocapillaria tomentosa*. Treatment of capillariasis with the anthelminthic fenbendazole at 0.267 mg/L per tank was started. Fish on the affected rack (about 500 total) are being treated with fenbendazole-treated feed for 3 consecutive days at 2-week intervals for a total of 3 treatment periods. Testing of treatment efficacy will commence upon completion of the treatment series. *Pseudocapillaria tomentosa* infections have been described in zebrafish colonies and are associated with wasting disease, increased mortality, and intestinal neoplasia. To date, few studies have described effective treatment for capillariasis, and treatment regimens described may have adverse effects on zebrafish fecundity and research outcomes. Appropriate measures, including health evaluations of incoming fish, proper quarantine, and appropriate surface disinfection of embryos, should be taken to prevent introduction of this parasite into zebrafish colonies.

P47 Supportive Care of Postoperative Hyperthermia in Swine (*Sus scrofa domestica*)

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The etiology of postoperative hyperthermia in swine remains unknown; however, the condition is similar to malignant hyperthermia in which rapidly elevating body temperature can lead to death if untreated. In our experience about 24% of postoperative Yorkshire swine (n = 16/65) show signs of hyperthermia (rectal temperatures >102.5F). The onset ranges from 2-4 hours following extubation and removal of IV catheters, making administration of cold IV fluids difficult. Medical management using steroids, NSAIDs, and/or sedatives has been described. Also advantageous are cooling methods, such as alcohol baths and application of wet towels directly on the torso. Unfortunately these methods are labor intensive, stressful, and not well tolerated by swine. Alcohol baths may also be painful if contact is made with the surgical incision(s). In addition to medical therapy for postoperative hyperthermia, refinements to nonpharmacologic methods of cooling should be considered. After an assessment for signs of pain and potential underlying causes of hyperthermia, our standard treatment of postoperative hyperthermia includes administration of 2.2 mg/kg carprofen PO or SC along with an edible chilled enrichment item, or "banana popsicle." We enrich with fresh bananas making the frozen version both familiar to the swine and therapeutic because they eagerly consume the frozen treat. Popsicles are made in advance using a 16-ounce plastic cup with lid. About 6 inches of a stainless steel chain is placed into the cup with a blend of banana and water. The remaining chain is fed through a hole created in the lid and the lid is secured. The entire assembly is then frozen. When needed to treat a postoperative hyperthermic swine the banana popsicle is easily removed from its container by running under warm water. The treat is anchored to the recovery stall in easy reach with a spring snap link. These treats cost about 50 cents each and take 5 minutes to produce. The banana popsicle provides a more positive interaction between the swine and staff than previously described direct cooling methods. Restoring normothermia in febrile, postoperative patients is vital to a smooth and successful recovery. We have found great success in supplementing our medical therapy with banana popsicles.

P48 A Survey of Spontaneous Disease in a Population of Wild-Caught Meadow Jumping Mice (*Zapus Hudsonius*)

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The meadow jumping mouse (*Zapus hudsonius*) is a small North American hibernating rodent belonging to the family Dipodidae, which also includes jerobas and birch mice. While species closely related to the meadow jumping mouse initiate hibernation as nutritive supply diminishes, the meadow jumping mouse enters dormancy mainly in response to a decreased photoperiod. As a consequence, when these animals are housed in a laboratory setting, torpor can be regulated via adjustments in the light-dark cycle. This characteristic, coupled with their small size, makes the meadow jumping mouse an attractive model for hibernation research. Between August and September of 2014, 13 meadow jumping mice (5 male, 8 female; various ages) were captured within the Bolton Flats Wildlife Management Area in Harvard, MA and subsequently housed at MIT with the aim of establishing a breeding colony. The health status of individual mice was routinely assessed, and identified pathogens and pathologies were recorded. Mice were screened on arrival for ectoparasites and endoparasites via hair-pluck, anal-tape-test, and fecal flotation, and were transported directly to a dedicated quarantine room. Until zoonotic pathogens could be excluded, enhanced personal protective equipment was required for personnel working in the quarantined area. Enteric organisms of note included coccidia in one mouse, a novel *Mycoplasma* species (closely related to *M. microti*), and a novel *Helicobacter* species (closely related to *H. hepaticus*). Adenoviral infection was also detected in one mouse by fecal PCR. Of particular interest, 3 mice exhibited hemorrhage in various joints on necropsy; histopathological analysis revealed a skeletal disorder consistent with hypovitaminosis C. Gross and histopathologic defects were absent in an additional 2 mice necropsied subsequent to colony-wide dietary vitamin C supplementation. Our health assessments revealed an array of pathogens and diseases that should be considered when establishing and developing health monitoring protocols for laboratory meadow jumping mouse colonies.

P49 Reduced Postanesthetic Complications in Stereotactic Guinea Pigs Given Prophylactic Metaclopramide

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Our facility has several research groups that use guinea pigs for the common application of hearing and stereotactic research. The guinea pigs used in these labs are a unique subset of over conditioned adults (BCS >4/5) who receive multiple anesthetic events throughout their long-term experimental use. Two of these research groups regularly experience a need to support their animals with subcutaneous fluids and twice daily syringe feeding several days postoperatively. Often these animals become anorexic, developing postoperative ileus, dysbiosis, and in severe cases, hepatic lipidosis and death postanesthesia as determined by diagnostic necropsy. To minimize the occurrence of ileus, the gastrointestinal motility stimulant, metoclopramide (0.75mg/kg SQ BID), was offered prior to and

postanesthesia for 2-3 days in a subset of 5 guinea pigs. All 5 animals that received metoclopramide were able to maintain their preanesthesia body weights and received fewer days of supportive care postanesthesia. The addition of prophylactic metoclopramide to guinea pigs undergoing anesthesia reduced the recovery period and decreased incidence of postoperative ileus. This preventative treatment may be a useful addition to stereotaxic procedures or other longterm anesthetic protocols in guinea pigs.

P50 Finding Your Dexterity: A New Approach to Tackling the Early Stages of Surgical Training and Practice

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It is not uncommon for experimental surgeries to be performed by individuals who have limited formal surgical training. These individuals often have no knowledge of basic surgical instrument handling and tissue manipulation. Trainers are faced with the challenge of ensuring that these individuals have the appropriate skills to perform live animal surgery. Often training must be conducted in a short amount of time and with limited budgets. Our goal was to create a curriculum that focused on the early stages of surgical training by creating exercises for teaching and practice of surgical instrument selection and handling, and development of the dexterity and skills that are essential to good surgical technique. We created a series of surgical classes that make use of various inhouse inanimate tools which we call "dexterity tools." The dexterity tools are designed to address many of the common repetitive hand motions required to perform surgery. The exercises we have created with these tools help to facilitate a variety of ways to practice similar hand motions and provide the option to adapt the exercises to the individual trainee's skills and needs. During the process, we do our best to alleviate some of the stress associated with the learning process and we provide take-home practice tools and exercises to our trainees. Since including the dexterity skills classes to our training program, we have had approximately 30 research personnel complete the series of classes and have hosted similar training for 32 attendees of a District 1 AALAS workshop. We have witnessed a transformation in these trainees' attitudes towards surgery and proper technique. Trainees express that they feel more comfortable and confident in their surgical skills and they show an appreciation for the need to practice and refine their skills. The proactive approach to tackling the early stages of surgical training using the dexterity tools and exercises has been a positive addition to our training program. We spend less time trouble shooting problems that result from inadequately trained people performing surgeries, as we instead focus our attention on the positive outcome of proper training.

P50A Porcine Aneurysm Model Provides an Objective Method of Brain Aneurysm Coil Selection

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Endovascular coil embolization has become a standard treatment option with proven efficacy for intracranial aneurysms. While most manufacturers produce similar coils, respective coils differ in terms of material, size of coil wire, coil shapes, and detachment mechanism. The choice of coil manufacturer is therefore often based on user preference and experience. However, coils are expensive and stocking a wide variety poses fiscal challenges. We sought to use a well-established porcine aneurysm model as a tool to establish an objective, reproducible, and standardized process for institutional procurement. Under continuous monitoring of vital signs, sidewall aneurysms were created by harvesting the external jugular veins via bilateral neck dissections. These veins were fashioned into blind-ended sacs. Both common carotid arteries were exposed, and venous sacs anastamosed end-to-side to create aneurysm sacs. Arterial access was achieved by femoral arterial puncture, and guide catheters advanced to the respective common carotid arteries. Standard microcatheters were used to catheterize the aneurysms under image guidance. Coiling of aneurysms was performed via microcatheter to the point of aneurysm occlusion. A 5-point Likert scale grading system was used to evaluate several predetermined criteria for each coil and manufacturer. Criteria included ease of coil navigation and deployment, mechanism of detachment, and ease of coil retrieval. Five aneurysm coil manufacturers were evaluated, with 7 neurointerventionalists evaluating each type of coil. Analysis of evaluations revealed 3 coil types with superior grades, and 2 with inferior grades. The 3 superiorly graded coil type manufacturers were awarded preferred vendor provider status for ongoing aneurysm coil treatments in brain aneurysm patients. This model provided an objective, safe method for coil selection for procurement. To our knowledge, this is the first published account using this method for a procurement process.

P51 Effects of a 21-Day Cage Change Schedule on Tumor-Burdened, Immune-Compromised Mice Housed in Ventilated Disposable Caging

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Technician time and disposable caging supplies are valuable and costly assets in a laboratory animal facility. Extending the length of time between cage changes from 14 days to 21 days would reduce both technician time and the amount of consumable supplies used. However cost effective, we have to consider if the animals would be adversely affected by this change in procedure. Since a majority of the mice housed at our facility are immune-compromised and used for oncology research, this study was performed with 2 groups of tumor burdened mice to determine if there are adverse effects of prolonged housing. Fifteen Athymic nude mice and 15 NOD/SCID mice were housed 5 per cage in clean disposable ventilated cages prebedded with 1/8" corncob bedding. Ammonia sensors were affixed inside each cage. Cages were monitored daily for 21 days to document general health, ammonia levels, cleanliness of the cages, and welfare of the mice. Cages displaying a dangerous ammonia level reading were changed immediately and the animals were euthanized and necropsied to check for any physical signs of ammonia toxicity. Body weights were taken weekly. At the end of the study the animals were euthanized and necropsies were performed. None of the animals displayed any physical signs of distress or illness over the course of the study. After day 15, the Athymic nude cages were considered dirty and had ammonia readings of medium-high to dangerous levels. The NOD/SCID cages were considered dirty by day 19, but none reached higher than a medium ammonia level. Although none of the cages ever reached a point where living conditions appeared unacceptable and no physical signs of illness presented, the dangerous ammonia readings of the Athymic nudes created unacceptable housing conditions. As a result, our current cage changing schedule remains unchanged.

P52 Evaluation of the 52nd Annual Meeting of the Society for Laboratory Animals GV-SoLAS in Frankfurt

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The 52nd Annual Meeting of the GV-SoLAS was held in Frankfurt from September, 10–12, 2014. It was the largest national event in Europe to date. A total of 819 delegates participated. The principal themes were the future of laboratory animal science, anesthesia and pain therapy, health monitoring, and neurosciences. The meeting included invited lectures, debates, oral presentations and posters as well as 13 theoretical and practical workshops. For the first time, the

meeting was evaluated. A bilingual online survey was conducted (German/English). Fifteen closed questions, in combination with 6 open questions, were asked. A total of 32.7 % of all participants took part in the survey (n = 268). The majority of participants (50.7%) stated that they had been working in the laboratory animal science field for more than 10 years. As a main reason for participation, 21.4% selected the practical training (workshops), whereas the theoretical training was selected by 65.3%. The exchange with colleagues was selected by 76% as the main reason for participation (multiple answers were possible) The results suggest that the opportunity of direct communication during the conference continues to play an important role for the participants. For a large number of participants the meeting seems also to become increasingly important as a training event. The latter must clearly be seen in the context of the stricter European regulation on continuous education.

P53 The Benefits of a Business Services Group in an LAS Organization

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A laboratory animal services (LAS) group has many moving parts due to the complex nature of the care provided. With so much time being spent with the animals in the facilities, LAS employees can find themselves scrambling to find the time for the necessary daily tasks to ensure animal health and welfare. With all of the responsibilities of vet checks, cage changes, housing and receiving, working with the researchers, and all of the other hats that members of LAS have to wear, one question remains. What about the administrative piece to the operation? How can it be ensured that metrics are collected, researchers receive the assistance that they require (especially with animal ordering and importing and exporting), and chargebacks and per diems are provided correctly and in a timely banner to allow for sufficient budgeting? In order to provide all the necessary services without sacrificing animal care, a business services group was formed. Business services consists of a team that provides support in the following areas: finance (animal vendor contracts, DA budget management, chargebacks, per diems, purchase order (PO) creation and management, and invoicing), projects (team collaboration software), animal procurement and the import/export program (animal ordering support for both research community and vivarium operations staff, logistic coordination of imports and exports), and administrative LAS support (IT, travel, LAS event coordination), as well as other one-off services. Once business services was created, both researchers and LAS staff had contact points for all administrative related services needed, In conclusion, a business services group allows for the research community to receive the assistance that they need while giving back time to the LAS employees that work in the vivarium so they may focus on the most important part–the animals!

P54 A Comparison of the Effectiveness of Harem and Timed Mating for Cynomolgus Macaques in a Preclinical Research Setting

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Mated cynomolgus macaques are often required to meet the goals of developmental and reproductive toxicology studies. A commonly used method to obtain pregnant animals is through a timed mating procedure, in which menstrual cycles are tracked, optimum ovulation period identified, and animals moved in to breeding pairs for a 3 day mating period. This one-to-one method of mating has been historically ~30% effective at our preclinical research facility. In order to attempt to increase pregnancy rates, a harem mating procedure was created using pen-style caging and 1 male to 5 females for a 2-3 week period. The 2 males selected for harem mating were new breeding males that had some experience with timed mating before transition to the harem style mating. Data was collected for 3 different harem mating groups for each male, in addition to all data from their timed mating sessions. After evaluating the pregnancy success rate of the 2 males in both types of mating arrangements, harem mating did not appear to be a more effective way to obtain pregnant females, nor did it significantly save staff time as initially postulated. While not superior to timed mating, the harem style mating did allow several females with irregular menstrual cycles to become pregnant, which may not have been achieved through timed mating processes. Therefore, harem style mating may be useful in some situations when pregnancy is unlikely to be achieved through more traditional timed mating methods, but would not be considered a better substitute for timed mating. Additionally, the exact gestation day cannot be calculated for harem mated females, so this type of breeding may not be appropriate for some projects where this discrete data is required.

P55 Establishing a Socialization Program for Rabbits

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Changes to the 8th edition of the *Guide for the Care and Use of Animals* prompted a rabbit socialization program to be developed. We were faced with the challenge of providing social housing to approximately 100 rabbits which had historically been singly housed. Our facility was unable to meet this standard with our existing cages. Purchasing all new caging was extremely cost prohibitive, so alternatives were considered. Retrofitting existing cages allowed us to achieve our goal of having cages suitable for social housing at the fraction of the cost of buying all new. Initially, standard operating procedures were drafted to detail the new housing policy. Investigators were notified of the new requirements and our plans for compliance. They were allowed to review the draft standard operating procedure and provide input. At the start of our program, only females from incoming deliveries were paired. Existing females and all male rabbits were exempt. Rabbit social housing compatibility forms were created to track results. Successful pairing and outcomes, including the difficulties involving separations and reintroductions, were well documented and tracked and our technicians have become more comfortable with assessing compatibility. Implementing this program has been a success with the majority of our qualifying animals, 80%, being successfully paired.

P56 Evaluation of 2 Types of Diets on the Reproductive Efficiency in a Colony of Knockout Mice with Profound Biotinidase Deficiency (Btd-/-)

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Biotin, Vitamin B complex acts mainly as a coenzyme in several metabolic processes such as gluconeogenesis, fatty acid synthesis and the catabolism of several branch-chain amino acids and odd-carbon fatty acids. In mammals, biotin deficiency can cause alterations in neurologic, metabolic and reproductive functions. The use of genetically modified animals has been useful for studying numerous diseases in both humans and animals. In order to understand many aspects of the pathophysiology of the disorder a group of scientists from the Henry Ford Hospital (Detroit, USA) recently developed a transgenic biotinidase-deficient mouse. When fed a biotin-deficient diet these mice develop neurocutaneous symptoms, reproductive disorders and teratogenic problems (microphthalmia, micromelia, cleft palate). The clinical features are reversed with biotin supplementation. The aim of this study was to compare two types of diets (defined purified diet with free biotin—Diet A and natural modified diet from a natural diet—Diet B) and their effect of reproductive

performance in males and females. From a colony of homozygous wild type mice (WT) and homozygous knock-out (KO) mice matings were carried out, 40 homozygous knock out animals were obtained in order to form 4 experimental groups; 1) monogamous matings with diet A, 2) mating in harem system with diet A, 3) monogamous matings with diet B, 4) matings in harem system with diet B. Five gestations were monitored and obtained measurements at several reproductive parameters (productivity efficiency index, average fertility rate, litter size at birth, litter weight at birth, litter weight at weaning, sperm concentration, sperm motility, and sperm morphology) were evaluated over a period of 6 months. Analysis of Variance (ANOVA) analyzed data. The results showed that weaning weight is the only different characteristic when the 2 diets are applied in females ($P < 0.05$). Regarding mating systems, the triplets are placed at producing more animals with better phenotypic characteristics. For males, there is sufficient evidence to conclude that sperm concentration, motility and morphology is different between WT and KO group ($P < 0.05$), regardless of the type of diet they received.

P57 Dermal Application Calculated Dose Site Size versus Calculated Body Surface Area in Rodents

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Dermal application for test article is a necessary and commonly used dose route in rodents. The standard area for dermal application sites is approximately 10% body surface area (BSA). This BSA represents the approximate surface area of a drug that will be used in humans (for example, the face for acne medication, the top of the head for hair loss drugs, etc.). Currently in some CROs, the standard for calculating this dose site area is done by mathematical equation to find the mean surface area for each sex/group. This area is then measured out on marking templates to mark the "four corners" of the dose site template on the animals. In addition to being quite time consuming, this method is also inaccurate and requires handling the animals more often than is necessary. In order to increase accuracy and simplify dose site identification, data was collected by measuring the anatomic landmarks. The area measured started from the scapulae to the wings of the ilium extending to the lateral midline of the animal. This surface area represents the approximate 10% BSA as required as a standard for preclinical toxicology studies. Various sizes and age groups of rodents were measured based off of these landmarks and a proposed dose area (PDA) was calculated and compared to the 10% BSA (based off of bodyweight). The findings for the PDA represented between 7-13% BSA, a 3% range of deviation from the approximate 10% standard. Dose site markings can now be eliminated and handling may be reduced. Additionally, accuracy is improved due to relying on the natural anatomic landmarks on our rodents.

P58 A Performance-Based Approach to Evaluating the Effect of Modification to Vivarium Room Air Changes per Hour.

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The primary purpose of ventilation is to provide appropriate air quality and a stable environment, according to the *Guide for Care and Use of Laboratory Animals* (the *Guide*). Modern systems allow for increased control over multiple variables independently, such as temperature, humidity, and airflow on a room by room basis. In an effort to "go green," we decided to take a performance-based, quantitative approach to determining the optimal air change rate to ensure appropriate air quality while using less energy and reducing stress on the ventilation infrastructure. We measured temperature, humidity, carbon dioxide, allergens, particulates, ammonia, and endotoxin levels in multiple rooms of our conventional facility. After collecting baseline data at 10-12 air changes per hour for 3 days in multiple rooms housing multiple species, we decreased the air changes per hour to 8 and then 6 air changes per hour over a similar period of time to determine the impact on those measured values. Animals were also monitored to ensure there were no visible effects on animal health or behavior. The measured values were generally unchanged relative to baseline, except for some increases in carbon dioxide and allergens in certain species, and no abnormal clinical signs were observed in the animals. After analyzing the data, we recommend decreasing the current 10-12 air changes per hour (as recommended in the *Guide*) to 8-10 air changes per hour, depending on species, room size, and other pertinent variables.

P59 Redesign of a Novel Nonhuman Primate Puzzle that Reduces Food Waste and Provides Environmental Enrichment

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We have redesigned and improved upon a novel primate feeder that was first designed in 2006. While the original design served the purpose of reducing food waste as well as providing environmental enrichment, the feeder could not be reconfigured or hold a variety of foods, and would frequently break if accidently dropped. During our redesign we encountered various familiar challenges, the feeder had to use the existing feed slot, hold the daily biscuit supply, not interfere with the squeeze mechanism, attach securely to the housing cage and stand up to demands of the cagewasher while being easy to manually clean. Our newly redesigned feeder can hold biscuits and a variety of enrichment, in a 4 level enrichment compartment. The feeders are now composed of stainless steel mesh and polycarbonate. The polycarbonate insert is removable allowing the body of the feeder and inserts to be cleaned easily through a standard tunnel washer. The primates forage for their food through a switch back design by inserting their fingers through the 1 inch stainless steel holes to move the food down the polycarbonate channels. The inserts can be reconfigured with different levels of complexity, so the feeder stays challenging to the primate using it. In addition, the enrichment food is picked at rather than being eaten all at once—allowing for longer foraging periods. With the polycarbonate insert removed the unit also allows access to primates without removing the entire feeder. With different levels of enrichment the primate can now make choices in its environment to either forage for biscuits or enrichment food. We have effectively used this feeder on single and paired housed macaques, and conclude the redesign is successful as a new tool for primate enrichment.

P60 Interventions that Minimize Food Chewing in Female CD-1 Mice

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CD-1 sentinels frequently exhibit excessive food chewing, which negatively impacts animal welfare. Mounds of chewed food may block access to water, cause flooding, reduce cage space, impair cage ventilation, may increase cage changes, and may confound dirty bedding sentinel exposure. We compared the amount of food chewed by 19 boxes of female CD-1 sentinels (vendor A) (4 mice/box) under 4 enriched conditions, a circular disk of paper strips, small paper rolls sprinkled on bedding, extruded diet, and standard housing (standard rodent chow and cotton square). All boxes received a cotton square in addition to any new enrichment tested. Each box served as its own control, being rotated between enrichments at cage change. New enrichments were followed by standard conditions for 1-2 weeks to allow boxes to "reset" prior to starting a new enrichment. Feeders were filled with a measured amount of food and the food remaining at cage change was subtracted from the starting weight to calculate the amount of food chewed. Mice receiving circular paper disks or extruded diet chewed less food ($p < 0.01$) than

mice under standard conditions or receiving paper roll enrichment. In a second experiment, the amount of food chewed under standard conditions by female CD-1 mice (4 mice/box) from 2 different vendors was compared (9 boxes from vendor A and 8 boxes from vendor B). Vendor B mice chewed less food than Vendor A mice ($p < 0.01$). The amount of food chewed by vendor B mice under standard conditions was not statistically different than vendor A mice receiving either extruded diet or circular paper disks. In conclusion, circular paper disks, extruded diet or changing vendors eliminated excessive food chewing by CD-1 mice.

P61 Increased Space Allows for a Less Stressful Breeding Environment for the Gray Short-Tailed Opossum (*Monodelphis domestica*)

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The gray, short-tailed opossum (*Monodelphis domestica*) is becoming increasingly important as a mammalian model for research. The opossums' year-round breeding, manageable maintenance, and sequenced genome have made it the most commonly used laboratory marsupial. For these reasons, we maintain a breeding colony of opossums for use in our research. In our original breeding protocol, 1 female and 1 male opossum (with their respective jar-nests) were switched into each other's cages. This provided an opportunity for the animals to be exposed to each other's pheromones, which is necessary for the female's induced estrus and ovulation. After 2 days, the female and male were placed together in the female's original cage (39.37 x 27.94 x 19.05cm) for 5 nights. The size of each cage allowed for 2 jars, 1 croc of food, and a water bottle. While 25% of paired opossums successfully mated (produced embryos or newborns) using this protocol (17 of 68 matings over a 5 month period spanning January to May), the rate of opossum mortality from aggression was significant (7.6%). We therefore employed a new protocol in which we placed the male and female cages beside each other with the plastic tops removed for 2 days, thereby allowing air exchange between the cages. Unlike our original method, opossums were not transferred into different cages during this time. After 2 days, the male and female opossums were placed together in a larger cage (50.8 x 40.64 x 20.32cm) that provided adequate space for the opossums to move around while containing two jars, both crocs, and a water bottle. Opossums are solitary animals, and the new cages not only allowed for a neutral mating territory but also more space for the mating pairs. Although mating success did not improve under the new protocol (16%, 24 of 148 opossums over a 5 month period), the rate of mortality was reduced by more than half (2.6%). These results are consistent with the new protocol providing a less stressful environment during mating. Moreover, the larger cages made is easier to view the opossums, and thereby facilitated the monitoring of animal health and mating activity. In summary, our new protocol positively impacts opossum health during mating and increases our understanding of opossum husbandry.

P62 Rack Exhaust Plenum PCR as a Means to Detect *Pneumocystis carinii* Outbreaks in Laboratory Rats

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Pneumocystis carinii is an opportunistic fungal organism often excluded from laboratory rat colonies due to its ability to cause interstitial pneumonia and incite an immune response. *P. carinii* can be challenging to diagnose antemortem due to delays in seroconversion and an inability to detect via PCR of feces or oronasal swabs. In fall 2014 we detected *P. carinii* via routine sentinel serology on a rack in a room containing 4 double-sided ventilated racks housing approximately 350 rats. To control this outbreak we needed to identify and remove infected rats. Accordingly, one rat per cage on the sentinel-positive rack was tested via serology, seropositive cages were removed, and the environment was disinfected. The seronegative cages were retested 6 weeks later and were found to be negative. Unfortunately, *P. carinii* was again detected via sentinel serology 3 months later on 2 additional racks. A new approach using rack exhaust plenum PCR in addition to serology was used based on our previous finding that rack exhaust PCR did detect *P. carinii*. After removing seropositive rats, all racks were washed and 6 weeks later all the racks in the room were retested by PCR and found to be negative, and sentinel serology also remains negative. Rack exhaust plenum PCR has not previously been published as a means to detect *P. carinii* or to verify successful eradication. In conclusion, we found using rack exhaust plenum PCR in combination with serology facilitates the identification and elimination of *P. carinii* positive rats. Rack PCR also has the potential to aid in colony health surveillance. We propose validation of the ability of rack exhaust plenum PCR to detect *P. carinii* in order to add it to the list of organisms known to be routinely detected via rack exhaust plenum PCR.

P63 Incorporating Wildlife and Field Studies into Your OHSP

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Field studies can present a vast array of hazards, some of which are difficult to identify, most of which are unique to wildlife work, and many which are not covered in the typical occupational health and safety program training. How should we tackle this problem to assure our field studies researchers and students stay safe and are properly trained? The OHSP at our institution has designed individualized, online training modules specifically for: laboratory, agricultural, campus facilities, and field studies/wildlife personnel. At our institution, OHSP training is required for those listed on ACUC protocols. Individuals have the freedom of choosing their hazard category, then completing a training module created specifically for their line of work. Our field studies online training course covers hazards specific to wildlife work in the field. Since the hazards of field studies can be quite broad, we have narrowed them down to reflect only regional hazards, located in and around the state of Missouri. We cover topics such as: basic wild animal handling, bites and scratches, surgery and obtaining tissue samples from a live animal, local insect vectors of zoonotic diseases (ticks, mosquitoes, triatomine bugs), zoonoses found in the Missouri area (leptospirosis, listeria, giardia, rabies, hantavirus, salmonella, plague), tetanus, and histoplasmosis. We also include a short field guide of local snakes, arachnids, singing insects, flora to avoid, resources you can turn to if you need more specific information, what to do if you get hurt, items to carry with you, and tips for staying safe in the field. Our field studies training has been edited by veterinary, wildlife, and safety professionals at our institution.

P64 From Infants to Prepubescent Juveniles: Managing Enrichment in an Active Baboon Nursery

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A well-established environmental enhancement program can serve as a great resource to NHPs and their different housing accommodations. At our facility, we have an active baboon nursery for ages birth to prepubescent years. The nursery is continuously establishing new ways to enhance primate life while helping the animals create healthy and species-specific habits. Manipulative and sensory enrichment are a daily part of primate life. Infant baboons start out with hanging toys, rattles, and chimes to better establish hand/eye coordination. Social group formation (SGF) playtime helps form healthy social habits, and promotes normal physical behavior (climbing, jumping, etc.). Fruit and other food items are introduced when infants near weaning age to encourage mastication. Sensory

enrichment such as mirrors, bubble baths, and finger paints are common favorites. The first year of life we use all senses. Cage furniture is placed inside of the baboon enclosures and changed biweekly. As the baboons progress in age, foraging and manipulative enrichments are some of the main focus. As they move to the larger housing unit, they start to receive more task-oriented feeding devices such as large foraging and manipulative boards, foraging barrels and buckets, and an abundance of other manipulative items, encouraging the baboons to use strategy and work to receive fruit or forage. The nursery is dedicated to enhancing primate life while keeping a professional atmosphere and following all of the appropriate guidelines, rules, and regulations.

P65 Refinement of an Animal Housing Room Decontamination Protocol

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Animal housing room decontamination (decon) is an important process to eliminate potential pathogens and reduce the biologic burden within the room. Our current decon method developed and used during a full facility decontamination in 2006 has demonstrated its effectiveness. However, we were interested in determining if all components of the decon process were essential to achieve room sanitation. During our full decon procedure, rooms are emptied of all mobile and detachable equipment, foamed with a germicidal detergent, all surfaces scrubbed with a firm brush, rinsed with water, and then fogged using a chlorine dioxide solution. On average this process takes 6.5 hours of physical labor over 2 days. To determine if foaming and scrubbing could be removed from the procedure and still obtain satisfactory results, half of an emptied rodent housing room that had been in service for the previous 6 months was decontaminated using our current decon method (side A), while the other half was only fogged and rinsed (side B). The decon methods were then evaluated by sampling the ceiling, walls, and floor with ATP swabs (n = 9/side) and trypticase soy agar contact plates (n = 9/side). The ATP swabs were read using a luminometer, which measures the ATP in relative light units (RLU). The contact plates were incubated at 35°C and bacteria growth was quantified in colony forming unit (CFU) at 72 hours. Post-decon, ATP swabs from both sides of the room resulted in 0 RLUs in all samples collected (n = 18). The post-decon contact plates from side A demonstrated no growth while contact plates from side B resulted in 1 CFU in total. The results from our trial indicate that chlorine dioxide fogging followed by a final rinse with water achieve a level of disinfection that is within our department's acceptable range. By implementing this change we save an average of 2 hours of physical labor per room decon which ultimately will decrease the cost and increase the rate of turnaround on the decon of facility rooms.

P66 What Worked for Us: Experiences and Pitfalls of Housing Tropical Frogs in Static Tanks

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Dynamic research projects often require flexibility to accommodate diverse animal models and quick timelines. A unique opportunity to acquire a warm water *X. tropicalis* frog colony arose with no opportunity to construct appropriate long term aquatic housing. To accommodate this import, we temporarily housed the frogs in static tanks. Although *Xenopus* species are widely used aquatic research models, there were very few resources that specifically outlined static housing husbandry procedures specifically for tropical frogs. Considering variables such as species-specific needs, husbandry, space, costs, and urgency, we established setup and husbandry procedures based on extrapolation of related cold water species. Repurposed feed bins and sediment-filtered conditioned tap water were used as containment. Readily available water test strips, reagent titration chemicals, submersible heaters and circulators were used to condition and monitor water quality. The static tanks were kept in a 76–80°F regulated room. Initially, we housed 3 frogs/l water with a ~25% water change 3x weekly 2–3 hours after feeding commercially available pelleted feed. Tanks were siphoned daily to reduce debris and organic buildup and water quality testing was performed weekly. Our extrapolated housing density, husbandry and water maintenance regimen proved to be problematic. Water balance was difficult to maintain and caused acute mortality from nitrogen and ammonia toxicity after several weeks. As a result, we adjusted our housing density to 2 frogs/l and implemented a minimum 80% tank water change 3 times weekly. Feeding and siphoning procedures remained the same. We experienced no further mortalities since these adjustments were made. Improved water quality was attributed to the decrease in housing density, increased water volume changes, and reduced fecal and organic load. Successful water quality management and maintenance of tropical frog static tanks can be problematic and requires a delicate balance between housing density and husbandry to create and sustain a viable environment.

P67 Going Above and Beyond for Head Implanted Rodent Models

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The exploration of autism, depression, and Huntington's, Alzheimer's, and Parkinson's diseases has led to various forms of head implanted rodent models within our company's neuroscience research unit. After surgery, these animals had been housed in traditional static rodent cages without access to liquid water as approved by the Institutional Animal Care and Use Committee (IACUC). Due to the size and positioning of the implant, traditional wire bar lids that house the water supply and food could not be used. They were offered various forms of a gelled hydration source and food on the floor of the traditional cage. With the mission of "flawless animal care" in mind, we set out to contact various companies to search for a more compatible cage option for both our head implanted mouse and rat models. We partnered with a respected animal caging vendor and set out to use and make specific modifications to one of their existing caging systems. The ultimate goal was to provide a caging system that provides optimum space for the animal and to design a water bottle and feeder to allow ad lib water and food consumption for the animal without compromising the head implants. We were also interested in providing individual ventilation to each cage as opposed to the traditional static housing in which we had historically housed this animal model. Design modifications were made and ultimately led to a successful implementation of this new caging system for these animal models starting in December 2013 through December 2014. Improved animal welfare for this animal model has resulted from the implementation of these modified caging systems. Continuous improvement to animal housing systems can be driven from within your program, not necessarily what's currently available in the marketplace.

P68 Techniques for Restraint and Vaginal Cytology Collection in the African Giant Pouched Rat (*Cricetomys ansorgei*)

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The African giant pouched rat (*Cricetomys ansorgei*, formerly *Gambianus*), a large rodent native to Sub-Saharan Africa, is of particular interest to the research community based on their trainability to detect both explosives and tuberculosis. They are rare in the United States research setting and there is a paucity of information on reproductive physiology within scientific literature. Our goal is to better understand the reproductive cycle of these animals

through vaginal cytology, while causing minimal discomfort and without using anesthesia or sedation. Our restraint technique involves guiding the animal into a handling bag. The bag is made of durable, synthetic cloth and is approximately 12 inches by 2–5 inches with a wider open end and a smaller closed end. Once the animal is in the bag with the tail protruding out the open end, a drawstring is cinched closed and tied with the handler placing adequate pressure on the rump to assure the animal will not turn in the bag. A micropipette with a filter tip is then placed into the vaginal orifice and the vaginal canal is lavaged 4–5 times with 80-100 µl of sterile physiologic saline; the slide preparation is then stained per standard practice. Handling is always a two person job to assist with closure of the bag and preparation of supplies for the handler. There is potentially large intraspecies variability for the initial restraint based on individual animal behavior, but handlers can safely perform vaginal lavage once the animal is restrained. Placement of the pipette tip centrally into the vaginal canal is necessary to avoid interference of the vaginal walls, which will block the pipette tip due to back pressure. The methods described provide quick, safe, and reliable means of restraint and collecting vaginal cytology samples from the African giant pouched rat with minimal discomfort.

P69 Litter Box Training of Rabbits Housed in Pens

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Recently, social housing of rabbits has been recommended as a default housing method by the American Association for the Assessment and Accreditation of Laboratory Animal Care, International. In the effort to increase social interactions and improve animal welfare by group-housing rabbits in pens, it was discovered that the sanitation procedure of these pens was time consuming and tedious. It was decided to attempt to litter box train the pen-housed rabbits with the expectation of reducing manual labor daily, decreasing the use of acid and disinfectant/detergent, and improving animal welfare. Two litter boxes containing irradiated corncob bedding were introduced in the corners of the pens where rabbits were housed and a sample of their excrement was added to each box. Over time, the less used box was removed from the pen. Boxes were emptied a minimum of every other day, and a sample of soiled bedding was taken from the dirty box and placed into the clean box. This helped the rabbits identify the area they marked previously for consistent use of the box. Used boxes were replaced with clean boxes a minimum of twice a week to prevent urine scale buildup. Litter box training of pen-housed rabbits was successful despite encountering several challenges that allowed for the opportunity for brainstorming and innovation. As a result, daily husbandry tasks were decreased by 13 minutes per room per day with the addition of litter boxes. Full room sanitization procedures were decreased by 1 hour per room. Along with this increased time efficiency, litter boxes also led to improved animal welfare by providing a new form of enrichment and deceasing chemical use around the animals.

P70 Nest Location Preference and Complexity in Mice Housed in Individually Ventilated Versus Static Caging

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Provision of nesting material to mice (*Mus musculus*) promotes species-typical behaviors such as foraging and nest building, as well as aiding in thermoregulation. Recent studies have investigated rodent cold stress and thermoregulation based on housing type, specifically focusing on whether offering nesting material may ameliorate those factors. The introduction of the individually ventilated cage (IVC) has had a significant impact on rodent husbandry in the laboratory animal community. However, it is unclear how airflow impacts the nesting behavior of mice. We examined nest location and nest complexity of mice housed in IVC versus static cages. Mice of mixed backgrounds, representative of a large scale academic institution (n = 14), were initially housed in static cages and provided with a 5 cm^2 compressed cotton square. Three days later, nests were scored and the nest locations (front, middle, or back third of the cage), cage temperatures and cage humidities were recorded. The study was then repeated with the same mice housed in individually ventilated caging, in which airflow was also recorded. There was no difference in either temperature or humidity in IVC versus static caging, but mice in IVCs tended to construct more complex nests. Mice constructed the majority of nests in the middle and back third of both caging types. Lastly, survey data on air flow and nest score and location was randomly collected on 1,769 ventilated cages 7 days post-cage change. We found that 65% of nests were located in the back third of IVCs, with the remaining 35% equally distributed between the front and middle cage locations. The nests built in the back third were the most complex, while the nests built in the middle third tended to be the least complex. These results indicate the presence of air flow in the cage does not influence nest location, but may impact nest complexity.

P71 Developing an Ergonomic Safety Risk Assessment and Identification Program for Workplace Injury in an Animal Care Organization

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The element of risk associated with animal care and workplace safety is as diverse to each individual animal research institution as the personnel working in those institutions. Assessment of workplace risks can be a challenge for an OHS department because of restricted access to animals and animal care personnel. Developing a program that would be effective for one group of animal care personnel may not be appropriate for another group for risk identification. Risk assessments in ergonomic and safety practices were needed in order to determine if a safer workplace for students and staff was necessary. This program was developed to observe and monitor the risk associated with individual tasks in caring and managing animals in a research and teaching environment. High incidence of skeletal muscular injury had been reported prior to these observations. In order to develop this program, identifying specific risk involved in animal care and maintenance equipment tasks were established. When observing the individual care staff and students in real time, this gave an overall view of risks associated with their specific tasks. By establishing an actual eye witness account of the activities performed during working hours and recording on forms the work effort, these reported injuries now have been discovered. With the use of several observational risk assessment task forms and reporting all observation to the IACUC, these groups now have the tools for risk identification. They will now take responsibility in monitoring appropriate safety levels and reportable near miss incidence. The development of these reports has helped with identification of problem areas and associated risk for the overall animal care groups. This program has provided a higher level of oversight in identifying ergonomic and safety risks in animal care.

P72 Cage Level Communication at a Large Academic Institution

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Within a large academic institution, communication regarding individual animal needs between the various stakeholder groups (animal care, veterinary, research staff) can be challenging. As a result of our geographically expansive program and a need to ensure continuity of care, we developed a method for personnel to easily identify cages undergoing unique care using a cage level system. The cage level system consists of color coded acetate films which are

placed in front of the cage card and reflect the category of care. Special labels affixed to the film provide basic information related to care of animals within the specific cage. Red: animal needs to be evaluated. Blue: animal is under monitoring or treatment; a label provides a summary of the physical exam findings and treatment plan while a separate animal observation report (AOR) maintained by the animal health technician is used to document care. Yellow: animal husbandry is not standard; a label provides details regarding the husbandry modification needed. Green: action is needed by the research team; animals require weaning or a cage exceeds acceptable density limits. Clear: used for documenting a variety of situations which do not require any action such as an animal that has a known physical defect (missing eye), or a temporary need for more intense monitoring associated with research activities. A label placed on the clear acetate film describes the specific circumstance. Sheets of transparent film are purchased and cut to fit inhouse. The film colors chosen can be easily distinguished by personnel and are durable enough for reuse, minimizing replacement costs. Over time, this system of communication has been refined as needed using input from all of our stakeholders to create a very effective method of communication, personalized to fit our program needs.

P73 Cross Fostering as a Method of Eliminating *Pneumocystis carinii* from Transgenic Rat Colonies

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In August 2013, a sentinel from a breeding colony of transgenic rats tested seropositive for *Pneumocystis carinii* following 3 months of exposure to soiled bedding. Multiple rats in the colony proved seropositive, and attempts to eradicate the infection from the colony via selective culling of seropositive rats were not successful. Because depopulation was not an option for these valuable transgenic rats, an alternative method of pathogen elimination was employed. Rats from the *P. carinii*-enzootic colony were bred in our quarantine facility. To reduce the possibility of transmission to pups, breeding rats were fed a commercial diet containing trimethoprim and sulfamethoxazole from 5 to 10 days prior to the expected parturition date until the end of gestation. Dams were removed from the sire and placed in a clean cage 24–48 hours prior to parturition, and the pups were fostered to clean, vendor-purchased females within 24 hours of birth using an iodex immersion technique. Successful elimination of the pathogen was documented by comprehensive testing of cross-fostered pups and their foster mothers. Testing strategies included: PCR of swabs from rack exhaust plenums at least 6 weeks post exposure, serology of pups at >14 weeks of age, serology of cross foster mothers at >7 weeks post exposure to pups, and histopathology and *P. carinii* PCR of lung tissue from 1 pup per litter. Two litters of pups (25 rats total) were successfully cross fostered using this program, and the colony was subsequently reestablished from this group of animals allowing depopulation of remaining infected rats. In conclusion, this cross foster program and multimodal testing strategy is a viable alternative to depopulation for eradication of the pathogen from valuable transgenic rat colonies.

P74 Computer Technology Assisted Weight Management for the Maintenance of Healthy Nonhuman Primate Research Models

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Weight management is vital to ensure the health of nonhuman primates. The identification, management, and tracking of excessively thin or obese individuals is a challenging task for veterinary care staff. We developed novel software that automates the collection and reporting of animal data. It selects and consolidates specific information from the animal's medical record necessary for accurate weight management. The program's capabilities include a weekly automated alert listing animals due for case review, a search function for the easy retrieval of information, a graph for visualizing growth curves, a tracking system for alterations to feed rations, and auto calculates an ideal weight range. Prior to implementation, reviewing each animal's weight management information required an average of 9 minutes. This has been reduced to 4 minutes; an improvement of 56%. Staff can evaluate factors ranging from an animal's gender, age, and species, to their pregnancy status, recent location transfers, and assignment to protocols on a single screen. Clinical staff can readily assess all aspects of an animal's weight management needs while simultaneously improving accuracy and consistency between animals, thus saving valuable clinician time. This program is an efficient, intuitive, resourceful tool for reducing the data management effort necessary for the attainment and maintenance of healthy body conditions among the animals in our nonhuman primate colony.

P75 The Bin System: How to Never Place an Order and Still Have Enough

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An ordering process for facility consumables must be reliable, efficient, accurate, and maintain an adequate inventory of items critical for operations. Consumables are single use items which are necessary to facility operations and may be directly associated, as are diets and bedding, or indirectly associated, as is the case for personal protective equipment, with welfare of the animals in our care. Facility procedures will either not be able to be performed (critical failure) or not be able to meet operational criteria (process critical failure) should the inventory of consumables not contain the correct items in sufficient quantities. Adoption of a six sigma DMAIC (define, measure, analyze, improve, and correct) methodology analysis of our existing ordering process resulted in the design of a modified two bin ordering system. The analysis included critical point to failure process outlines (fishbone diagrams), value added step analysis, sessions, and inventory usage data collection through the use of kanban cards. Invoices were analyzed over comparable time periods before and after the bin system implementation so that process control and expenditure data could be obtained. The bin system combines two existing just in time inventory strategies: kanban card systems; and two bin point of use systems. In the bin system, specialized storage bins are employed as multifunctional control elements. Bins are circulated through the ordering chain and function as order signals, transport containers, storage containers, and point of use distribution containers. The bin system has reduced the ordering process to three steps. Two of these steps are value added. On hand consumable Inventory has been reduced from a month's supply to a 2 week supply. The system is self-maintaining and self-regulating, and it has eliminated the potential for critical failure supply chain errors.

P77 Employing Best Practices for a Guinea Pig Health Surveillance SOP

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An increasing use of guinea pigs in our facility led us to reevaluate our guinea pig health monitoring standard operating procedure (SOP). While considering best practices, relevant guidelines, and standards, we reviewed methods for blood sampling in the guinea pig in addition to methodology for diagnostic testing. In our review, we noted a lack of information regarding optimal minimally invasive blood sampling techniques in the guinea pig. As part of our evaluation, we compared techniques for blood collection from the

lateral saphenous vein to blood collection from the marginal ear vein. We compared ease of blood collection (success rate and time taken for a single sample) as well as amount of animal handling, restraint, and supplies required to perform the technique. After performing multiple trials, we determined that use of the marginal ear vein was superior to the use of the saphenous vein for our purposes. Sampling from the marginal ear vein resulted in less stress associated with handling and restraint, required fewer supplies, and could be performed more quickly than sampling from the saphenous vein. Sampling from the marginal ear vein was also less technically demanding resulting in higher success rates. In concert with recent changes to our rat and mouse heath surveillance program, we chose to adopt a commercially available dried blood spot technology for our guinea pig colony. The dried blood spot technology utilizes a single drop of whole blood thereby eliminating the need to anaesthetize or euthanize animals and decreasing the time required to gather and prepare samples. It has also significantly decreased costs associated with shipping our health surveillance samples. In conclusion, we established a new SOP for health surveillance of our guinea pig colony. By adopting a minimally invasive blood sampling technique (marginal ear vein) and implementing dried blood spot technology testing, our new SOP incorporates the principles of refinement and reduction and also represents significant advantages for our animal care program in terms of savings in labor and materials.

P78 How Do You Know Which IVC System Best Meets Your Needs?

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Our university animal care department was provided with funding to purchase new rodent caging and we decided to transition our rat caging to individually ventilated caging (IVC). There are many benefits of IVCs to personnel, animals and facilities as a whole. Benefits of IVC are; higher levels of biocontainment, reduced exposure to laboratory animal allergens (LAA), increases cage capacity without building renovations, and decreases in operational costs. As we have not used this type of caging for rats, we wanted to conduct comparisons for different IVC systems. In an ideal situation, we would conduct a side-by-side comparison of all systems but, we were only able to obtain one caging system at a time. Prior to evaluating the systems, a team consisting of cagewash, maintenance, and husbandry personnel created a scoring matrix encompassing factors from cagewashing; animal care personnel, and research staff working in the rooms and with the caging; animal behavior. This would allow us to evaluate each IVC using the same parameters. The team came up with five evaluation sections: animal environment, husbandry care, maintenance, procurement and other factors. Each section was further broken down into specific areas that were rated from 0–10. Systems were tested for a 1 month period and scoring was completed at the end of each trial. Using the matrix, we were able to do a side-by-side comparison of each system to assist us in making our final decision. We believe that by using this scoring matrix, we were able to devote the time needed to evaluate each IVC system on its own merit; allowing us to pick the IVC system that best fit our needs.

P79 A Unique Variation on Providing Enrichment to Research Dogs

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The *Guide* states that environmental enrichment must enhance animal wellbeing by providing animals with sensory and motor stimulation, through structures and resources that facilitate the expression of species-typical behaviors and promote psychologic wellbeing through physical exercise, manipulative activities, and cognitive challenges. While it may be normal procedure to provide chew toys, beds, and shelves inside a kennel or cage area, it is harder to meet their species-specific requirement of exercise and play. Due to our mild weather and space available, the decision was made to close off a grass area between 2 buildings that contain our kennels and create an exercise yard. Custom made fencing was installed to match the ends of the building to provide an exercise area. Ramps were designed to fit over drainage areas and corners, for the safety of our staff and the dogs. The dogs were trained to exit and enter through one doorway in each building that led out to the exercise area. The area was provided with toys to help with socialization and play. The exercise area was big enough that a group of 6-8 beagles could be let out at one time. All dogs, either in pairs or groups, were given access to the yard except on rainy days or when the exercise area was compromised and a potential health risk was involved. This summer, when it gets very hot, shade areas and kiddie pools will be put out to prevent heat exhaustion. The area is also big enough for dogs of all sizes to run around, dig, and play. This area has exceeded our expectations and made all of our dogs very happy and healthy.

P80 A Novel Approach to Training Parenteral Injections and Oral Gavage in Mice

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Accurate dosing technique during in vivo research is vital to the welfare of the research subject, the quality of the data, and the integrity of the study being performed. Thorough training is essential for technicians to become competent at each technique, thereby reducing the number of mice improperly dosed on studies. When training technologists to perform subcutaneous, intraperitoneal, per os (oral gavage), and intravenous injections, sterile saline is often used as it is a benign solution. Unfortunately, sterile saline is difficult to visualize once administered and poses a challenge for both a trainer and trainee to check the accuracy of the procedure. Inadvertent esophageal perforation from oral gavage dosing, for instance, may not present with clinical symptoms for up to 24–48 hours, far too long to provide useful feedback. Furthermore, it is hard to assess the depth of a subcutaneous or intraperitoneal dose with colorless saline. A simple and effective method to provide clear, visible feedback on each technique utilizes human-grade food coloring for injection training in mice. Concentrated dye provides instantaneous feedback for intravenous dosing. Following humane euthanasia, the accuracy of intraperitoneal, subcutaneous, and oral gavage dosing is easily visible at 1-10% dilution in sterile saline. However, higher concentrations can lead to difficulty assessing the dose volume in a syringe. This visible feedback improves the productivity of practice and the trainer's confidence in assessing a technician's new skills. In addition, the use of food coloring can eliminate some of the technician's stress of learning a new technique by adding an element of enjoyment. For example, correct intravenous dosing using 100µL of concentrated food dye will temporarily change a mouse's skin color. No adverse clinical signs have been noted from using either concentrated or dilute dye when administered at standard dose volumes. Within 24 hours, all food coloring will be eliminated via urine and feces. Therefore, using food coloring as a training aide is an inexpensive, straightforward, and humane way to refine and assess correct dosing technique and to decrease risk of inaccuracy in the future.

P81 Preventing Process Drift While Improving Animal Welfare: Impact of Procedures Improvement in a Cat Colony

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Process drift occurs when a procedure becomes altered as it is passed down through laboratory personnel. Reviewing and changing

procedures can significantly improve processes, animal welfare, and provide quality benefits. Quality benefits include reducing injury to staff members, increasing efficiency, and reducing stress. Variations between staff members were recently identified with regards to blood collection techniques within our feline colony. We realized the importance of continuing to improve our procedures for the welfare of the animals, as well as preventing process drift amongst personnel. Our goal was to allow for collection of multiple samples in a timely manner without causing undo stress to the animals, while improving efficiency. To reach our goal, we had to evaluate and improve our procedures. We started by identifying what techniques were being used, where the variation occurred, and specific causes of stress within the cat colony. Our next step was planning, creating, and defining our new procedures. Planning the new procedures included the following: training/assessment of staff, reducing animal stressors, as well as procedure and husbandry modifications. The results from continual improvement of this procedure have been beyond expectations. The time spent on blood collection was reduced by approximately 50%. It was also discovered that simple, minor improvements to our procedures also influenced better behavior from our cat colony, and lead to a decrease in injury to our staff members. The behavior of our cat colony and stress level has improved substantially. We have also had an unexpected improvement in technical conduct confidence and morale among staff members.

P82 Using Security Video to Monitor Research Facility PPE Compliance

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Proper protective equipment (PPE) compliance is a common issue managers of research facilities monitor on a daily basis. During a research facility visit outside of normal business hours (off hours), the director noted that a member of the lab staff was not wearing a lab coat. This incident raised the question of how compliant personnel are with the PPE requirements during off hours and whether this posed a biosecurity risk. To address this concern, we reviewed video from the security cameras located within the hallways of the animal facility and the facility access records derived from the security entrance to the animal housing area. The access records indicate the time of entry of personnel. The entry times were reviewed along with the video to determine how many people entered and how many donned the required PPE prior to entering the animal holding rooms. These reviews were done during two different 2-week periods to include normal business hours, after hours when care staff is not present, and weekends when there is a limited number of animal care staff working. After the first 2-week review period, 93% of people entering the animal holding areas donned appropriate PPE. During the second 2-week review period, the compliance rate was 94%. An allowance of ± 2% is estimated for people who did not initially use proper PPE but who had dedicated PPE in the rooms upon room entrance. The combined use of facility security cameras and access records were useful tools to track facility PPE compliance rates during off hours.

P83 Establishing a Successful Program for Retirement and Rehoming of Research Animals

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Animals in research have significantly contributed to the advancement of science. It is our responsibility as an industry, to promote the retiring of animals from the laboratory whenever possible. Rehoming animals can be a challenge for most facilities but in our experience, there are more benefits than challenges, and establishing a retirement program is an obtainable goal. Many facilities have had limited success with establishing a program due to reservations including; confidentiality concerns, animal adaptation to the environment outside of the laboratory, and especially funding. Nonhuman primates pose the greatest challenge as their rehoming options are especially limited. Steps for establishing a retirement program include an internal written procedure, screening of adoption organizations; implementation of legal protections, and creating a confidentiality contract and release of liability. Over 300 animals have been rehomed through one company's program. Before relinquishing the idea of implementing a program, consider the 3 original Rs, and add 3 new Rs; retirement, rehoming, and responsibility. Establishing a program can reduce the total number of animals euthanized and boost employee morale. Now is the time for the industry to give back to these animals that have been such a vital part of science.

P84 Evaluation of Laundered Versus Disposable PPE

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All animal facilities require some level of personal protective equipment (PPE) to be worn. Because our facility is located in California, where water, energy, and land (including landfill) costs are at a premium, we wanted to review our PPE in terms of both cost effectiveness and environmental impact. In 2014 we did a comparison of disposable versus laundered lab coats and coveralls. Our evaluation took into consideration standard PPE, which everyone wore when entering our facility, as well as added PPE worn by individuals entering our BSL2 areas. All waste that comes out of BSL2 rooms is considered biohazardous and has to be disposed of in that manner. In working with EH&S, comparative medicine analyzed supply chain, direct costs, and the environmental impacts of both laundering garments and sending disposable garments as BSL2 disposal. After thorough consideration, we chose to purchase laundered lab coats and coveralls rather than use disposable PPE.

P85 Creation and Application of Rodent Cage FLAGS

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Breeding colony management for biomedical rodents maintained within a large academic institution requires constant oversight by personnel. Within our animal housing areas, handwritten sticky note messages were often the sole means of communication between animal care staff and researchers regarding pups found, wean dates, and overcrowding. Due to potential for misinterpretation of handwriting, and potential for notes to be removed from or fall off the specific cage, a collaborative departmental team created a rodent breeding management solution that simplifies communication, saves time, and is securely attached to the cage. This color-coded card system, called "For Labeling Animals–Guided Shorthand" or FLAGS, entails a preprinted message tag (1x2 inches) affixed to the cage card by paperclip for animal care staff and research staff to correspond on cage needs. The FLAGS system was piloted in certain animal rooms known to have high volume breeding colonies and potential management issues. For the launch of the FLAGS to the research community at our institution, we used web-based training, reference documents, instructional signage, and informed researchers during user facility group meetings. Animal housing areas have been stocked with storage containers to hold the colored tags, and the low cost materials used allow for single-use FLAGS application to minimize risk of potential cross-contamination from tag reuse between housing rooms. The positive outcomes have been that these disposable tags are minimal in cost, cage needs are made clear, and there is consistent information system disseminated across campus; in addition, the creation of this unique FLAGS system has been one of our most rewarding internal projects due to the departmental and cross-institutional collaboration partnership toward a consistent

communication solution.

P86 Quantifying Environmental Contamination from Feed in Farming-Style Housing for American Mink (*Neovison vison*)

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The American mink, *Neovison vison*, is a unique animal model in toxicology and biomedical research. Industry guidelines suggest that mink housing units be cleaned as needed through washing with an appropriate detergent. In our setting, mink are housed in galvanized mesh cages with wire bottom floors and solid-bottomed nest boxes for whelping. Mink are fed a balanced raw protein diet once daily that is mixed and refrigerated weekly in sealed feed bins. Uneaten feed from previous day is scrapped daily before new feed is placed on cage top. Cleaning involves power washing of cages every 6 months, disinfection of nest boxes prior to whelping, and quarterly removal of manure under cages. The purpose of this study was to assess environmental contamination of the cages at the level of feed placement in an outdoor mink facility. Environmental testing was conducted within the facility at 2 seasonal time points. Cages were defined as occupied, previously occupied, and never before used. Luciferase testing demonstrated the presence of ATP on all cage tops, as would be expected given the placement of feed on the tops, the constant exposure of all tops to the animals within the cages, and the outside environment through natural air flow. For CFU, the highest counts were noted in 2 occupied and 2 never before used cages. Most cages demonstrated a decrease in CFU counts over time. When present, bacterial growth ranged from rare to moderate on cage tops. The majorities of isolated bacterial species were commensal organisms of mink or other animal species from which the diet originates and are unlikely to cause disease in animals or humans. By assessing performance-based standards of clinical health of the animals, we feel that the cumulative data represent acceptable low levels of environmental contamination associated with feeding of mink in outdoor research housing facilities.

P87 A Scripted Approach to Improve Rodent Procedural Record-keeping Compliance

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Medical records are a key element of the veterinary care program and are considered critical for documenting animal wellbeing. All those involved in animal care and use must comply with federal laws and regulations regarding human and veterinary drugs, to include drug records, treatments, and storage. The Institutional Animal Care and Use Committee (IACUC) semiannual inspections and post approval reviews have identified chronic problems with maintenance of post procedural records as one of the top 5 items of noncompliance in research laboratories at our institution since 2008. The institution's first organizational-wide effort to resolve the non-USDA rodent recordkeeping concerns occurred in 2012 when the IACUC required an online module (*VU ACUP Recordkeeping: Non-USDA Rodent)* to be completed by all researchers and staff prior to protocol approval. The module highlighted regulatory requirements and basic principles of recordkeeping. The IACUC and regulatory team dedicated more attention on records during semiannual inspections and post approval monitoring visits and noted a significant increase in noncompliance issues involving a failure to record anesthesia or postprocedural analgesic administration as described in the IACUC-approved animal protocol. The Office of Laboratory Animal Welfare (OLAW) at NIH directed the IACUC to develop a systemic strategy to reduce the number of reported recordkeeping violations related to anesthesia or analgesic administration. A "Rodent Procedure Record" institutional outreach was implemented in 2014 to effect transformative change where the institution used a scripted approach, or research method, as explained in Heath & Heath's (2010) book, "Switch: How to Change Things When Change is Hard". Since the implementation of the "Rodent Procedure Record" outreach in October 2014, over 700 researchers representing 161 laboratories have been visited. The January 2015 semiannual inspections resulted in zero surgical and/or postoperative/procedural care recordkeeping deficiencies for non-USDA rodent users trained during the 2014 outreach.

P88 Quantifying *Xenopus laevis* Swimming Behavior through Infrared Technology

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Xenopus laevis has emerged as a preeminent amphibian animal model in fields of vertebrate cell and developmental biology and more recently oncology as well as drug discoveries. Although we have a good understanding of their laboratory husbandry and care conditions, we still need to better elucidate *Xenopus* behavior in captivity if we wish to use their behavior as a research paradigm. Using infrared technology, we tracked a mature female's swimming distance and water column preference from 16:00 in afternoon to 10:00 the following morning within a 10 gallon aquarium. Results showed that the female swam 259 horizontal meters in 18 hours; 16.4 meters per hour during the 12 hour dark phase and 10.3 meters per hour during the 6 hour light phase with 93% and 7% of time on water column bottom and top, respectively. We expanded this investigation by tracking mature females' swimming distance over 200 hours within converted standard polycarbonate rat cages. We provided husbandry (feeding and physically moving frogs to cage with clean water) at hour 135. For the first 135 hours, frogs again demonstrated higher nocturnal activity with an average of 86.8 meters per hour during dark phase and 36.8 meters per hour during room's light phase. Interestingly, dark and light phase swimming distance plummeted dramatically to 23.1 meters per hour and 12.1 meters per hour, respectively, for the final 83 hours after physical manipulation (husbandry service). Results clearly demonstrate this specie's nocturnal nature in the laboratory and its propensity to "lay low" after physical handling. Although we've gained additional insights into *Xenopus* frogs' swimming behavior, one should exercise caution when using this paradigm if the study involves frog handling.

P89 Affectionate, Loyal, and Loving: Adopter Survey of Rehomed Laboratory Dogs

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Former research beagles are a vital teaching resource in veterinary medicine and veterinary technology schools. Purpose-bred dogs join our teaching colony after use in noninvasive research projects. Dogs arrive at 1–3 years of age, and remain for 2–3 years before adoption to private homes. Dogs are fed in the morning and turned out 45 minutes later for 30–45 minutes of group exercise. To accustom dogs to home life, students, faculty and staff take the dogs on leash walks most days and provide basic obedience training. The dog walking program is mandatory for first year veterinary students. We surveyed 48 owners of 49 beagles adopted since 2004 to determine owner satisfaction levels and behavioral issue persistance to determine how our program could better prepare beagles for home environments. We received completed surveys for 31 dogs, a 64.6% response. The most persistent problem was house soiling, 22.6% of owners reported their beagles urinated indoors and 16.1% defecated indoors. Accidents occurred at least weekly for 19.4% of owners and monthly for 16.1% of owners. Indoor continence occurred sooner for crate-

trained dogs. Of the 51.6% of adopters who crate trained their beagles, 75% were house-trained within 6 months or less. Of the 48.4% who did not crate train, 47% were house-trained within 6 months or less. Other behavioral concerns include fear of loud noises (66.7%), reluctance to walk on a leash (32.3%), and open spaces (16.1%). All behaviors improved over time. Barking was not a concern for most owners; 67.7% reported their beagles never or very rarely barked. Aggressive behavior was rare (6.5%) with 1 dog being aggressive towards cats and 1 to people with a "scary toy." When asked for 2 positive outcomes of adopting a beagle, an overwhelming majority (87.1%) reported their dogs were affectionate, loyal, and loving. Reported behavioral problems were manageable and likely reflect results of early conditioning. House-training was the most common challenge (58.1%). These data demonstrate the feasibility and animal welfare benefits of rehoming former laboratory dogs. Based on these results, we believe early introduction of leash walking and obedience training as well as a routine schedule will facilitate the transition to home life.

P90 Strategies for Successfully Social Housing Incompatible Cynomolgus Macaque Triads

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At our company we are committed to ensuring all animals have the highest level of care and welfare. At our facility, over 70% of our studies require primates to be housed in groups of 3 (or triads). While we have a near 100% social housing success with the majority of our cynomolgus macaques (*Macaca fascicularis*), aggressive behavior can occur within the triads, leaving 1 or more animals singly housed. If the social unit breaks down midstudy, regrouping is rarely possible due to study design constraints. We adopted a round-robin system within the triad to facilitate continued social housing and minimize single housing. This social housing schedule has been successfully implemented in all of the triads in which it has been attempted (n = 8 triads). To maximize success, the behavior staff identifies the animal who is most compatible with the other animals in the triad and that animal spends 3–4 days fully socially housed with 1 partner and then switches partners. Meantime, the less compatible partner remains at a reduced access level (for example, grooming access) on days when they are not fully socialized. We have found this minimizes aggression and injuries within the triad and leads to greater social success. While this social housing paradigm represents only a small percentage (0.7%) of our total socially housed population, we have found it to be a useful tool in keeping animals socially housed thus meeting their behavioral needs. This system eliminated the need for a singly housed animal to have a behavioral exemption due to incompatibility, and has improved welfare for many animals in our facility.

P91 Using Performance Standards to Promote Socialization on Chronic Rat Studies

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For years animal research facilities have followed the recommended minimum space guidelines for commonly used laboratory rodents housed in groups. However, transitioning from wire bottom to solid bottom caging for various organizations and clients, coupled with a variable growth rate of a diverse range of rodent strains (and particularly amongst different suppliers), can challenge resources to universally accommodate all scenarios. To strictly adhere to recommended space allocations on long term studies, we initially housed 2 rats per solid bottom cage. However, with normal unscheduled rodent mortality, many studies resulted in singly housed animals. Hence, the conundrum is whether it is more beneficial to the rodents to follow the strict spacing guidelines or use performance standards to determine the number of animals housed together and promote socialization whenever possible. To assess the benefits and risks when considering performance based standards, a number of criteria were used to examine large rats (greater than 500 grams) specifically. First, body weight data, used as a primary health indicator, was collected and compared on large, commonly used rats from studies up to approximately 120 days in duration, ranging from 1/cage to 5/cage. Additionally, large, naïve rats were cohoused 3/cage and 4/cage in housing slightly smaller than the recommended guidelines, and video footage was recorded to monitor the behaviors and general observations over an extended period of time without human presence. Review of the data showed that body weights were higher when fewer animals were housed together, but the rats showed the same relative growth curve regardless of the number of animals per cage, and the video documentation demonstrated normal social interactions and space utilization with no adverse behaviors or observations detrimental to animal health. Therefore, housing more than 2 rats per cage (n = 3 or greater) is recommended to reduce the chance of singly housed animals and help ensure socialization whenever possible by taking performance standards into consideration.

P92 A Six Sigma Approach to Resolving Customer Concerns about Guinea Pig Water Bottle Levels

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Refilling guinea pig water bottles is a time consuming, ergonomically taxing task. Researchers at our facility have expressed concerns in the past about guinea pigs emptying their water bottles overnight and in response we implemented full refills once or twice a day, and added a second bottle to many cages. This did not, however, alleviate the occasional concerns being expressed, nor prevent water bottles from being found empty, and caused an increase in staff workload. We evaluated this problem using the Six Sigma DMAIC process (Define, Measure, Analyze, Improve, Control) with the expectation of being able to implement changes to improve customer satisfaction and staff efficiency. We defined that guinea pig water bottle refills were a point of customer dissatisfaction, and established a Six Sigma team including management, the training coordinator, and animal care technicians. A measurement system analysis was used to determine how accurately our staff can judge the volume of a partially full water bottle and found it was a difficult judgement call that would require both training and mistake proofing. We analyzed our processes and determined that our staff was wasting a significant amount of time and resources refilling bottles that contained more than enough water to last until the following day. We selected and implemented our improvement strategies which included identifying a defined point at which water bottles should be refilled including mistake proofing with visual markers and conducted staff training. A repeat of our measurement system analysis was performed to determine how well staff was identifying water levels after training. We controlled the process by communicating with investigators the daily water needs, requirements for refilling bottles, and evaluating guinea pig hydration. The outcome of this project was very positive as we resolved the customer concerns, and significantly improved the efficiency of the animal care staff.

P93 Effect of Nesting Material on Aggression in Diet-Induced Obese C57BL/6 Male Mice

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C57BL/6 (B6) male mice have been observed to display conspecific aggression. This aggression necessitates separation and individual housing to prevent serious injury, eliminating the ability for mice to be social housed and consuming valuable space and caging resourc-

es. For diabetes and obesity studies, male B6 mice are routinely used for efficacy studies. Mice are received at 6–8 weeks of age and fed a high fat diet for 10–12 weeks. Due to aggressive behavior many mice are separated after several weeks of housing to reduce injury and stress on the mice. If a strategy could be applied that could reduce or eliminate this conspecific aggression, mice could be socially housed, providing them with social conspecifics, as well as saving on space, caging, and labor resources. We tested 3 different types of cage enrichment strategies on male B6 mice from 4 different vendors fed high fat diets to determine if enrichment and/or vendor strains had an effect on aggressive behavior. The mice were examined and weighed weekly for 12 weeks. At the end of the study the mice were euthanized and the pelt removed for scoring using the PALS scoring method. Based on the observations, the vendor strain played a role in aggressive behavior. However, there was no significant reduction in aggressive behavior from the different enrichment strategies tested.

P94 If You Measure it, They Will Come To Work: Transparent Tracking of Staff Attendance Reduces Unexpected Absences

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From 2011–2014, 3 facilities within the our hospital's comparative medicine department, had on average 18 "call outs" a month from ~38 total staff members. Call outs are the notification by a staff member on the morning of a scheduled shift that they will not report to work. Their impact include: 1) uneven workflow throughout that day as well as the rest of the week; 2) the inability to meet just-in-time (JIT) requests; and 3) increased stress levels for the staff who did report to work. A spike of 30 call outs in December 2011 prompted a deep dive into the attendance management process. Compliance with the department's attendance policy was reviewed and the data reporting system that was used for tracking attendance was also reviewed. At that time, facility managers tracked overall staff attendance over an entire month recording the number of people present for work each day, but did not have a standard process for tracking call outs by specific staff members. A second spreadsheet was added which focused on call out dates specific to each staff member as well as highlighting the corrective action time point and actions taken. This sheet allowed the managers to see the call outs by individual staff members over the 12 month period. The spreadsheets were stored in a shared folder which increased accountability as other members of management were able to see if corrective action was being delivered consistently. By the end of FY14, the campus averaged ~10 call outs a month, which was a 45% improvement over the FY11 results.

P95 Suture Boards for Training Surgery Students

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In August 2011 the IACUC at our university mandated that a training program be implemented for all researchers performing survival surgery on any vertebrate species. As part of this training, a suture board was developed that could be economically produced, sturdy enough to last for months of repeated practice, and mimic features that the students would encounter prior to practice on a live animal. The boards contain suture, needle drivers and forceps, in addition to pictures of a proper square knot and suture placement. The boards use a nylon backed neoprene material featuring precut "incisions" to be closed, and an "artery/vein" to tie off. These are used during in class training as well as checked out for 2 weeks by the students to facilitate practice prior to the second surgery class involving a live anesthetized animal. The boards have proven to be a useful tool to teach suturing and instrument handling in the surgery classes at the university.

P96 Establishing Proficiency in Retro-Orbital Injections and Bleeds in Mice

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In August of 2010, the Animal Use Training Program at the university reevaluated the criteria for competency and certification of retro-orbital eye bleed and injection in rodents. Competency evaluation prior to this time consisted of observing an individual's technique and visually examining the eye and orbit following the procedure. The animal was then euthanized before it regained consciousness. If the individual performed the procedure smoothly, with no apparent damage to the eye or tissues around the eye, the person was certified. Because of observed complications in animals that had procedures performed by certified researchers, it was decided to change the certification process. The first change was to require an individual to perform the procedure perfectly 3 consecutive times with no apparent damage to the eye or surrounding structures. The second change was to examine the 3 consecutive mice that had had the procedure the day of the procedure and 48–72 hours later. If damage was observed, the individual was not certified. The results of these certification events were maintained in order to determine the failure rate of individuals that were perceived to have perfectly performed the technique when watched by the trainer (3 different trainers participated), but actually caused damage. Certification of individuals performing 3 different techniques were tracked. Orbital bleeds on awake mice had the highest failure rate (13/61, 21.3%), while orbital bleeds on anesthetized mice a lower failure rate (25/196, 12.8%). Orbital injections on anesthetized mice had the lowest failure rate (7/119, 5.9%). These data indicate that for these techniques, competence cannot be determined strictly by observation, but rather a delayed outcome measurement is needed to determine competency in all cases.

P97 Detection of *Pneumocystis* by Environmental Air Dust Testing of IVC Rack Plenums

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Environmental air dust (EAD) testing is emerging as a sensitive method to detect pathogens and can enhance the detection of pathogens not readily transferred via dirty bedding. *Pneumocystis* is a fungus that causes chronic pneumonia in immunocompromised mice, is not detected consistently by dirty bedding sentinels, and is generally diagnosed via postmortem analysis. While investigating PCR of EAD as an addition to our rodent sentinel program, rack exhaust plenum dust was submitted for PCR testing of rodent pathogens. Via the EAD PCR method, *Pneumocystis* was detected on one IVC rack within the housing facility and was placed on quarantine. To assess if *Pneumocystis* is reliably detected via PCR of EAD, all cages on the quarantined rack were placed on a freshly cleaned, *Pneumocystis* negative rack with new sentinel mice. Monthly EAD samples of vertical and horizontal plenums were submitted for 6 months. Within 1 month, *Pneumocystis* was detected via vertical pooled plenum EAD PCR testing, and one individual row was positive via EAD PCR testing. *Pneumocystis* was detected within 2 months on a second row via horizontal plenum testing. The rack and positive rows remained positive for the duration of the 6 month study. PCR of oral swabs collected from mice on positive rows, and GMS staining of lung sections from mice displaying clinical signs of pneumonia were negative for *Pneumocystis* at all collection time points. Positive EAD PCR results were confirmed positive via PCR

testing of pooled lung tissue from 10 mice on each positive row at the end of the 6 months. Further, PCR testing of lung tissue from dirty bedding sentinels did not detect *Pneumocystis*. This study demonstrates the ineffectiveness of dirty bedding sentinels in detecting *Pneumocystis* and suggests that EAD PCR testing can be utilized for surveillance of *Pneumocystis* in mouse colonies housed on IVC racks.

P98 A Quick Assessment Testing Method to Assess Rodent Cage Ammonia Levels

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Excess cage ammonia, if inhaled, can cause respiratory mucosal irritation and cause laboratory animals to be unsuitable for research. The objective here was to develop an easy performance-based approach to detect adverse ammonia levels that could have broad application in research, training, testing, and biocontainment situations. We hypothesized that premoistened commercial water ammonia test strips designed for fish could be adapted to provide a quick assessment of ammonia levels in individual rodent cages. The time for a complete test strip color change was measured at different defined cage ammonia concentrations in vitro. Observations were also made on 5 replicate static mouse cages holding 3 adult mice each, and measured daily for 2 weeks by test strip and by independent analyzer detection. A standard curve (x = actual ammonia ppm, y = time to strip maximum color change in sec) was fitted to a negative exponential function ($y = 116.61x^{-0.837}$) with an $R^2 = 0.9527$. Time to complete color change of 10–15 sec represented 10–20 ppm, < 10 sec represented ≥ 20 ppm, and < 5 sec represented ≥ 40 ppm ammonia. Observations from replicate mouse cages monitored daily for 2 weeks, as confirmed by both methods, indicated 3 cages out of 5 went from levels < 5 ppm to over 20 ppm by 10 days, and 2 cages exceeded 20 ppm at 8 days. Our results indicated use of water test strips are convenient and reliable for quick assessment testing for ammonia under a performance standard.

P100 Developing a Quality Assurance Program for Cagewash Equipment

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Our institute manages aging cagewash facilities using rack washers, tunnel washers, autoclaves, and bottle filler stations. Although many have built in mechanisms such as temperature displays and fail-safe alarms, they were not always accurate and we continually experienced increased breakdowns, which impacted performance of cagewash tasks. A lack of sustainability led us to reevaluate processes to ensure caging materials were processed efficiently. Collaborating with our facility service and environmental health and safety departments, we developed a comprehensive quality assurance (QA) program to implement routine, simple, cost-effective and reliable processes to validate our operations and prevent disruptive mechanical failures. We assessed the value and role of temperature sensitive tape, microbiological monitoring with ATP luminometers, chemical titrations, sterilizer control tubes, and biologic spore ampules. Daily temperature tape was the first line of verification in washers to confirm temperature reached 180°F during the rinse cycle. We performed periodic titrations to ensure correct chemical concentrations. Microbiological ATP testing was found to be rapid, cost effective, and accurate and replaced traditional CONTACT PLATES assays. Autoclave loads were processed with steam indicator tape to assure thorough sterilization. Sterilizer chemical tubes were used to further verify sterility for all autoclave cycles. Biologic spore ampules were added to biologic waste cycles to maintain compliance with regulations. We also increased preventative maintenance with our service provider to help prevent equipment failure before it occurred and implemented an electronic tracking system to monitor repairs. By implementing these multifactorial assessments in our QA program, we have been successful in rapidly identifying equipment malfunctions and addressing them early to ensure continued high-level performance. The comprehensive program helped cut costs on unplanned maintenance repair, maintained desired set points, provided a steady source of clean caging supplies and materials for animal housing environments, and maintained compliance with EPA/local requirements.

P101 Physiologic Effects of Common Procedures in Male C57BL/6 Mice

GLAS: Yes
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In day to day activities mice are exposed to a variety of experimental and husbandry procedures. We examined the impact of each of these standard husbandry procedures on the physiologic parameters of mice. We hypothesized that mice would return to baseline physiologic status within 30 minutes of cage change, in house transportation, weighing, or scruffing. In addition, we also hypothesized that mice in the same room as other mice being euthanized would not have any alterations in their physiologic status. To examine the effect of the common procedures, 16 week old male C57BL/6 mice were implanted with telemetry probes that measure heart rate, blood pressure, and activity. Mice were tested at least 7 days after implantation to allow the mice to recover from the telemetry implant surgery. Prior to testing there was no activity in the room for at least 30 minutes and all mice were resting in their nest at the beginning of all procedures. As expected, all procedures led to a significant increase in heart rate, blood pressure, and activity ($P < 0.05$), except for observation of euthanasia ($P > 0.38$). The physiologic values for carrying the mice or pushing the mice down the hall on a cart while in a cage, scruffing a mouse, and weighing a mouse all returned to baseline within 30 minutes. There were no significant differences between the physiologic values of a male and female scruffing a mouse. Changing the cage led to a prolonged increase in values for over 60 minutes. In summary, mice showed no physiologic response to other mice being euthanized in the room suggesting they are not a sensitive species. Mice return to baseline following standard procedures in 30 minutes except for when they are moved to a new cage.

P102 Comparison of Nesting Material Use by Female C57BL/6 Mice

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The *Guide for the Care and Use of Laboratory Animals* provides recommendations on mouse husbandry. Two key factors for oversight of good animal welfare are the need to visually ensure animal health every day and to provide an environment that has enrichment. It has been previously established that nesting material is an acceptable form of enrichment for laboratory mice. A common complication of using nest material is the inability to visualize mice when they are in the nest. To provide both an enrichment and to allow visualization of the mice, we hypothesized that clear cellophane shredded nesting material would be a suitable replacement for the normal white shredded paper nesting material. To evaluate this, 6 pairs of 5 week old female C57BL/6 mice were examined for 9 weeks. Their ability to make a quality nest, whether they used the nest, body weight gains, and the technicians ability to see the mouse in the nest were evaluated. Using the naturalistic nest scoring system we showed no difference in the nest quality between white paper and clear cellophane nests (average nest score was 2.73± 1.08 and

2.73±0.87 respectively). In addition, there were no differences in body weight gain or use of the nest. There was a significant improvement in the ability to see the mice in the clear cellophane nest compared with the white paper nest (97% versus 63%). We propose that using a clear cellophane shred for nesting material may be an alternative to white shredded paper.

P103 Dry Mist Hydrogen Peroxide Sterilization of an Automated Tattoo Machine for Use with Immunodeficient and Defined-Flora Mice

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Sterilization of equipment and supplies is a critical component of a biosecurity program to prevent entry and spread of adventitious infectious agents in research vivaria. Ethylene oxide gas, chlorine dioxide gas, and vaporized hydrogen peroxide (VHP) are commonly used to provide sterilization of equipment containing embedded electronics with integrated circuit boards. This study evaluated the effectiveness of a hydrogen peroxide dry mist system to sterilize an automated mouse tattoo system. To meet internal biosecurity requirements, the tattoo system required frequent sterilization for use on groups of defined-flora and immunodeficient mice. A semi-rigid isolator equipped with a portable blower was modified to allow connection to a customized dry mist hydrogen peroxid generating unit via a flexible hose and extended delivery nozzle. Three sterilization configurations of the tattoo unit were evaluated: 1) housing cover off/cooling fan off, 2) housing cover on/cooling fan off, and 3) housing cover on/cooling fan on. Sterility was evaluated using biologic indicator (BI) strips containing $1x10^6$ *Geobacillus stearothermophilus* spores placed inside the tattoo unit and on the exterior of the unit. Hydrogen peroxide mist was introduced into the isolator, and the isolator then sealed overnight. The BI strips were cultured for 7 days, and included positive controls. Initial exposure with the cover off/fan off resulted in complete kill of all BIs. Due to the impractical nature of removing the cover, this configuration was abandoned in further testing. Exposure with the cover on/fan off configuration failed to kill BI spores placed inside the tattoo unit. Exposure of the cover on/fan on configuration resulted in complete kill of all BIs for 5 exposure cycles. The tattoo unit functioned properly following all hydrogen peroxide exposures. This evaluation illustrated that dry mist hydrogen peroxide delivered within a flexible-film isolator provides effective disinfection and sterilization of an automated tattoo unit when the fan was active during the exposure period. Use of this process may also provide a simple method for sterilization of other electronic devices used on rodents that pose a risk for exposure to infectious agents.

P104 A Study of the Environmental Conditions of NSG Mice Housed for 14 Days in a Disposable Caging System

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Our company continually strives to optimize the housing conditions of mice to maximize animal health and performance while minimizing costs. Previous internal studies using CD1 mice in individually ventilated cages (IVCs) indicated that 70% of the cages maintained suitable environmental quality (ammonia (NH_3) <50 ppm) by day 14. Additionally, data identified the positive impact on cage environment and associated animal health conditions when nude mice were maintained in autoclaved caging systems. The question arose if disposable caging with irradiated bedding, feed, and water would show any significant change in the microenvironment over a 14 day time period. The NOD SCID gamma (NSG) strain was chosen for evaluation as they account for approximately 25% of all mice in the vivarium. Ninety NSG female mice (average weight 18.2 g) were housed, 5 per cage, in 18 cages symmetrically located on both sides of a double sided disposable caging rack. Ventilation was set at 70 air changes per hour (ACH). Irradiated bedding, feed, and water were provided for all cages. Ammonia levels were used as the main criteria of environmental quality during the 14 day test. Daily temperature, relative humidity, and luminosity levels were also measured within the microenvironment. Weight gains, feed and water disappearance, and bedding weight increases, averaged 10.5, 210, 230, and 106 g/cage, respectively, during the 14 day test. All 18 cages had 0 parts per million (ppm) of NH3 by day 14 of the test. Results indicate it is possible to extend cage change interval to 14 days with no adverse measurable change in caging conditions.

P105 A Study of the Environmental Conditions of NSG Mice Housed for 14 Days in a Reusable Caging System

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Our company continually strives to optimize the housing conditions of mice to improve current standards of health and performance, while creating cost efficiencies. Previous studies evaluated the effect of cage change interval on cage environmental conditions in both immunocompetent (CD1) and immunocompromised (nu/nu) mice in reusable individually ventilated cages (IVC). Quality of the cage environment, was acceptable as determined by ammonia levels (NH_3) <50 ppm. In previous studies with immunocompetent mice this level was reached in 7 days. Conversely, immunocompromised mice attained 8 ppm in only 1 out of 10 cages in a 14 day study. We have now investigated the effects of using sterile feed, sterile bedding, and automatic watering on the environmental quality of a 14 day cage change interval study with immunocompromised NOD SCID gamma (NSG) mice. Ninety female mice (average weight 17.8 g) were housed 5 per cage, in 18 cages symmetrically located on both sides of a double sided IVC rack. Ventilation was set at 75 air changes per hour (ACH). Ammonia levels were used as the main criteria of environmental quality. Daily temperature, relative humidity, and luminosity levels were also measured within the microenvironment. Animal weight gain, feed disappearance, and bedding weight increases averaged 4.5, 200 and 258 g/cage, respectively, during the 14 day test period. Only 28% of the cages developed detectable ammonia levels (≤14 parts per million (ppm)) by day 14. Under conditions of this study, cage change interval can be extended to 14 days with NSG mice housed in reusable IVC caging.

P106 A Multipurpose NHP Transfer and Weighing Unit

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Transfer units are routinely used to move monkeys into clean caging every 2 weeks. Depending on protocols and studies, monkeys are required to be weighed at least monthly, but sometimes as often as weekly. Our original process of weighing the monkeys required that each monkey be placed into a restraint chair and wheeled onto the scale individually. This transfer unit has incorporated scales so that monkeys can be weighed when they are changed over, thereby preventing the additional work required to take out and weigh each monkey individually in a chair. When staff need to weigh animals between changeovers, they have the animals jump into the unit to get weights and jump back to their home cage without having to place them into chairs and take them down the hallway to the floor scale one by one. These units increase efficiency, minimize risk, and improve animal welfare because they reduce the amount of handling required of the monkeys. An added benefit is that since they are made of clear polycarbonate resin rather than stainless steel, we are able to get a good look at the monkeys during changeovers. These units can also be used to easily perform a 360° veterinary evaluation

of the animals if there is a specific health concern or need for closer observation (potential lesion on animal's back, surgery site evaluation, etc.) without having to chair them. These units have been successfully used in BMS facilities.

P107 Unforeseen Enrichment Benefits of Innovative Shelters Built During a Weather Crisis for *Macaca mulatta* in a Captive Group Setting

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Indian rhesus macaques are found in a wide range of habitats, including a wide range of temperatures. Several breeding centers in the U.S. have indoor/outdoor corrals and field cages which house rhesus social groups. In South Carolina, until recently, the housed rhesus did not benefit from heated shelters, as the lower temperatures, even the extremes, fall within the ranges of their natural habitat. In 2014, extreme temperatures were forecasted and added shelters seemed necessary for the field cages. The facility decided to use horse hay ring feeders as an already made structure that could be capped with HDPE (high density polyethylene) sheets. Three of the four parts of the hay rings were joined together and placed sideways. We observed added benefits right away attracting juveniles (1 to 3 years old) that used the inner structure as "monkey bars" and the outer structures as slides. Time use of these structures declined over a week, but then stabilized and still were used by juveniles for the majority of their time at play. After a week, total play time was still increased in juveniles and adult females. It remained the same for infants and adult males. Aggressions resulting in traumas decreased. Shelter use has remained the same through the following 3 seasons and have shown to provide benefits far beyond simple winter shelters.

P108 Understanding the Laboratory Animal Workforce: Personnel Management Implications

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Understanding employee motivations and attitudes are key components of effective personnel management. In all industries, including the laboratory animal field, organizations strive to engage their employees to increase job performance and productivity. Presence of a calling has been related to increased work commitment and satisfaction. A calling can be described as a person's belief that she or he is called upon (by the needs of society, by a person's own inner potential, by God, by higher power, etc.) to do a particular kind of work. An online survey was constructed to gain an overall understanding of the current laboratory animal worker mindset. The survey was completed by more than 800 people comprised of research personnel, office staff, animal care staff, managers, and veterinarians. Three hypotheses were tested: individuals who indicate that their work is a calling would be more likely to 1) be older, 2) have more education, and 3) feel supported by their organization. Results of our survey indicate that having a calling is not related to age or level of education, but is related to job satisfaction and organizational support. Additionally, our results draw attention to a subset of employees. Presence of a calling is not related to job classification except among the laboratory animal technicians. Within this job category, facility managers, supervisors, animal health technicians or veterinary technicians, and senior laboratory animal technicians are more likely to have a calling than mid or entry level technicians. Similarly, presence of a calling is not related to salary except among the laboratory animal technicians where individuals with higher median salary are more likely to indicate that they are working in their calling. With the recent relative decline of available funding in our field, an engaged staff is essential to the continuity of work flow within an organization for continued growth and advancement of scientific discoveries. Our findings may identify areas that management could target to improve productivity and increase worker engagement and satisfaction.

P109 Intra-Articular Dosing Technique in Mice for the Delivery of Contrast Agents for MRI Imaging

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Magnetic resonance imaging or MRI has long been an established method to provide physicians and researchers a noninvasive method of obtaining detailed images of internal structures of the body, including organs and tissues. This is beneficial in the diagnosis and treatment of a wide range of diseases and injuries. To aid in the generation of the image, it is sometimes necessary to add contrasting agents to the patient to allow tracking of specific cells and their dispersal within the area of the body undergoing observation. At our institution, investigators studying the healing process of articular cartilage in mice required a technique to inject contrast agents into the intra-articular space. The ability to precisely inject contrast agents such as gadolinium, iron oxide, or methotrexate is necessary to yield images that show immune cells vital to the healing process in cartilage. Obtaining images of these cells through periodic MRIs provides an indepth look into the progression of the healing process. We describe the process to accurately perform intra-articular administration in the mouse. Also, a training process was developed that allows individuals learning this technique to determine the success and quality of their injections.

P110 Human Environmental Enrichment: A New Twist on Human-Animal Interactions

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New and inventive approaches to provide value-added environmental enrichment for nearly every species used in biomedical research have been discussed in numerous public forums. But what about environmental enrichment for those humans involved in the same research? Research has shown that focused attention on activities that contribute to healthier and more active lifestyles have a positive impact on how people feel about themselves and impact work performance. A new approach to using the human-animal bond was explored through a program called Canine Unwind. Through a partnership with a local humane society, adoptable dogs were allowed to visit our campus over lunch hours and interact with interested employees in a controlled setting. The program offered employees a new opportunity for physical activity and stress reduction and a sense of community between employees and partnering humane societies. Employees without experience with dogs attended training by C. Swaim, author of "Puppies for Dummies" and were partnered with experienced volunteers. Many volunteers were animal care personnel who facilitated the human-animal interactions. Adoptable dogs in the program met health requirements determined by the veterinary staff. Selection was also determined by behavior assessment to reduce the risk of negative behavior. Volunteers from the humane society transported dogs and assisted with initial introductions of the dogs to volunteers. In return, the program provided the humane society an opportunity to increase their exposure in the community and increase interest in adoptions and volunteerism. Although participation in Canine Unwind was initially limited by invitation only, the activity attracted employees who observed the activity and came out to learn more. Photographs captured smiles and collegial interactions as people and dogs walked around a designated path. After the event, an online survey collected feedback: 100% responding saying the program should continue, 74% wanted to volunteer for future events, and 85% cited stress reduction

as their favorite outcome. The Canine Unwind program provides human enrichment through animal interactions and encourages physical activity with the added advantage of stress relief and new personal interactions.

P111 How Mouse Infusion Led to Microsampling

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At our company, we are constantly looking for new ways to meet the needs of our clients and refine our current procedures and techniques. As is often the case, opportunities to improve processes present themselves when you least expect them. While we were honing our mouse tail catheter placement procedure, we realized it might be possible to repeatedly collect a clean and precisely measured amount of blood from the tail vein. We can now routinely collect blood samples varying from 10 µL up to the maximum blood volume allowed from mice via the lateral tail vein, without the need for anesthesia, or other limitations and risks associated with repeated retro-orbital blood collection. We can successfully use this microsampling technique not only in mice, but also in rats and are hoping to expand this technique to other species as well.

P112 Implementation of Tools to Increase Communication Surrounding the Housing Status of Nonhuman Primates

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Recent updates to the *Guide for the Care and Use of Laboratory Animals* and the Animal Welfare Act have elevated guidelines and expectations for social housing of nonhuman primates. The veterinary sciences team at our company constantly strives to identify innovative and efficient strategies for communicating the pair housing status of nonhuman primate macaques that minimize risk, reduce stress, mitigate animal welfare concerns, and maintain study integrity. The success of the most recently implemented strategy depends upon effective communication between the designers (vet staff) and the hands on implementation team (care staff). We present an overview of successes and challenges serving to highlight effective communication methods including, but not limited to, cage labeling devices, room schematics, and group face to face meetings.

P113 Infectious Aerosol Containment in a Mouse Running Wheel Caging System

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Innovative research projects may pose challenges in housing animal research model systems. Researchers on our campus studying the interaction between exercise and immunity required that mice be infected with a zoonotic virus and housed within a running wheel apparatus for an extended duration. By experimental design, mice required free access to cage running wheels attached to computer monitoring equipment. The challenge was to find a mechanism to safely contain the housing and potentially aerosol generating agent in an animal exercise environment under ABL2 conditions. The manufacturer for the running wheel cages did not offer a premade filter cage lid which could fit on top of the running wheel. Fabrication of these lids was possible, but cost prohibitive. In a collaborative effort between the division of animal resources and the division of research safety, a modified caging system was developed. The modified system allowed for the mouse running wheel cage to sit inside a secondary enclosure created from a modified rat cage with a microisolation top, which contained the entire running wheel apparatus. The process involved creating an extension of the rat cage with a second rat cage, nesting the 2 rat cages together to extend the cage height allowing free movement of the running wheel, an opening for the exit wires, and sealing the gaps. We have successfully implemented the use of these cages in this research application. We believe this simple, cost effective modification could have broader applicability to other institutions with research programs investigating this emerging area of the interaction between exercise and immunity.

P114 A Versatile, Economical, and Ergonomic Approach to Door Seal for Room Decontamination

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Biosecurity and the protection of the health status of rodent colonies is an essential component of a program of animal care and use. Specialized research equipment shared between researchers, and core facilities using equipment which cannot be moved or readily sanitized by traditional methods, present challenges in preventing the spread of contaminants. The university has selected a portable chlorine dioxide gas generator to decontaminate equipment and rooms—the process is effective and compatible with many materials (including electronics associated with a majority of imaging, behavioral, and telemetric equipment). The process of decontamination of a room and the equipment contained within that room involves creating a seal around all access points including the air ducts and doors. The room is maintained at a slightly negative pressure relative to the surrounding areas to prevent the potential escape of dangerous gas. Facilities which have not been designed or retrofitted with special door seals and ports place tubing under the existing door and seal the door with tape or custom rigid door plates taped around the door. Our facilities would require multiple door plates due to different door sizes and we wanted to investigate alternatives. Vinyl shower curtain liners are readily available and an inexpensive flexible material we found to use in place of a rigid door plate. They can be easily transported and rapidly cut to fit different door sizes and are easier to use. When sealed around the door the relative pressurization of the room is evident (by the movement of the material) and provides a continuous visual monitoring of the room pressurization as an additional safety measure. We have successfully used these inexpensive liners on multiple types of doors and found them easy to use and adapt to multiple configurations.

P115 Venous Catheters in Guinea Pigs for Longitudinal Studies

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In starting a study that involved positron emission tomography (microPET) and computed tomography (CT) imaging in guinea pigs, both of which would require an IV catheter, work had to be done on how to do this successfully. Online research was somewhat helpful but the guinea pig itself provided more direction. Most information suggested a cardiac puncture, but since a reliable bolus infusion of contrast was needed, this was not an option. The most promising paper found online suggested using a 22 or 24g in the lateral foot vein, a 24g catheter was able to be placed twice in 100's of catheter insertions and the lateral foot vein itself, maybe 20% of the time. For this study 2 injections are needed, sometimes hours apart, a small 30g homemade mouse tail vein catheter is used for the isotope injection and a 26g commercially available polytetrafluoroethylene catheter for the contrast infusion portion of the CT study, which involves 2 contrast injections, while the scan is running. For most of the guinea pigs, the tarsal vein, as it comes up from the toes, is used. The saphenous is a possibility, but since the imaging is of the hind leg muscles, using that is a last chance option, saved for the more difficult veins. The guinea pigs do occasionally have a lateral foot vein that is large and straight enough for a catheter placement, and when seen this way, it is generally an easy insertion. There are several

other things learned that helped along the way, prewarming with a hot glove, prepoking a hole with a 22g needle, otherwise their tough skin would just accordion the polytetrafluoroethylene catheter, and most of all, tape.With all these things, getting a catheter in these tailless rodents has become relatively easy, making the continual imaging work well for this study.

P116 Identification and Classification of Singly Housed Animals

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As regulatory requirements increasingly emphasize social housing as the standard for all social species, the need to be able to quickly and accurately identify why a given animal is not socially housed is becoming increasingly important. This identification process draws attention to and minimizes unnecessary social isolation and helps highlight which animals are candidates for social housing. The reason an animal is singly housed must also fit into an approved exemption to social housing category; these include veterinary, protocol, attrition, or fighting. To standardize this process, we created an identification and classification system. This process requires participation by both husbandry and laboratory staffs as both engage in activities that may result in an animal becoming singly housed. Singly housed animals are identified by placing a blue tab on the top of the cage card. Two pieces of information are recorded on the tab: an approved exemption code and the date on which the animal became singly housed. If husbandry staff cannot identify the reason the animal is singly housed, the technician places a vertical blue flag behind the cage card, the flag is marked with the date the cage was identified. The laboratory staff then have 1 week to record the appropriate code on the blue identification tab. If the lab staff does not assign a code within 1 week, husbandry staff reports the issue to their supervisor and after 2 weeks it becomes a noncompliance, which is then reported to the IACUC. Signs hung in each animal room display the correct usage of the tabs and explain the exemptions codes; in addition, an email notification was sent to all animal users describing the process prior to implementation. This system provides an easy way to quickly identify singly housed animals, in addition to when they became singly housed and why.

P117 Improving a Large Animal Enrichment Program

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At our university, we are committed to upgrading our large animal enrichment program. We are constantly reviewing available literature, visiting websites and encouraging our own staff to come up with innovative and practical ways to offer a variety of enrichment to multiple species. This strategy has allowed us to improve the quality, and build up the menu of methods available, which avoids frequent repetitions and monotony. To achieve this we offer special enrichment several times a year. These offerings require extra effort, but function for multiple species. For each of these, we will describe preparation, species use and participation, and evaluation of reactions. The special enrichment offerings fall into 2 categories: seasonal and recurring. The seasonal offerings include pumpkins in the fall and hard boiled eggs in the spring, which the animals have never or rarely experienced before. They spend a great deal of time engrossed in these objects, which decreases monotony. The recurring enrichment includes fresh wheat grass in soil and bags of popcorn. These allow animals to have a novel experience and in subsequent administrations, they become very excited about certain objects. For example, the wheat grass gives our NHPs, pigs, and rabbits something to play with, forage through, and eat. The addition of special enrichment to our program has led to an increase of novel objects offered to our animals. While we have a wide offering of standard enrichment devices and treats, these new additions allow us to further promote species appropriate behaviors and psychologic wellbeing.

P118 Use of Video Remote Interpreter for Hearing Impaired Employees in a Laboratory Animal Program

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Approximately 500,000 Americans are classified as deaf or hearing impaired and use American Sign Language (ASL) as their primary mode of communication. Eighteen percent of the husbandry staff in the Bioresources Department at Henry Ford Hospital is classified as deaf or hearing impaired and have limited options for communication in the workplace outside of a certified in-person ASL interpreter (ASLi). ASLi is advantageous in the workplace to ensure the accurate exchange of information and improvement in employee engagement. In our department the use of ASLi was also associated with a significant wait time and subsequent disruption in work schedule as interpreter availability varies. To better support our deaf and hearing impaired employees, and to assure compliance with all federal and state employment and disability regulations, a pilot study was conducted to assess the quality, efficacy, availability, and cost of a video remote interpreter system (VRI) over a 6 month period. VRI is a fee-based service using video conferencing technology to provide real-time interpretation via offsite interpreters. During this 6 month trial 122 VRI calls representing 1,488 minutes were completed. Daily rounds were conducted during the first week of the pilot to address problems and obtain initial feedback from end users. A 6 question survey was completed by the end user at the conclusion of each VRI call. All survey questions were associated with a subjective response (excellent, very good, good, fair, poor or yes, no). A cost analysis of VRI compared to ASLi was also performed. The survey results showed that VRI is a preferred method of ASL communication for our department in terms of quality, efficacy, and a reduction in wait time. The cost analysis demonstrated that the monthly use fee of VRI was similar to ASLi, however a significant savings was found for calls less than 30 minutes in duration. The results of this pilot study show that the implementation of a VRI system for deaf or hearing impaired laboratory animal employees is an acceptable and preferred alternative to ASLi, and can be easily implemented in a laboratory animal program to improve comprehension and engagement of deaf or hearing impaired employees.

P119 Designing, Implementing, and Sustaining a Successful Animal Care Technician Journal Club

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During an acute and substantial increase in labor associated with a multi-facility disease outbreak, animal care technicians' morale and career engagement decreased, and job attrition was markedly higher than average. To address these issues, we implemented a technician lead journal club. This initiative required managements' approval for holding journal club during work hours, supervisory support to generate attendance, and use of teleconferencing to ensure that remote facilities were able to participate. Initially, two topics were selected for each meeting based on the staff's reported interests, and to demonstrate the important outcomes of their work efforts, at least one topic specifically referenced research outcomes from our institution. To maintain journal club attendance, we routinely surveyed staff over time. Based on their suggestions, we included video media during presentations, and after discussing relevant articles, we incorporated hands-on activities, such as fabricating enrichment items. Following a recent decrease in attendance, we solicited investigators to present on their research projects, and included facility tours. As a result, attendance increased by 333.33%, when compared to the previous meeting. Our efforts to increase technicians' autonomy through peer lead journal clubs have been a success. Technicians report more interest in laboratory animal

science, and concurrently, longevity with the department has increased. Compared to the previous 6 months, the average technician attrition rate decreased from 6.28% to 2.72%, after journal clubs were initiated. To maintain success and attendance over time, we will continue to survey staff, incorporate their suggestions, and modify meeting content and structure.

P120 Wellbeing of Laboratory Rats Housed in Pet Style Caging

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Husbandry standardization for lab animals has historically focused on ways to minimize variations to serve the needs of science. Although standardization may be required within a study, it is not necessarily appropriate to hold a single standard across an entire facility housing many studies. Our institution houses a colony of conventionally housed rats for the sole purpose of training animal care and research staff. As there was no reason these animals must be kept in standardized caging, we decided to work on the feasibility of housing this group in pet style cages to improve their wellbeing. Twelve rats (6 male, 6 female) were double-housed in standard cages (approx. 10 x 18 inches). Six males and 6 females were group housed according to gender in 2, 3-level pet cages (approx. 30 x 20 x 40 inches H) that each contained 2 pod shelters hanging from the ceiling. Behavior and interactions with conspecifics were assessed at least once daily. After 1 month, blood was collected for prolactin and CBC. Behavior assessment demonstrated a strong gender effect on behavior within the cage; males hid and were inactive, but females were highly social with each other and humans and very exploratory. Stress markers from blood seem to support that in this study, the males did not adept well to the open style pet caging as their prolactin and neutrophil:lymphocyte ratios were significantly increased as compared to the standard housed controls ($P = 0.0035$ and $P = 0.0574$, respectively), while females had no significant differences. Adapting pet style caging to a laboratory environment was not without complications for the male rats; however, everyone on the team was so rewarded by the pro-social and exploratory nature of the animals that we continue to evaluate the advantages and disadvantages of this housing style.

P121 Evaluation of 6 Industry Diets on the Reproductive Success of DBA-2 Mice

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This study expands on previous findings that evaluation of 3 types of rodent feed showed no distinction of effect on the breeding success C5Bl/6 mice. Here, we used a more poorly breeding strain of DBA-2 mice and expanded the possible dietary strategies to 6 options. Six breeder pairs each were maintained on: 1) a standard control diet; 2) a commercial breeding diet with increased fat; 3) a supplement given 3 times weekly in addition to standard control diet; 4) sunflower seeds 3 times weekly; 5) supplemental gel cups given 3 times weekly; or 6) a pre-market total replacement diet. A dietary effect on breeding was quickly demonstrated. Breeders successfully produced significant increases in the proportion of pups successfully weaned from dams fed the sunflower seeds ($P = 0.0593$), while there was a significant decrease in the proportion of pups weaned from dams fed the breeder diet ($P = 0.0104$). There were significant increases in the cannibalization of pups by dams fed the breeder diet ($P = 0.0082$), while there was a significant decrease in the cannibalization of pups fed the love mash diet ($P = 0.0335$). Fatty lipid profiles of each diet were analyzed, identifying the dietary fats used in these diets for a more specific understanding of the role they play in the mouse life cycle from conception, fetal development, lactation, and maintenance of weight gain. This study will be of interest to veterinarians and technicians who manage breeding colonies.

P122 Intra-Cage Environmental Conditions in Individually Ventilated Cage (IVC) Systems

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Recommendations regarding environmental conditions suitable for housing laboratory animals are usually based on the conditions prevailing in the macroenvironment and not those in the microenvironment. This study compared the intra-cage microenvironment of 2 different IVC systems. We measured intra-cage temperature, relative humidity (RH), and light intensity for 2 IVC systems using a small self-contained data logger placed inside of the cages. In a preliminary test, we found small but consistent differences due to the position of the data logger within the cage (left, right, front, and top) and observed a trend for systematic variations in the parameters related to the cage position within the rack. We then conducted studies with both reusable and disposable IVC systems. Eighteen cages were used in each study. Nine cages were placed on each side of a double rack in a 3 rows (top, middle, and bottom) and 3 columns (far, midpoint, and close to the blower) configuration. Each cage contained 5 female NOD SCID Gamma (NSG) mice initially weighing 18 g. Data loggers were affixed to the inside front of the cages and recorded temperature, Relative humidity and light intensity at 1 hour intervals during the 2 week test period. An additional data logger was placed inside the room, near the rack, and recorded the same parameters at the same time intervals. The results of both tests indicated significantly higher ($P < 0.01$) temperatures and light intensity in the cages in the higher rows and in the columns farther from the blower. RH followed the opposite pattern. Large variations were observed with the time of the day and from day to day within the experiment. In general, the room temperatures were about 3 degrees lower, and RH about 10% lower, than the values measured inside the cages. This was confirmation that the microenvironment, although different from the macroenvironment, still remains within the *Guide* recommended parameters.

P123 Optimizing Stocking Densities to Reduce Cold-Stress in Laboratory Mice

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Mice housed under routine vivarium temperatures (21°C) are chronically cold-stressed. This cold-stress, which varies by housing type, can alter experimental results. Mice prefer to defend their body temperature through behavioral adaptions, such as nesting and social huddling. Huddling is the most frequent eusocial behavior of mice driven primarily thermal regulatory needs. In this work we aim to determine the optimum stocking densities in modern individual ventilated caging based on thermoregulatory needs of mice. Mice were stocked at various densities (1–5 mice per cage) in individually ventilated cages using a complete factorial design: female and male; C57BL/6J or BALB/c; and nude or hirsute (n = 5). We measured brown adipose tissue mediated nonshivering thermogenesis with thermography as a proxy for cold-stress. Single and paired housed mice were significantly more cold-stressed than mice housed in groups of 3–5 ($P < 0.01$). Paradoxically, male hirsute BABL/c mice at stocking densities of 4 and 5 were under significantly great cold stress than at stocking densities of 3. The reductions in nonshivering thermogenesis from socially huddling were less than those seen under thermoneutral conditions described in previous studies, implying social huddling behavior is only a partial solution to cold-stress under modern housing conditions. Social huddling narrows the physiologic gap between the tested phenotypes (nudes and hirsute). These findings provide specific stocking density recommendations for individually ventilated cages and conform to

the social housing guidelines defined in the *Guide for the Care and Use of Laboratory Animals*.

P124 Paperless Vivarium: Transitioning from Paper Based Forms to a Paperless Vivarium

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Our company is committed to the ethical treatment and high-quality care of all animals used in research. To fulfill this commitment, our comparative medicine (CM) complies with all laws, regulations and accrediting standards related to the use of animals in research. In addition, we constantly strive to provide flawless animal care, as well as maintain compliance with external, internal, regulatory, and AAALAC standards. In order to facilitate this compliance and maintain a flawless animal care and use program by improving business processes, CM created a collaboration team that drove the efforts to transition from paper based forms to a paperless environment within the vivarium. The collaboration team reviewed all paper based forms within the vivarium such as room logs, vet forms, procurement forms and converted the paper forms into electronic forms. The vivarium was outfitted with tablets so that individuals could access the forms from any animal holding room. The electronic forms were created using preexisting commercially available software that the company utilized on a daily basis. After gathering requirements for each form and creating the electronic version, training then took place to ensure all colleagues understood how to use each form. Documents are kept in one centralized location making the accessibility easier to locate. Colleagues enjoy the simplicity of the forms as they look similar to the paper based forms. The forms live on a secured server that only authorized users have access to. Paperless vivarium is an effective and efficient approach to our facilities with limited resources and no additional staff needed. Through this novel approach, we are meeting CM strategic imperatives to execute flawless animal care and welfare, engage colleagues, and harmonize and create efficiencies and flexibility.

P125 Improving Animal Procedure Safety by Reducing Isoflurane Exposure Effects

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Isoflurane is a common inhalation anesthetic used in laboratory animal science and veterinary clinics. The release and exposure of waste anesthetic gases in the work environment is an occupational hazard for staff working with the agent. As part of a lab risk assessment, the environmental health and safety department monitored several large-scale animal procedures that utilized isoflurane to characterize exposure levels of isoflurane to in vivo research personnel and addressed the issue of reducing this exposure. Through a series of large-scale animal surgical procedures, industrial hygiene monitoring was conducted to characterize isoflurane exposure among the surgical staff. Three surgeons anesthetized more than 100 mice in a surgical setting. The workstation consisted of a portable isoflurane/oxygen anesthesia system. The entire set up was in a Class II A2 biosafety cabinet that was ducted to the building exhaust system. A portable local exhaust unit was also used. One employee worked at the induction chamber anesthetizing mice and transporting cages in and out of the biosafety cabinet. The other 2 employees performed the surgeries while the mice were administered isoflurane. Air sampling for isoflurane was conducted using commercially available assay technology halogenated anesthetic gas monitors. The monitor samples workplace air by diffusion of halogenated anesthetic gases. The halogenated anesthetic gases are collected on the media via adsorption. Three personnel samples and 1 area sample were collected along with a field blank to test for possible contamination. Samples were collected immediately following in vivo surgical procedures. The start time and end time that each monitor was exposed to the environment was recorded. This data was reported to an external analytical laboratory. Based on the results, the use of both an externally vented biosafety cabinet in conjunction with the use of a supplementary exhaust system can substantially decrease the anesthesia gas exposure levels during anesthetic procedures. In vivo staff has now been supplied with the necessary equipment to limit anesthesia waste gas exposure.

P126 Does Position on the Rack, Location in the Room, or Proximity to the Animal Transfer Station Affect Illumination inside the Mouse Cage?

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We studied the effect of room position, location on the rack, and proximity to the animal transfer station (ATS) and room door on light levels within cages in a mouse room containing 14 commercially available ventilated racks. Light measurements were taken with a onset datalogger 1 inch inside the front of 4 cages on each of the top, middle, and bottom rows of all racks. We evaluated the effect of room location, row height, and horizontal position in low (daytime) and high (override) light conditions. Results demonstrated a consistent light gradient from the top to the bottom of racks in both lighting conditions: top row (12.1 – 3.3 lum/ ft²), middle row (4-8 – 1.8 lum/ ft²) and bottom row (3.3- 1.1 lum/ft²). Rack location and horizontal row position had minimal effects except for cages on the end of rows nearest to the overhead lights, which consistently had light levels higher than other cages on the row. We then determined if light from the ATS increased light levels inside the cage. We found the ATS had no effect on cage illumination during either the high or low daytime light conditions, even for racks placed within 4 feet of the ATS. However at night, an average increase of (1.6 lum/ft²) was noted on the middle row of racks placed within 4 feet of the ATS. Finally we noted that when the room door was opened during low daytime light, brighter light from the corridor increased light levels in cages as far away as 10 feet. We suggest that for rodent models requiring consistent low light, cages should be placed in the interior lowermost rows of racks furthest from the room door. Further, breeding cages should be placed > 4 feet away from the ATS to avoid reproductive difficulties due to interruption of the dark cycle when the ATS is used at night.

P127 Repurposing a Used Street Sweeping Broom as an Enrichment Device for Dairy Cows at a Large Academic Institution

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Brushes, especially motorized versions, are commonly used in dairy cow housing facilities as enrichment devices. However, these devices are usually only placed in lactating cow pens due to financial constraints as they cost approximately $2500 per unit. This leaves other groups of cattle on the operation, typically dry cows and heifers, without access to an enrichment device. Repurposing used street sweeping main brooms, which are otherwise discarded after normal wear and tear, is a cost effective way to achieve access to an enrichment device for dry cows and heifers. These brushes can be found in city and university maintenance garages and are usually free of charge. Our institution received 3 used main brooms from our city's maintenance garage at no cost. Brushes were power washed on arrival to remove debris collected during street sweeping. The brushes are 5.5 feet long, 25 inches in diameter with a 8.5 inch diameter central steel support tube. A simple mounting system was created from scrap material using a 7 feet long 3 inch diameter galvanized pipe and a 3 inch wide 1/8 inch thick steel plate, also 7

feet in length. A hole was drilled into the concrete pad of the dry cow lot in a central location of the pen. Two feet of the pipe was placed into the hole, and the steel plate placed next to it to stabilize the pipe. A skid-steer with pallet forks was used to lift the brush and place it onto the mounted pipe. Immediately after installation, they cows began interacting with the brush. Behaviors observed included chewing on the bristles and aggressively rubbing their faces, necks, and sides on the brush. Repurposing used street sweeping brooms provides an extremely low cost, sustainable resource of engaging environmental enrichment devices for all of the dairy cattle at our institution.

P128 The Other Rs in Research: Reduce, Reuse, and Recycle—Repurposing Existing Equipment to Meet Guide Recommendations for Rabbit Housing

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Social housing, environmental enrichment, and adequate vertical space are all key features for rabbit housing recommendations made by the 2011 *Guide for the Care and Use of Laboratory Animals*. The *Guide* states, "housing enclosures that allow greater freedom of movement and less restricted vertical space are preferred" and the suggested height for rabbit caging was increased to 16 inches. Additionally, the *Guide* emphasizes social housing and proper enrichment, which includes vertical space, suggesting that it provides animals with "a degree of control over their environment which allows them to cope with environmental stressors". Historically, the rabbits under our care were singly housed in stainless steel cages slightly under the current height standard. By using existing space and equipment, as well as considering the *Guide* recommendations, we were able to improve rabbit housing without replacing equipment by 2 methods: modifying cubicles for floor housing and repurposing existing finch aviaries. This allowed us to introduce group housing and caging systems that did not limit height available in the primary enclosure while conserving resources, along with increasing enrichment opportunities within the housing space. We have successfully housed same sex groups (2–3 individuals) of both males and females for up to 6 months.

P129 Single-Use Huts and Exercise Wheels: Cost Considerations and Effects on Stereotyped Behaviors in Mice

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We evaluated product cost, sanitization frequency, and behavioral effects in mice, when using a single-use, plastic, recyclable hut and exercise wheel as environmental enrichment. We assessed two stereotyped behaviors: excessive food gnawing and barbering (N = 15 cages per group), which both require enhanced oversight, and increase labor costs. We measured baseline behavior with a nesting square in each cage, and then measured changes to behavior over 4 weeks, when a disposable plastic hut and wheel was added to the cage. In the presence of huts and wheels, we observed an immediate and sustained reduction in food gnawing ($t(14) = 3.580$, $P < .01$), and severity of barbering was significantly reduced over time (Wilk's Lambda = .002, $F(51, 63) = 8.274$, $P = .001$). Next, we addressed cost. Our institution's per diem rate included the option to use opaque, disposable cardboard huts, but these devices compromise staff's ability to visualize animals. They are also quickly destroyed, limiting their enrichment value and requiring weekly replacement. To implement the more durable, transparent, plastic hut and wheel, while negating their comparatively higher costs, we extended the duration of use. To establish an acceptable duration, we left the devices in cages for 4 weeks, transferred them weekly during cage changes, and concurrently performed quantitative and qualitative bacterial assessments. Aerobic and anaerobic cultures yielded commensal bacteria only, and in insufficient quantity to negatively impact animal health. Replicate organism detection and counting plating demonstrated a significant increase in bacterial colony forming units between weeks 1 and 3 ($t(21) = -1.937$, $P < .05$). In accordance, we extended use of plastic huts and exercise wheels to 2 weeks, which reduced our enrichment costs by 7.9%, when compared to using the cardboard product. We also successfully reduced the severity of some stereotyped behaviors in our colonies, and the associated labor costs.

P130 An Evaluation of Best Practices for Zebra Finch *(Taeniopygia guttata)* Euthanasia

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Although zebra finches *(Taeniopygia guttata)* are increasingly common as animal models in biomedical research, very few published reports are available on euthanasia techniques for small avian species. At our institution, an intracoelomic injection of pentobarbital in unanesthetized, adequately restrained zebra finches has been the preferred option for euthanasia. This procedure involves a caudal midline injection to avoid the air sacs, and it appears to cause minimal pain and distress. The 2013 AVMA Guidelines for Euthanasia do not endorse intracoelomic pentobarbital in conscious avian species, but do consider carbon dioxide (CO_2) asphyxiation as a conditionally acceptable method. The concern with using CO_2 in avian species is that their highly efficient respiratory systems could make them more sensitive to its toxic effects.The primary aim of our study was to determine the optimal method for euthanasia of laboratory zebra finches. Healthy male and female adult culls were euthanized by 5 different methods (n = 50, 10 birds/group): intracoelomic pentobarbital (19.5 mg) alone, 4% isoflurane anesthesia followed by intracoelomic pentobarbital, and CO_2 at three displacement rates (20%, 40%, and 80% volume/minute). Videorecording each procedure allowed for the quantification of time to recumbency and respiratory arrest, as well as the onset and duration of any behavioral indicators of stress (open-mouth breathing, head shaking, wing flapping, and neck hyperextension). Results to date (n = 20, 4 birds/group) indicate intracoelomic pentobarbital and isoflurane followed by intracoelomic pentobarbital methods of euthanasia lead to fewer stress-related behaviors than CO_2 asphyxiation at any displacement rate. Isoflurane followed by pentobarbital has a significantly ($P < 0.05$) longer latency to respiratory arrest than euthanasia by 20% and 40% CO_2 displacement rates. We also found that an 80% CO_2 displacement rate is associated with significantly ($P < 0.05$) less gasping compared to 20% or 40% CO_2 displacement rate. Our results indicate that intracoelomic pentobarbital is an effective and humane method of euthanasia and may be less stressful than CO_2 asphyxiation.

P131 3D Digital Modeling as a Novel Approach to the Analysis of Mouse Nests

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3D digital modeling is a complex and broad technology, utilized by many industries and becoming more widely available. In recent years, it has gained popularity in both human and veterinary medicine with uses including modeling and printing surgical devices and prosthetics for individual-specific or personalized medicine. Our interest in 3D digital modeling lies in its novel application for analyzing quality of nests built by laboratory mice. While several studies have investigated the efficacy of various nesting material as environmental enrichment for mice, 3D digital modeling will allow

us to further understand the dynamics of nest building and the importance of providing appropriate nesting material. One of two types of nesting material, crinkle and rolled paper, was provided to mice. Photogrammetry, the practice of obtaining information about physical objects through the process of recording, measuring, and interpreting photographic images, was then performed to analyze nest quality. With this method, digital images of the nests were taken and uploaded to a software program to create 3D models of the nests. We were able to produce high-quality 3D images of the crinkle paper nests that were taken out of the mouse cages. The 3D images of the rolled paper nests were of medium quality because the nests were less robust and compact, making it impossible to take the nest out of the cage without destroying its structure. This presentation will show how to use photogrammetry as a performance standard tool on analyzing nest material provisions to mice.

P132 Cloudy Cages: Is Autoclaving the Only Culprit?

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Standard practice is to implement a more rigorous cleaning regimen for cages exposed to unwanted pathogens. During outbreaks in our facility, exposed cages are autoclaved, submerged in chlorine bleach, and processed through the tunnelwasher to kill and prevent further spread of suspected agents. We have noticed our rat cages (blue) including water bottles have become so cloudy after this regimen that animals are no longer easily seen through cages, making daily health checks difficult and cages unusable. We recently ordered new rat cages (green) and wondered if we would observe the same issue. The purpose of this study was to determine which factor in the cleaning regimen caused the cloudiness and to see if we could recreate the cloudiness in the green cages to determine certain preventative treatments. In our study we autoclaved blue and green cages. Then submerged the cages in 3 different chemicals: chlorine dioxide, hydrogen peroxide, and chlorine bleach. We used 2 different types of facilty bedding. Cages were autoclaved twice. We also wanted to determine if there were rat specific effects on the clarity of the cages so some cages housed rats throughout the study, resulting in the autoclaving of cages with dirty bedding. Others were autoclaved with clean bedding. Using damaged blue cages, we developed a cloudiness grade that was used for the analysis of the autoclaved cages in this project. Relative to the new green cages, the old blue cages were more damaged and discolored. There were no differences between chemical treatments and bedding conditions, suggesting that the cloudiness is a result of the autoclaving process. This study has important implications for facilities undergoing an outbreak, as understanding the appropriate way to disinfect cages without damaging their clarity can maintain optimal care level and save money, which would otherwise be spent on purchasing new cages. For future implications, we will explore the different types of autoclave settings to determine an optimal setting that would not damage cages. We will also explore the use of the facility water softening system, which supplies water to the autoclave system. Lastly, we will conduct a similar study with our mouse cages to see if the same results would be observed.

P133 Extended Use of Pure Nylon Rodent Chew Resulted in Cost Savings with No Detrimental Effects in Sprague–Dawley Rats

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Environmental enrichment for laboratory animals has beneficial effects on their welfare and health. The data generated from environmentally enriched animals has been shown to be less variable and of higher quality. Rodents rely in large part on their olfactory cues to navigate in their natural environment. Enrichment devices such as commercially available pure nylon rodent chews, wood blocks and durable chew bones have been shown to reduce anxiety and stress in rodents. In this study we evaluated whether the use of pure nylon rodent chews can be prolonged without affecting the health of the rats. Sprague–Dawley Rats (5-6 week old) were pair-housed in cages with commercially available alpha cellulose bedding and ad-libitum diet with portion controlled bedding material. We provided pure nylon rodent chews (1/cage) to 3 groups each containing 8 cages (n = 16 rats) and assessed the pure nylon rodent chews at 4, 8, and 12 week intervals for their bacterial load, debris accumulation, and percent usage. We also examined rats for their CBC/chemistry at the above intervals and measured their body weights weekly. The bacterial load as well as debris accumulation on pure nylon rodent chews peaked at 4 weeks and dropped to lowest levels by 8 weeks. The percentage use of pure nylon rodent chews as measured by their weight peaked at 12 weeks. In addition, we saw no significant changes in RBC, WBC, and liver enzymes as well in their body weights. Therefore, we suggest that pure nylon rodent chews can be safely used for extended periods up to 8 weeks as enrichment devices for rats.

P134 PCR Testing of Soiled Bedding Collection Cages, without Live Animals, as Part of a Routine Health Surveillance Program

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With the advent of microisolation ventilated caging and affordable, high throughput PCR testing for excluded agents, improvements to the traditional soiled bedding live sentinel model for routine health surveillance are being pursued. Though soiled bedding sentinels have been the gold standard for many decades, this method depends on infection and colonization of sentinel animals and it is known that many agents do not transmit readily. PCR testing of dust samples has been shown to be effective in detecting potential pathogens and can eliminate the need for live sentinels; however, limitations such as distance of infection from the sampling site, access to exhaust plenums, and obstruction of the passage of organisms to the plenums by filtered lids have confounded the adoption of reliable sampling methods. Also, unless racks are decontaminated after a positive result and a baseline test is performed, false positives in future testing may occur. We proposed the use of a soiled bedding collection cage, without sentinel animals, as a method of routine health surveillance. We compared PCR testing of fecal pellets from traditional soiled bedding sentinels to PCR testing of a swab placed in a soiled bedding collection cage, with no animals present. Results demonstrated that the collection swab identified known bacteria in the colony reproducibly. While further evaluation will be conducted, the use of a nonanimal collection cage may provide a surveillance method which eliminates the variables of dust swabbing, while providing advantages over the use of live animals. This method eliminates the need for active infection and colonization, captures data from an entire testing period, can be used on any housing system including static housing, starts each testing period with a clean sample without the need for baseline testing, and fulfills a 3R commitment to reduction in animal use by eliminating or reducing live sentinels.

P135 Evaluation of Animal Identification Methods for SPF High-Throughput Animal Vivariums

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Animal identification methods have long been a topic of discussion and debate throughout the years. Commonly used animal identification methods include toe clipping, ear notching, ear tagging, tattooing, microchip transponders, and hair dyes. The methods used within the lab animal community varies from institute to institute based on the needs of the vivarium. Some animal identification methods may make sense for one vivarium but could negatively

affect the work flow of another. High-throughput facilities especially have a difficult time selecting an identification method as they must walk a fine line between addressing the needs of the facility and taking into consideration the humane treatment of the animals and impact on employees. It is therefore important for each facility to properly evaluate their needs and choose a method that is suitable for their institute. The transgenic technology department at our company has conducted a small study in which various animal identification methods were evaluated on mice and rats based on the needs of an SPF high-throughput vivarium. Evaluation was based on usability, readability, durability, capacity, cost for both product and start up, impact on animal health and welfare, and user ergonomics. The results from the study have shown that while various methods are feasible for most facilities, toe clipping and ear tagging remain top contenders of animal identification in mice for our high-throughput facility. While we found that ear tagging in rats was best done at wean age to ensure the lowest risk of tag loss, it was by far the most efficient method (besides toe clipping) that was tested. Both methods are user friendly, cost effective, durable and allow one technician to identify hundreds of animals in a matter of days.

P136 Environmental Enrichment Intervention in the Retired, Singly Housed, Adult Male Rhesus Macaque

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Two, singly housed, adult male Rhesus macaques (*Macaca mulatta*) at our university, completed their given behavioral experiments causing a decrease in positive human interaction during their day. Husbandry and veterinary technicians noticed one of the males in particular losing interest in his environment (decreased technician interaction, prolonged sleep, and decreased foraging). Under the established environmental enrichment schedule, various manipulations were given twice a day (music, movie, scent, and food). In response to the change in demeanor and activity level, an enhanced enrichment schedule (EE) was created that would expand these enrichment activities to 3 times per day, maintaining a unique schedule in 2-week intervals for both monkeys. We hypothesized that by increasing the number of enrichment encounters per day, would decrease abnormal behaviors. Focal video recordings were performed once a week for 4 weeks to observe and quantify behavior. Four categories of behavior were scored: sleeping, inactive sitting (for example. void of interest in surroundings), self-grooming/alert sitting (for example, watching roommate/movie/technician), and foraging/eating. Over the 4 week EE intervention, the duration of self-grooming/alert sitting increased 26%, whereas sleeping decreased 12.5%. No significant changes in inactive sitting and foraging/eating durations were observed. In conclusion, we found that EE treatment was successful at improving the monkey's interest in environment and normalizing behavior; however, the greater frequency of food enrichment increased both macaques' body weights.

P137 Institutional Animal Care and Use Committee (IACUC) Administrators Practice Network Fosters Business Acumen in Comparative Medicine

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In May 2014, the IACUC Administrator (IA) Practice Network was created and endorsed by a corporate comparative medicine executive team. The IA Practice Network is a committee that consists of the five IACUC Administrators from each comparative medicine site within the corporation and one sponsor from the executive team. The purpose of the committee is to give the IACUC administrators an opportunity to collaborate, network, and focus on improvements and efficiencies to processes and procedures across sites. Monthly teleconferences are held which have resulted in a greater understanding of IACUC business processes across respective comparative medicine (CM) sites. By sharing IACUC administrative information, efficiency gains related to IACUC member training, principal investigator support, documentation, semi-annual program reviews, and facility inspections are probable. Whether the CM site has 30 or 130 active animal use protocols (AUPs), regulated or nonregulated species, 10,000 sq. ft. or 150,000 sq. ft. of animal facility space, the programmatic requirements are the same. Each IACUC administrator frequently coordinates multiple IACUC activities concurrently and having a resource like the IA Practice Network to engage, educate, and collaborate has resulted in value added and more efficient IACUC business practices.

P138 Developing a Method to Actively Monitor Waste Anesthetic Gasses from Anesthesia Systems with Integrated Internal Carbon Filters

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Our facility uses several different anesthetic system configurations, some of which employ integrated internal activated carbon filters to capture waste anesthetic gasses (WAGs). Regular maintenance of the system is key to ensuring breakthrough of WAGs does not occur and potentially expose workers to permissible exposure limits greater than those recommended by OSHA. Individual machines are used with varying frequency depending on the research unit and study design. Each machine is configured with an internal activated carbon filtration system that requires a somewhat time consuming process to access the filters and replace the bulk activated carbon. Historically, this had been performed as part of routine maintenance on a predetermined interval throughout the facility. The actual carbon replacement process is labor intensive and can result in variable distribution of the adsorption medium within the receptacle and less than optimal scavenging of the WAGs. Furthermore, many filters could be changed out either well before they reach their expected adsorption maximum, or worse, not until they are close to saturation risking breakthrough exposure to employees. Prefilled activated carbon filters are available to simplify the process, but the trade-off for ease of use entails an increase in operational costs and generation of unnecessary waste. A more appropriate replacement method should take into account that the frequency of use of the equipment determines the effective lifespan of the carbon filtration system and thus drives replacement intervals. In the spirit of continuous improvement, we established a method of continuous in-life monitoring of WAGs and optimized the replacement frequency of integrated internal carbon filters.

P139 Rodent Drinking Water: An Assessment of Microbiological Testing Systems

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The *Guide* indicates that laboratory animals should have access to potable, uncontaminated drinking water. Four various types of treated water are used in our animal facilities: city water filtered through a 0.45µm filter (filtered tap), deionized (DI), reverse osmosis (RO), and reverse osmosis-deionized (RO-DI). Water is autoclaved if being provided to immunodeficient and immunocompromised animals in some animal facilities. Other facilities provide autoclaved water to all rodents. The current gold standard for water testing requires using R2A agar to grow bacteria that may inhabit potable water; yet this is a cost, labor, and time intensive process, especially as the procedure requires plate incubation up to 7 days. The objective of this study was to validate the use of standard, non-water specific real-time adenosine triphosphate (ATP) swabs as an alternative to performing bacterial colony counts. The study also investigated whether there was a difference in water microbiological quality based on water type. Swabs of water samples in bottles post-autoclave were

collected twice a week; concurrently, water samples were collected for plating on R2A agar. Our data shows that ATP levels did not directly correlate with the number of bacterial colonies on the R2A agar. However, when the ATP value was zero, there was usually no bacterial growth on the agar. Interestingly, we also found our autoclaved DI water to have high colony counts and ATP levels, and these only increased with each time point post-autoclave. In the process of troubleshooting the contamination in the DI water, we found that autoclave cycle time might not have been sufficient, which we have started to investigate together with the efficacy of our DI water system. In summary, non-water specific ATP swabs should be used cautiously for water microbiological quality monitoring. Our experience also highlights the importance of the implementation of a water microbiological program.

P140 A Global Enrichment Committee

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Being part of a company with global sites, it is important that all the sites are working together to achieve success. Before the formation of the global enrichment committee, each site had its own enrichment guidelines. The sites did not communicate what enrichment items worked or didn't work. Each site was working independently of each other for enrichment. In order to communicate better among the sites, a global enrichment committee was created and comprised of at least one person from each site. The committee wrote global enrichment guidelines for all of the sites to use. A list of all enrichment items used at each site was compiled. The committee researched the enrichment items to ensure that the item satisfied at least one of the species specific behaviors. A centralized internet hub was created for information on enrichment items, which is shared among all 4 sites. Having a centralized team to oversee the enrichment program, has had many positive outcomes. With a unified enrichment program, each site has been able to consider new and/or different enrichment items. The sharing of information provides scientific rationale for the benefit of each item in terms of species specific habits and behaviors, thus promoting an environment that is optimal for animal health and wellbeing.

P141 Certified Professional IACUC Administrator (CPIA) Program Demographics

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The Certified Professional IACUC Administrator (CPIA) program was created to provide a formal recognition of an Institutional Animal Care and Use Committee (IACUC) professional's broad knowledge of IACUC functions and expertise about animal care and use programs. In the 7 years since the first test was administered, 475 individuals have become certified, demonstrating steady growth of the program since its inception in 2007. On their examination applications, candidates have the option to answer a series of demographic questions. This information provides critical insight into those in the field who seek professional development opportunities. At the time they sat for the exam, most CPIAs were 30-50 years old, female, and white. The most common degree obtained by CPIAs was a Bachelor's degree, followed by a Master's degree. In order to sit for the exam, candidates must have IACUC administrative duties, and either 2 or 3 years of relevant experience, depending on whether an educational requirement has been met. At the time they sat for the exam, most CPIAs had at least 6 years of relevant experience as IACUC administrators, managers, or staff members. However, many also served in other capacities. CPIAs are affiliated with a wide range of institutions and organizations: 57% of CPIAs worked in academia at the time they sat for the exam; 15% worked in biotech/industry, and 7% for the government. Additionally, at that time they sat for the exam, 54% of CPIAs worked in IACUC offices that have less than 3 full time equivalent staff members. This data suggests that there is a diverse demand for the CPIA program, and it is a desired professional development opportunity.

P142 Traditional and Nontraditional Continuing Education

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Ensuring that members of the research teams are appropriately trained is a requirement of the Animal Welfare Act, the *Guide for the Care and Use of Laboratory Animals*, the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the United States Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training. Institutions are also responsible for providing continuing education of research teams. Some institutions simply focus on conventional means such as subject focused online training. However, one very effective means is educating research teams is education through noncompliance. Our university employs the AALAS Learning Library and noncompliance issues of the research community to provide annual continuing education. This is attained through the AALAS custom course feature. Custom courses are designed to help research staff learn from the errors of others and has found this to be an effective means of decreasing noncompliance issues.

P143 Environmental Impact of an Animal Facility: Life Cycle Assessment of a Mouse Facility

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In 2008 a new life sciences campus building opened. In the context of this sustainable campus, the monitoring of energy data showed a significant increase of energy expenditure correlating with the opening of this building. We suspected that the animal facility was the main contributor of this change. This was the starting point to launch a life cycle analysis of our animal facility. The aim of this study is to analyze the environmental impacts of the campus' mouse animal facility. The analysis included the following activities: administration and back office; mice husbandry; cages and racks washing; ventilation; import and export of animals; scientific procedures; waste. For all the activities, the energy expenditure, the infrastructures, and goods consumption were considered. The data were processed and analyzed. The collected data were combined with a worldwide-recognized database ecoinvent. Three environmental impact measurements were considered: climate change (CO_2 footprint), human health, and ecosystems quality. They were assessed by using the IMPACT 2002+ LCIA method. The results show that cages and racks washing are the main contributors to the impacts on climate change and on human health, mainly due to the steam produced by gas for autoclaving and water heating. If mice husbandry is the second contributor for those indicators, it is the main cause of the impacts on ecosystems quality, mainly due to cereal cultivation for mice feeding. This analysis will allow giving some recommendations for improving the environmental performances of the animal facilities. We used these data to simulate what would be the environmental costs to run our facility with disposable cages.

P144 Environmental Impact of an Animal Facility: Modeling the Environmental Impact Scenario of a Change from Washable to Disposable Cages at a Mouse Facility

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The first goal of the study was to evaluate the environmental impact of our facilities in order to improve the environmental performance of the current facilities.Second, taking this environmental impact as a baseline, we simulated the replacement of all washable cages currently used by disposal ones and assessed whether one strategy would be superior to the other in terms of environmental impact. The same methodology as for the first study was applied. For all the activities, the energy expenditure, the infrastructures, and the goods consumption were considered. These data were combined with a worldwide recognized life cycle inventory database ecoinvent. Impacts on climate change, human health, and ecosystems quality were analyzed, using IMPACT 2002+ life cycle impact assessment (LCIA) method. We concentrated our analyses on climate change and human health because no difference was found for ecosystem quality. The simulation shows, globally, climate impact is lower for disposable cages than for washable cages and human health impact is higher for disposable cages than for washable cages. In conclusion, there is no clear best environmental option between washable and disposable cages. To reduce the animal facility environmental impacts, it is better to try to reduce the impact of each alternative rather than changing the alternative (washable or disposable). A few other conclusions can also be drawn from this case study. In view of the major impact of animal facilities on the environmental and financial budget of an institution, the LCA of animal facilities should be a must. Such approaches help to refine the energy budget of current facilities and to take strategically oriented decisions when an animal facility has to be remodeled or when a new one is planned.

P145 Development of a Volunteer Dog Socialization Program for an Established Colony in a Pharmaceutical Research Setting

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Dogs are one of the most highly domesticated and social of the common laboratory animal species. Human-dog socialization creates attachment and trust, which assists in the development of coping strategies that serve to bridge periods of adaptation to new procedures and environments. This reduces stress and experimental variability. Providing additional human-dog social interaction within a research colony can be a challenge. To address this issue, a volunteer dog socialization program was developed using nonhusbandry staff from within the organization to provide additional social interactions. In consultation with staff from within our department and occupational health and safety, a formal training program was developed to prepare nonhusbandry staff to enter the facility and work with the dogs within the colony. The initial pilot project would explore the feasibility of training nonhusbandry staff along with quantifying any benefits provided to the dogs in the colony and the staff caring for them. For the initial pilot project, 12 individuals participated in the socialization program over a 3 month period. During that time, 30 hours of human-dog socialization were provided. This was comprised of 265 individual human-dog interactions averaging over 6 minutes each. In comparison, the technical staff support for these activities was a total of only 6 hours. Surveys completed by both volunteers and animal care staff indicated that all involved felt the program was beneficial for the dogs and the training requirements were useful and easily completed. Based on these outcomes, we feel that a structured volunteer socialization program will be beneficial for our dog colony. Additionally, it is well established that humans can receive stress relief benefits from interactions with dogs, and this has been our experience as well. Expanding the program to additional organizational staff will allow us to increase the amount of socialization opportunities for our dogs, and potentially offer stress relief outlets for our staff.

P146 Enhancing Your Canine Socialization Program Through Volunteer Participation

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Most beagles are highly motivated by food rewards. However, this is rarely the case with our purpose bred beagles; they respond much more enthusiastically to petting and praise. Focusing on this characteristic was key in creating a socialization program that could improve their physiologic and social wellbeing while in our care. The enhanced canine socialization program was created to promote animal welfare by expanding conspecific interaction, offering novel and interactive enrichment toys, and increasing positive human interaction. Opening the socialization program to volunteers within our organization allowed us to increase the amount of human interaction the beagles receive without having a significant impact on staffing and operations. The canine animal technicians are trained to work with and supervise volunteers. To promote awareness of the program and recruit volunteers, flyers are distributed by email and information is presented during organizational meetings to eligible colleagues. Interested parties can attend a socialization session prior to committing to the program; and all volunteers are required to complete online training modules as well as hands-on training prior to joining. Once training is complete, volunteers work one on one with husbandry technicians during socialization sessions and their participation is tracked using online calendars. Increasing positive human interaction and expanding conspecific socialization has resulted in a more sociable and cooperative research animal while allowing for species specific behavior. Furthermore, by opening up the program to include volunteer participants, an opportunity for outreach and greater awareness of our efforts to provide flawless animal care and welfare was created. Future plans of integrating positive reinforcement training into the enhanced canine socialization program will develop the program further and shape behavior to promote positive handling and manipulation.

P147 Behavioral Changes Due to Social Separation in Female Cynomologus Macaques

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Primates housed in laboratory settings are required to be socially housed. However, separation of paired animals can sometimes be unavoidable. The purpose of this study was to investigate the possibility of behavioral changes in female cynomologus macaques (*Macaca fascicularis*) following a separation event. Six pairs, who had been co-housed for at least 6 months, were observed while co-housed and then at 3 time points following social separation. Behavioral observations were taken every 30 seconds with observation sessions lasting 10 minutes per pair. All behaviors observed were mutually exclusive. Analysis of behaviors across all subjects found that abnormal behaviors sharply increased during the observation 5 minutes after social separation. Additional, but smaller, increases in abnormal behaviors were observed the day following separation as well as 1 week following separation. These results indicate that social separation is a stressful event and attempts must be made to reduce frequency of animal separations.

P148 Behavioral Changes Due to Socialization in Adult Male Cynomologus Macaques

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The Animal Welfare Act requires that all social primates be socially housed unless exempted by a veterinarian or for scientific justification. Adult male macaques are often difficult to socialize due to high rates of aggression and subsequent injury. In this study, socialization attempts were made with 6 pairs of adult cynomologus macaques (*Macaca fascicularis*) and their behaviors were observed. Of these 6 pairs, 3 pairs were removed from study due to social injury. Pairs were observed prior to socialization, at grooming contact, and at full contact using scan sampling. Results indicate no significant behavioral changes due to socialization. While no abnormal behaviors were observed in the no contact observations, abnormal behaviors were observed during grooming contact and on the initial day of full contact. However, 1 week after socialization, abnormal behaviors were absent. Additionally, rates of foraging were similar between no contact and full contact indicating that food consumption was not impacted by socialization. Overall, these results indicate that while socialization may initially have negative effects in male animals including risk of animal injury and increased abnormal behaviors successful pairing of male nonhuman primates is attainable.

P149 Successful Relocation of a *Xenopus laevis* Colony

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Stable, reliable housing is a vital factor for successful animal husbandry and research. Although every species has specific requirements, amphibians in particular are sensitive to their environment and water quality. In our situation, a frog colony (*Xenopus laevis*) was being housed in a standard housing space within the facility. Remodeling plans presented an opportunity to move the frogs to a new, more species-specific location. Moving animals, while attempting to keep their macro and microenvironment stabilized, is an extensive project in itself. To create a more desirable housing space, an attached surgical suite and anteroom were included in the new design. This new surgical suite gave researchers a convenient space to perform their oocyte harvest, while the anteroom provided a quieter, more isolated environment that minimized the prevalence of nose injuries previously observed. The project involved the collaborated efforts of the husbandry, operations, and veterinary teams, as well as the research group. Everyone worked together to schedule and stage the move in a manner that least impacted the animals and research. The husbandry and veterinary teams worked closely to monitor the frogs and their environment while the operations team aided in transporting the frog equipment and housing unit. Preservation of the bio-bed, while critical, was only one of many precise steps taken to maintain the overall environment for the frogs. To preserve the water quality, water from the bio-bed was retained and then reintroduced once the system was constructed in the new location. For the first month, water quality testing was done twice daily and necessary adjustments were made to maintain balance and recreate a stable housing environment. Overall, the current layout of the new housing space allows for increased efficiency, improved animal health, and enhanced convenience for oocyte harvest.

P150 Evaluation of a Sentinel Cage Filter Sampling Process for the Detection of Rodent Infectious Agents by PCR

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PCR-based pathogen screening programs provide the potential for improving detection of agents that may not transfer to bedding sentinels and simplifies the sample collection and shipment process. Our facility adopted a PCR-based program which incorporates combined survival samples collected directly from the sentinel and resident mice for PCR and dry blood spot testing of sentinels for virus antibodies. Because the cages on our individually ventilated cage (IVC) racks have efficient cage-level filtration, which blocks the majority of exhaust air dust from exiting the cages, plenum testing is not possible. Based on a previous report which suggested sentinel cage filters can be used to monitor an IVC rack, we evaluated the use of a sentinel cage filter sampling (10 rack sides during a quarterly monitoring period) and compared test results with our current PCR-based method and historical data for soiled bedding sentinels. Although the number of agents detected were few, the sentinel cage filter results mirrored the results obtained for the survival samples collected directly from sentinels and resident mice. The only exception was *S. aureus*, which was only detected in the survival sampling and not the cage filter or by historical data. The sentinel cage filter sampling process was easier than direct sampling, but still required the transfer of soiled bedding to a sentinel cage. Findings of this study suggest that PCR testing of sentinel cage filter material, in combination with dry blood spot testing of sentinels for virus antibodies, may be a viable option over sentinel use by traditional nonsurvival screening methods.

P151 A Simplified Method to Identify Rodent Cage Status with Reusable Signage

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In many laboratory animal facilities the task of rodent husbandry practices is the responsibility of the animal care staff (ACS). With generally multiple staff on the ACS team overseeing the same holding area on a rotational basis, a facility-wide rodent cage status identification system is highly beneficial to the success of the husbandry program. This is especially important for individuals that may perform husbandry services at times in an area that is generally not assigned to them on the daily basis. Having a facility-wide identical process to identify the current status of the cage is crucial to efficiently provide the appropriate service to ensure the health and wellbeing of the animals. Our program has developed a simplified method for labeling rodent housing cages with reusable cage identifiers that can easily be implemented in any facility in order to streamline the process of daily husbandry practices and unsure conformity when communicating the current cage status within the ACS team and to the research staff. Previously used systems containing one time use handwritten sticky notes and various bulky index cards in the cage card holder were replaced with custom reusable static cling stickers containing precise words or numbers. Stickers clearly identify the current cage status, such as “pregnant,” “tentative wean date,” or simply notify of a special condition or status, such as “diabetic,” “ABSL2,” or “surgery.” By uniformly implementing this identification system throughout the facility, we have been able to effectively streamline the communication regarding the current cage status between the ACS performing husbandry and the research staff. The results of the new process are: (1) clean consistent signage, (2) easy identification directly on the cage, (3) identifiers are simple to remove and reuse, (4) faster turnaround time, and (5) well cared for animals. Upon implementation, the laboratory animal research program experienced a decrease in follow-ups needed in regards to husbandry services and a quicker completion of daily tasks due to clear and consistent cage status identification. The administrative requirements are minimal in order to implement these steps for faster cage status identification.

P152 Using Tablets to Reduce Paperwork and Streamline Data Entry in the Vivarium

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The research vivarium at our hospital recently introduced a grassroots initiative to implement lean principles within the department. Focusing on continuous improvement and respect for the people doing the work, the goal is develop critical thinking skills

about how work is being done and continue looking for ways to make it better. In this case study, you'll hear about how the department took a simple idea—to equip technicians with tablets to facilitate communication with researchers while in animal rooms—to the next level by developing a series of custom apps that eliminate a bulk of the paperwork within the department. By having technicians enter billable information, like technical assistance and supplies, and request cage cards directly into the vivarium business system, month end invoicing accuracy has improved and over 300 hours of administrative time and $10,000 in efficiencies have been freed up annually.

P153 Using Disposable Prefilled Water Bottles in Reusable Mouse Caging as an Alternative to Mixing Acidified Water for Reusable Bottles

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We needed to provide acidified water to a specific breeding colony, but the number of cages to be used did not justify investing in the typical resources for acidified water use. A technician had discovered that a particular kind of pre-filled disposable water bottle fits into our standard ventilated mouse cage setup (a polycarbonate mouse cage, wire lid, and filter top), which is kept on a ventilated rack. It may be more cost effective to use the pre-filled bottles and will eliminate all the risks associated with having to store, mix, autoclave, lift, transport, and dispense acidified water in reusable bottles. A group of immunocompromised mice were provided the pre-filled disposable water bottles at cage change. The biggest concern was that the animals would chew through the bottle leading to moist cages and possible health concerns such as hypothermia. After a month of testing and no signs of distress or loss of life, the entire colony was provided these bottles. The cost of using the pre-filled disposable bottles is a bit higher vs. the labor and supply cost of reusable bottles. However, there are unanticipated costs such as bottles and tops that need replacing, loss of animal life due to water leakage, and the cost of ergonomic injuries incurred by staff. We have documented reports of injuries incurred while assembling and using reusable water bottles and water pouches. The pre-filled bottles are an improvement in the process in terms of reduced labor time, improved ergonomics and employee safety, and quality assurance from the manufacturer; as compared to the potential for inconsistency when prepared inhouse. The reliability in quality of the pre-filled acidified water bottle is also an important factor for this research study involving special-needs animals, where consistent quality is imperative to good lab practices and study results. Therefore, the pre-filled disposable bottles are an improvement over reusable bottles for acidified water in terms of ergonomics, good laboratory practices, and animal welfare.

P154 Embryo Disinfection Protocols Used by Research Institutions Housing Zebrafish: Survey Results

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Zebrafish are increasingly utilized as animal models in a variety of scientific disciplines; however, standard biosecurity and husbandry practices for this species in the research laboratory are still being determined. Embryo disinfection is used by aquaculturists to reduce or eliminate pathogens from the surface of fish embryos to improve embryo survivability and overall colony health. Disinfection may eliminate extraovum pathogens discharged from fish during spawning and those present in the aquatic environment. While various disinfectants are employed, sodium hypochlorite solutions (chlorine solutions) are most commonly used due to availability, low cost, and reported efficacy. Unfortunately, protocols for surface disinfection are not standardized and toxicity has been reported for some concentrations of chlorine solutions. In order to understand common practices for surface disinfection of embryos and to assess the variability in embryo disinfection protocols among zebrafish users, we developed and circulated a computer-based survey on the Comparative Medicine listserve. There were 58 respondents from different academic, government, non-profit, and commercial institutions both in the U.S. and abroad. Respondents included veterinary and aquatics staff, facility directors and managers, and investigative staff. Chlorine solution was used by 51/58 respondents (89%) for embryo disinfection, but there was significant variability in the duration of chlorine solution exposure, the concentration of chlorine used, embryo stage during chlorine exposure, type of rinse solution used, and the use of additional agents, such as sodium thiosulfate or methylene blue. For example, 43.5% of respondents used a 10-minute total duration of chlorine solution exposure, 30% used a 5-minute exposure and 19.6% used less than 5 minutes of exposure. Most respondents (77%) disinfected embryos between 6 and 28 hour post fertilization. This variability supports the need for additional controlled studies to determine optimal embryo exposure time to chlorine solutions, the potential toxicity/value of additives, and possible alternative disinfectant solutions.

P155 Speaking of Research

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Speaking of Research (SR) aims to provide accurate information about the importance of animal research in medical and veterinary science. Informed discussion is imperative to understanding differing points of view, but all too often the voice advocating the value of ethically conducted scientific research involving animals is absent. Scientists and laboratory animal science professionals (LASP) each have a crucial role in educating the general public and policy makers regarding the importance of this work. Scientists are able to provide unique insights about how and why they use animal models. Why is it important? How will animals and humans benefit from the knowledge that is gained? LASP are able to communicate the conditions in which the animals in scientific studies live. How are they cared for? Who looks after them? Are they treated with compassion and respect? SR believes that animal research should be conducted with the utmost care, responsibility, and respect towards the animals. Another important avenue is describing the current threats to scientific research. Activist infiltrations of animal facilities often misrepresent conditions in the laboratories they film, significantly undermining public trust and support for scientific research. Animal rights groups have been known to exert pressure on individuals and companies in an effort to stop animal experiments. Recently this activity has moved into the political arena with animal rights groups lobbying for legislative reform or challenging research in courts. Another significant threat to scientific research has been the progressive decrease in funding for fundamental or basic research. At the same time, increasing cost and regulatory burden, sometimes without evidence of meaningful improvement in animal welfare, challenge the conduct of science. All of these threats require proactive discussion between scientists, LASP, policy makers, and the general public. SR believes that accurate information is necessary to underpin honest discussion surrounding the role of animals in science.

P156 Blood Collection and Restraint for Swine in a BSL3-Ag Environment

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Blood collection from swine is a routine task for animal laboratory technicians. Restraining swine in a conventional environment for blood collection can be a challenge due to the size and strength of the animal. The task becomes even more difficult inside a containment environment, as personal protective equipment (PPE) restricts movement and vision. The need to maintain the integrity of the PPE also becomes a factor. In addition, because needle stick injuries are a greater concern in a containment environment, finding the safest technique is paramount. We evaluated 3 restraint methods during a study where we collected blood from the anterior vena cava in swine in a BSL-3Ag animal room. One method involved the restrainer inverting the swine and lifting each of the fore limbs up and apart while the person drawing blood controlled the head. In the second method, the restrainer placed the hindquarters of the pig between their knees while the forelimbs were held with one hand and the head controlled with the other. The third method used a bell-end snare to restrain the swine and the samples were taken with the animal standing. The second form of restraint was preferred over the first. Because the rear legs were not restrained in the first method, they could compromise the PPE by tearing the coveralls or the powered air purifying respirator (PAPR) hood. The second method eliminated the hood issue, but if the pig's rear legs were not placed properly between the restrainer's knees, the animal's hooves could catch and tear the coverall PPE. There was also a greater chance of being stuck with the needle in the first method compared to the second as the person drawing blood also had to help restrain the animal's head. The third method, with the bell-end snare, was the safest because restraining the animal did not present risk for compromising the PPE. The swine's natural instinct to pull away from the snare provided the needed restraint. Also the needle stick could easily be done with one hand. Of the three methods, the snare method worked best in the containment setting due to the lack of risk to PPE and the safety of the needle stick technique.

P157 Surgical Animal Models in Support of the 3Rs

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The 3Rs (replacement, reduction, refinement) are the foundation of animal welfare in research. Animal models are critical for studying xenobiotic metabolism, biomarkers, and pharmacological/toxicological effects on the body. Alternatives for the replacement of animals in research are invaluable; however, there is still a need to use live animal models and refinement of methods which reduce the number of animals used are of critical importance. Acute surgical models are available which can be used for sampling of various matrices nonterminally, but these are limited in number of samples and robustness/duration of the model. We have developed surgical techniques for portal vein- and recirculating bile duct-cannulation in dogs and lumbar laminectomy in nonhuman primates (NHP) resulting in maintenance of animals in a colony setting for reuse without compromising animal welfare. We have combined multiple models (portal vein-cannulation and laparoscopic liver biopsies) to further decrease animal use while maintaining the highest quality standard of animal care. Portal vein-cannulated dogs have maintained bidirectional patency for >1 year; recirculating bile duct-cannulated dogs have maintained patency for 6+ months. Lumbar laminectomy NHPs have maintained bidirectional patency for up to 6 months. In all cases, animals recovered completely following surgery, are BAR (bright, alert, responsive), eating and mobile on the same day of surgery, and display no signs of pain or distress, indicating exceptional pain management. Animals are commingled in accordance with SOPs and there have been no significant changes in body weight or clinical pathology and no surgical complications. Study designs allow for serial samples of each matrix, as applicable, resulting in complete data sets for individual animals and overall animal use has been decreased substantially. Ultimately, these models provide refined surgical methods to reduce overall animal use without compromising welfare providing a significant advantage over current techniques and supporting the 3R philosophy.

P158 Hung up on Training? Calendar Reminders for Animal Care Procedures

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In our search for innovative and effective training tools for animal care staff, an adjunct to printed standard operating procedures and hands-on sessions was desired. Within animal facilities, reminder signs and postings are placed with best intentions, but often users become "sign blind" and the information may no longer be recognized as current or pertinent. Using principles of effective frequency, an initiative was undertaken to deliver information in a novel manner. Effective frequency is a quantification of the number of times a person receives a message until they take action, but prior to the message exposure becoming wasteful. With the start of a new calendar year (2015), single-page training aids were inserted into a hanging calendar template, with additional departmental reminder dates included. New illustrations featured various topics with emphasis on critical areas, for example, topics have included: breeding notification flags, feed storage logs, monitoring water bottles, reporting veterinary concerns and disinfectant use. Using 11 x 17 inch sheets of paper, a single month's calendar is printed on the bottom half and the training topic is printed on the top half of the page. A survey was sent to all staff and 89% responded (n = 19). The most commonly liked features were the illustrations and one-page summaries. Individual topics consistently scored highly for helpfulness, ease of understanding, and having enough detail. Overall, respondents agreed that a one-page training topic with illustrations is helpful. Based on the feedback we have reduced the number of calendars printed for every room and placed them in more centralized locations (by phones and in break rooms) for team discussions. The decision to release calendar pages separately by month has proved beneficial, allowing updates as we find errors in certain facility operations, or as training priorities change based on new staff hires and new initiatives.

P159 Training by the Book: Weekend Binders for Improved Communication

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Campus animal resources (CAR) includes a dedicated weekend animal care staff that oversees health and wellness checks within housing rooms (n = 86) across 9 distinct decentralized vivaria. Each housing facility has specific requirements; including the level of PPE, the type of rodent caging, altered light cycles, specialized feed, biohazard usage, and husbandry for a variety of species. We noticed that key details of specific rooms and experiments were not being communicated clearly to the Saturday/Sunday (weekend) staff. To resolve this in a consistent manner, we created a detailed weekend binder with one assigned supervisor to assist with questions each weekend. Binder updates are prepared weekly and include contact information, SOPs, instruction sheets by building, by room, and by individual investigators that may have certain husbandry requirements. The binder is designed to be interactive, as we expect staff to record notes, questions, and issues for review during the next week by the supervisory team. The weekend binder travels with the team of staff that work together to cover the animal rooms across campus and is returned to a centralized location at the end of the shift. The collective total labor for weekend teams went from 26 hours to 17 hours, a reduction of 9 labor hours per day, and 18 hours per week. Communicating updates and changes to weekend staff is now a regular part of meetings and all staff assist in weekend preparations. The weekend binder has become an essential part of our communica-

tion between supervisors and staff and assists with instilling departmental confidence that all animals are well cared for under the same husbandry and experimental conditions both during the week and on weekends. In addition, weekend binders are now used for holiday and vacation coverage, and are available for essential personnel that work during emergency weather closings.

P160 Development, Implementation, and Training of a New CO_2 Cabinet

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Recently, in one area of our vivarium, the need for an updated, high capacity, easy to operate euthanasia station that met the 2013 AVMA Guidelines for the Euthanasia of Animals was identified. Commercial euthanasia stations were considered due to their ease of operation, high capacity, and immediate availability but expense prohibited purchase. Therefore a multi-unit, vertical cabinet system with independent CO_2 flow was designed based on our estimated capacity, to fit the available space within the room, and for operational ease. The design included 4 cabinets that would fit up to 13 mouse shoeboxes at one time. Each of the 4 cabinets was equipped with its own flowmeter that was set to the appropriate flow volume and a separate valve to turn flow on or off at the cabinet level. After calibration, the flowmeters were locked out by covering them with a transparent plastic container to allow visualization of the flow but the inability of users to change it. Only authorized individuals are able to open this chamber if calibration becomes necessary. The unit is easy to use and requires only that researchers turn on the appropriate flow valve and then turn on the CO_2 at the supply tank level. We also developed a mandatory training program for all users that incorporates all aspects of the euthanasia process. To prevent use of the cabinet by untrained individuals, the valves on the CO_2 supply tanks were locked out with a combination lock system and users only gained access to the combination after completing the mandatory training class. This allowed us to not only ensure proper use of the cabinet system, but it also helped us develop a contact list for users in the event of problems or to update procedures. To date the system remains functional, PI feedback has been positive, and continued training has been ongoing to catch new employees and those wishing to be retrained.

P161 An Approach to Alleviate Food Aggression in Socially Housed Dogs

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In the animal resources group there have been ongoing efforts to continually improve the animal programs and facilities. Recently at one of our newly renovated dog facilities, a new caging system was designed by a group of animal care, scientific, and veterinary staff. One of the primary objectives was to provide the dogs with additional visibility within their cages to contribute to social housing. To achieve this goal, dividing doors between kennels were designed with clear tempered glass panels to allow better visualization when separated from cage mates. An unforeseen negative outcome to this design however, was that dogs were able to view each other while separated for feeding time. This led to expression of food aggressive behaviors by some dogs within the colony. Several ideas for solution were considered, including replacing the doors with tinted or opaque glass, using different door panels, or using magnetically attached covers to block visibility between cages. These solutions all presented a variety of other challenges. Since the adverse behaviors were restricted to certain animals within the cohorts, the solution to be implemented needed to be low cost and thus requiring minimal manufacturing. We found a simple solution by using a repurposed rabbit divider that allowed for the temporary placement of a visual barrier between potentially food aggressive dogs during feeding time. This method utilized minimal modification of equipment already present in the facility. The panel was easily movable, could be placed or removed as desired, and was easy to sanitize. In the areas where implemented, the dogs have ceased displaying food aggressive behaviors towards their neighbors during feeding time. They have not shown any destructive activity towards the visual barriers. We have concluded that it is very practical and cost effective to design and use dog kennels with visibility between individual animals, while at the same time having the means to control adverse food aggression behaviors.

P162 Uptake of the ARRIVE Guidelines in Scientific Reporting: How Well Are AALAS Journals Doing?

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The Animals in Research: Reporting In Vivo Experiments (ARRIVE) guidelines were first published in 2010 by the NC3Rs to encourage improved reporting of animal research to enhance experimental reproducibility and emphasize the 3Rs in experimental design. The checklist outlines the minimum information to be included in all publications reporting research results, such as experimental design, animal number considerations, husbandry, etc. Following publication, the ARRIVE guidelines were endorsed and adopted by more than 300 international scientific journals and funding agencies, including the AALAS journals. Despite widespread support, adoption and actual use by scientists, reviewers, and journals is thought to be limited, but no study has formally investigated this. The current retrospective study was undertaken to assess the implementation of the ARRIVE guidelines by AALAS journal (Comparative Medicine [CM] and JAALAS) authors and reviewers. To account for a possible lag from ARRIVE guidelines publication to uptake, only papers published in 2013 and 2014 were assessed. Further, only hypothesis-driven, original research in which in vivo studies were conducted were included, for a total of 132 papers (82 JAALAS, 50 CM). Each item was converted into a binomial (yes/no) format and subjective questions were graded on a numerical scale (0=not addressed, 1=partially, 2=fully addressed). When evaluated out of 67 total possible points, mean scores were 62% for JAALAS and 57% for CM, with no articles from either journal addressing all items. In terms of missing information, items pertaining to animal numbers (including randomization and statistics) were most frequently missing from both journals, with scores of 63% and 54%, respectively. Both scored highest (~90%) for inclusion of items in the title/abstract section. In summary, there is good uptake of the ARRIVE guidelines in scientific publications within AALAS journals but there is room for improvement. In general, details pertaining to animal numbers are often incomplete. To ensure consistency in reporting and experimental reproducibility, AALAS journal authors and reviewers should be encouraged to more closely adhere to the ARRIVE guidelines when submitting or reviewing papers, respectively.

P163 Sex in the City: Housing Strategy and Reproductive Performance in C57BL/6J Mice

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Continuous housing of a mouse breeding trio (2 females and 1 male) with up to 2 litters present in a small shoebox cage (total floor space <68 in^2) would exceed space recommendations listed in the 8th edition of the *Guide*. Our lab has previously reported that in colonies housed in small shoebox cages, breeding strategy (pair versus trio breeding) may not alter reproductive performance in some geneti-

cally engineered mouse strains. Recently, after obtaining IACUC approval, we evaluated if C57BL/6J mice reproductive performance for a continuous breeding trio is altered by cage size (small, total floor space of 67.6 in^2 [436 cm^2] versus large, total floor space 112.9 in^2 [727.8 cm^2]). We also evaluated whether reproductive performance is altered by breeding strategy in small shoebox cages. Age-matched breeders were evaluated for 6 months and included 5 pairs in small shoebox cages, 3 trios in small shoebox cages, and 5 trios in large shoebox cages. Data was collected to evaluate pup survival rate, production index (number of pups weaned per female per week), birth and weaning weights, and the number of male and female offspring weaned between 21-25 days of age. There were no differences in pup survival rate, production index, and weight at birth and at weaning. The sex ratio observed at weaning was even between male and female offspring from trios housed in large shoebox cages (n = 146 progeny from 10 female breeders). However, we observed some variability in the sex ratio at weaning between breeding pairs and trios housed in small shoebox cages, with 6% more females being weaned from breeding pairs (n = 172 progeny from 3 female breeders) compared to trios (n = 110 progeny from 6 female breeders). Although our data suggests that neither breeding strategy nor cage size may alter reproductive performance in C57BL/6J mice, some interesting trends regarding a greater ratio of female to male offspring at weaning were observed. Interestingly, researchers have observed the opposite finding in ground squirrels, where more male than female offspring were weaned. Future studies are needed to confirm these observations and to determine what husbandry factors (diet, housing density, preweaning mortality, health status of dam) may be contributing to sex ratio at weaning.

P164 Most Common Challenges with Cleaning and Decontaminating Laboratory Animal Cages

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Washing and sterilization systems are often used for critical cleaning, drying and decontamination of cages, racks, and other items associated with the care of laboratory animals. Optimizing cycles and loads to obtain the best out of these pieces of equipment can sometimes be a challenge. A good understanding of basic principles of washing and sterilization can help avoid typical mistakes which can lead to inconsistent performance, lower productivity, and increased operation and maintenance costs. Such knowledge represents an important step toward operational excellence. This poster reviews the various challenges faced by facility managers when trying to reduce energy consumption, improve safety and reliability, and optimize performance of their washing and sterilization systems. Best practices for overcoming these challenges and avoiding typical mistakes will be presented.

P165 Containment, Enrichment, and Source: Maintaining Happy, Healthy Crayfish

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Maintaining large numbers of crayfish for research purposes recently became a challenge for our small animal facility. We currently house 3 species of crayfish, *Procambarus clarkii* from a southern supplier, a self-sustaining breeding colony of *Cherax destructor,* and *Marmokrebs,* a parthenogenetic animal model. We house the *Marmokrebs* in a 50 gallon recycling aquarium with a small substrate, clay builder's bricks with multiple holes, plastic plants, PVC tubes and aquarium ornaments. By providing numerous hiding places, we have reduced mortality and allowed for higher density populations. The *C. destructors* are maintained in a large plastic trough fitted with an external pump. These animals also have a gravel substrate, PVC tubes, broken crockery, and plastic plants for enrichment. The need to separate shipments of *P. clarkii,* as well as experimentally manipulated animals, led to the conversion of two 100-gallon former lobster holding tanks to fresh water tanks. Additionally, a third system was constructed on a movable rack with 3 fiberglass shelves and 2 external filtered pumps. Crayfish thrive equally in the 5 inch water depth of these shelves as they do in our deep water tanks. The presence of plastic plants, PVC tubes, and other hiding places such as coffee mugs and broken clay pots allow the animals to establish their territory and reduces cannibalism. Discovery of a quality supplier coupled with the conversion and utilization of other various sized tanks has enabled us to consistently maintain large, healthy colonies of crayfish for our research program.

P166 Lab Animal Technician: The Dual Vocational Training Concept in Germany

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Germany has a long standing tradition in its dual vocational training which is unique when compared internationally. This dual vocational training system is based on compulsory schooling, including 13 years of education in general. Lab animal technician training represents an excellent example of this dual concept in Germany. In general, this education is comprised of 3 years of training, split between vocational training school (1/3 of time, theoretical based) and on-the-job training in a company or institute (2/3 of time, practical based). Additionally, the trainee has to follow comprehensive practical courses organized together by the training companies and institutes. In spite of the great benefit of the practical and theoretical training of young trainees, this dual training path does uncover disadvantages. The very strict and regulated curricula of the classic dual vocational education guarantees high quality levels, however, due to the vast restriction (up to 90%) of training conducted with transgenic mice under access restricted SPF conditions, it is overly time intensive. This negatively affects the capacity for educating trainees to meet the overall high demand for lab animal technicians, and often results in a situation where the majority of animal facility personnel has other professional backgrounds. For example, veterinary technical assistants will receive on-the-job training. For this reason, our institute established additional job training, based on AALAS' educational training, including a curriculum with more orientation to our individual needs in laboratory animal science. Through different training opportunities, the lab animal technicians following our training form achieve a balanced training that is equivalent to the traditional dual vocational training.

P167 Determining True Cost of Services Provided by a Transgenic Core: A Simple yet Powerful Approach

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"Why are your facility service rates so high?" is a common question from investigators. It is important to have a defensible answer based on actual cost analysis. Many programs, however, set rates based on cost estimates. Consequently, true costs may not be known. It can be challenging and time consuming to accurately evaluate true costs for services. In order to overcome these difficulties, we utilized an effective and efficient approach using a time-driven activity-based costing (ABC) model to determine cost of services provided by a resource laboratory at our institution. The time-driven approach was implemented by evaluating 2 parameters: 1) the time required to perform an activity and 2) the unit cost of the activity based on employee cost per minute. This method allowed us to rapidly and accurately calculate the true cost for services provided. We calculated

the quantity of activities and the time needed to perform activities such as microinjection of DNA construct or embryonic stem cells, embryo transfer, and in vitro fertilization (IVF). A time-driven ABC model was easily and successfully implemented to evaluate the cost of these services and the capacity of labor utilized to deliver these services. Calculations of the costs for a standard IVF using this model demonstrated that the true costs are roughly equivalent to the current service rates. Costs and rates for other services will also be discussed. We also determined that the labor supplied to conduct all services (10,860 min/wk) exceeded the practical labor capacity (10,080 min/wk) indicating that the laboratory team was highly efficient. In addition, our calculations indicated that more capacity may be needed if there is an increase in demand for services. Importantly, this time-driven ABC approach allowed us to establish a baseline model that can be simply updated to reflect operational changes or changes in labor costs. We demonstrated that the time-driven ABC model is a powerful management tool that can be applied to other core facilities as well as to entire animal programs, providing valuable information that can be used to set rates based on actual true cost of services and to improve operating efficiency.

P168 Standardization of Assessment of Veterinary Technician Workloads to Ensure Equitable Distribution

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Veterinary technicians are a critical component of the clinical team. Quantifying the workload of the veterinary technician group can be more challenging than that of other animal care and use program (ACUP) team members. Cage census is a useful measure that enables institutions to quantify and distribute the workload to animal care technicians and cagewash technicians. This method uses statistical averages to distribute the workload based upon a maximum and minimum number cages assigned to an individual within an 8 hour work day, leaving time to complete additional duties as assigned. However, cage counts alone are not always indicative of the number of clinical cases within an institution or a given vivarium. Different clinical cases may require more effort than others. This could be due to the complexity of the case, the setup of the vivarium, or an individual's experience with the case type. In an effort to distribute workloads in an equitable fashion, quarterly snapshots of clinical data, in addition to census counts, are reviewed and compared to facilities of similar sizes within our ACUP. This data is used to analyze how many active cases there were within a specified time frame, how many new cases per day occurred, and what types of clinical cases and treatments were recommended. After the data has been collected, it is paired with the census count and analyzed to determine if the facilities are equitable in workload. This information, in addition to personnel experience level and travel time between facilities, is used in scheduling veterinary technician work assignments. At our institution, we have found it valuable to periodically evaluate this data and make scheduling adjustments accordingly. Conducting quarterly analysis of this data helps identify trends and ensures an equitable workload throughout the year.

P169 Utilization of a Self-Assessment Instrument to Facilitate Cross-Training Opportunities and Enhance Training Proficiency

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Institutions are responsible to provide appropriate education and training to all personnel involved with the care and use of animals. Managers should be familiar with their employees' individual skill sets and ability to train others. Even those employees that have training experience may lack comfort with certain subjects or techniques, or have a phobia of public speaking, which can lead to inability to provide proficient instruction. If managers do not have this information and understanding of their team, this may result inadequate provision of resources to facilitate successful training. In order to gauge the comfort level of the trainers for rodent techniques courses at our institution (for example. handling, restraint, injections, and blood collection), a self-assessment instrument was developed to survey the training team. This survey, using a Likert scale, analyzed the comfort level of the employee performing, understanding, demonstrating, and instructing techniques taught in the training classes. Individuals that were not comfortable performing techniques were cross-trained with staff members that indicated they were proficient performing and instructing that technique. After the employee was trained in areas marked with low comfort level, their comfort levels in those areas were reassessed. Marked improvement in confidence and proficiency was noted in all employees that were trained on techniques and skills with which they previously felt uncomfortable. Building on the progress of the cross-training and improved metrics, a technical task list was implemented, which requires the staff to practice each technique monthly. This ensures that the team stays familiar with the techniques they do not routinely perform. In the future, any changes to the staff or training courses will result in a new survey that will be used to determine the need for cross-training and reassessment of proficiency.

P170 The Role of Veterinary Support in the Animal Biosafety Level 3 and 4 Laboratories

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The support role of the veterinary branch in infectious disease research has proven to be invaluable, and has supported many important missions, including evaluating new innovative vaccines and therapeutic strategies to combat the recent Ebola outbreak. However, as biosafety levels increase, the complexity of the roles of the veterinary branch also increases. To support these studies in animal biosafety level 3 and 4 (ABSL-3 and 4) laboratories, every function of the group has to be reevaluated to allow the husbandry and technical staff, veterinarians, and pathologists to work safely, efficiently, and effectively in these unique environments. To accomplish this, teams were formed from the veterinary branch, scientific staff, and biosafety to evaluate the training programs, caging systems, husbandry practices, refinement techniques, clinical evaluations, data collection, necropsies, and sample processing. The teamwork between the different entities involved in the high and maximum containment laboratories enabled our veterinary program to establish safe and workable solutions to support studies in the complex environments of ABLS-3 and ABLS-4. Research investigators recognize and appreciate the skill and value added by well-trained animal care staff and this collaboration has contributed to improved study design, data collection, and published manuscripts.

P171 Canine College: Socialization of Teaching Dogs for Animal and Human Welfare

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Veterinary students participate in a variety of skills laboratories that provide hands-on experience in the handling and physical examination of dogs. A stock colony of purpose-bred research dogs is maintained to help meet this learning objective. Several years ago, concern was raised following receipt of complaints from students and instructors that some of the dogs were fearful, hard to examine, and/or uncooperative during the labs. In response, the Canine College program was started, with the following objectives: condition stock dogs to the tasks asked of them during teaching labs; train dogs to leash-walking and other simple commands (for example, sit, stand, down); enhance enrichment of stock dogs by

increased human contact and activity; and promote behavioral modification and humane handling techniques. The Canine College is a veterinary student-run program with participation of both laboratory animal medicine and veterinary behavior faculty. Students must attend large group and hands-on orientation sessions that provide a framework for the Canine College objectives and positive reinforcement based training approach, an overview of the regulatory environment, and an introduction to biosecurity and the health and safety issues associated with working with animals. Following orientation, students sign up for 30 minute sessions (one dog at a time) that involve walking, behavioral conditioning, appropriate play, and/or physical contact (petting). Students document their participation using a group access Google Drive document, including progress and challenges for each dog they interact with; this serves as a record of improvement, as well as working notes for the next person who works with a given dog. The Canine College program has had a significant impact on the laboratory experience for both students and dogs, and has had the added benefit of helping to prepare dogs for adoption following their service to the teaching program.

P172 Handling the Feisty Ferret

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In order to properly house and interact with ferrets, their naturalistic attributes should be taken into consideration. These include their curious behavior, desire to hide and burrow, and their tendency to bite. Our enrichment program incorporates enrichment in their housing that caters to their specific behaviors. To help dissipate the ferret's natural instinct to indiscriminately bite from fear of being handled, we implemented an acclimation program followed by ongoing reinforcement of behaviors. Upon arrival at the facility, up to 3 ferrets are socially housed along with prepopulated enrichment items. The enrichment items consist of one hammock, at least two items for gnawing and playing, and one item for hiding and sleeping. These items are changed out once a week on a rotational basis. A paper bag is placed in each cage once a week for at least 24 hours for the animals to shred, hide, and burrow. The husbandry staff is also tasked with providing socialization time with each ferret. Each animal is taken out of its cage one at a time once a week for 7 minutes. The process consists of handling, restraining, nail trimming, weighing the animal, and play time. After a few weeks of acclimating these animals, there is a significant reduction in the amount of biting and less stress for the animals and the husbandry/research staff. The animals are more comfortable and come up to the front of the cage when the handler opens the cage. It is a huge improvement from when the ferrets first arrived and they appear well adapted to their environment. A well-developed relationship between humans and ferrets encourages behaviors that ensure the health and wellbeing of both. The reduction of stress by an acclimation program, followed by regular staff interactions with the ferrets, reduces the chances of bites. The research staff is able to do their job easier and also get quality data.

P173 A Creative Approach for Reducing Cost of Delivering Medicated Water in a Mouse Facility

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Medicated water is used for mice in several projects/studies including, but not limited to, inducible gene expression, pain management, antibiotics, and preventive care. Our facility requires antibiotic water for immune deficient mouse colonies (NSG/NRG) at every 3–4 months based on inhouse colony performance and clinical signs. Traditionally we used enrofloxacin injectable as solution in water bottles; however we did not calculate the costs, as the need was sporadic. With current increase in our NSG/NRG colony we realized that injectable enrofloxacin was expensive. In addition, we currently have a pouch system for water delivery that gave us flexibility and an opportunity for alternative methods. We made 2 different changes for reducing costs: 1) we switched from injectable to oral enrofloxacin tablets that were crushed and dissolved in normal saline as drug source; 2) we reduced the water pouch size from 500ml to 200ml minimizing wastage of unused drug. The drug was administered at 200 mg per liter of water and injected into pouches using a silicone patch, this mechanism is also cost effective from labor perspective compared to water bottles. Decreasing the pouch size and therefore the medication amount resulted in an immediate savings of $5,952 per year. Switching to oral tablets (crushed and dissolved) from injectable drug resulted in savings of another $3,033.60 per year. Overall, with the new process, we saved $8,985.60 per year from drug costs alone. Labor, water savings, and cage wash savings are not factored at this time, if we had to deliver in water bottles.

P174 Evaluating Maternal Competency and Infant Vitality in Baboon Mother Infant Pairs

S Doan*

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A system to monitor, score, document, and evaluate maternal behaviors and infant vitality was developed for the baboon breeding colony. This system generates scores for maternal behaviors and infant vitality by comparing observed mother-infant behaviors with specific behaviors that have been demonstrated by successful mother-infant pairs. This scoring system was needed to help technicians calculate the expected survivability of baboon infants and, thereby, decide whether or not it has become necessary to remove an infant from maternal care. This technique may also help to illuminate any behavioral changes of maternal aptitude and any effects that infant vigor might have on her maternal behaviors. This presentation is intended for animal care technicians and NHP colony managers. It will provide them with a method to score maternal competency and infant vitality in a template that can be tailored to their own animal health surveillance program.

P175 Low-Cost Enrichment Items Bring Unintended Benefits for Staff and Subjects

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During an annual review of our enrichment program, management challenged the staff to identify novel enrichment items without increasing our budget. The staff worked together and found a source of nesting material in the inner lining of our bedding bags. We found that this paper was autoclavable, easily shredded, and sufficiently available to provide nesting material. Other than the purchase of a midgrade shredder to produce strips, our supply cost was zero. Management identified this project as a suitable task for technicians on light/modified duty. Staff developed several approaches to molding ~7grams of shredded strips into paper balls that could easily be distributed into cages at the time bedding was dispensed. The compression molding of the shredded paper increased the quality of nests and the time spent building the nests. Mice have built nests scoring from 1–4 based on the naturalistic nest scoring system, implying that a good nesting material has been provided. Several hyperactive strains of mice showed a decrease in stereotypies and ceased chewing on mouse huts and balconies, saving funds spent to replace damaged enrichment materials. This successful initiative prompted all of our staff to become more involved and interested in participating in the enrichment program. More importantly, the staff developed an increased knowledge of normal animal behavior, and the project has spurred conversation about wild mouse behavior in order to better replicate it in the research environment. We are now

considering phasing out the use cotton pads in favor of the shredded paper, further increasing our cost savings.

P176 3D Printed Custom Wrenches Improve Disassembly Time and Reduce Worker Strain while Cleaning Rodent Drinking Valves

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One year after opening a new animal facility, our husbandry technicians were tasked with disassembling the systems engineering rodent drinking valves for annual cleaning. However, staff reported hand and wrist strain when attempting to pull the nosecone from the body of the valve. Our facility guidelines require the cleaning and inspection of approximately 2000 valves annually to comply with the vendor recommended maintenance. The rotation system requires approximately 240 valves to be disassembled, cleaned, inspected, and reassembled most months. Our team set out to develop an inexpensive tool to decrease wrist and hand strain without damaging the valve. Initially the team requested the biomedical engineer to help construct a tool similar to a common electrician tool to grasp the valve nosecone and base before pulling the two apart. With further input of the animal technicians, we were able to use a 3D printer to make two separate wrenches that achieved our goal. One wrench acts as a stabilizer for the body and the other allows the user to twist and remove the nosecap from the body. We reduced disassembly time by approximately 45 seconds per valve and have not received any reports of wrist or hand strain with the new wrenches. A key benefit to using a 3D printer was the lightweight yet strong design of the wrenches that prevents bending or scratching the nosecone. Each wrench has a material cost of $0.75 and disassembles approximately 300 valves before showing signs of wear. The biomedical groups continue to make adjustments to the design to help improve the lifespan of each wrench.

P177 Troubleshooting Aggressive Behaviors in Pair-Housed Rabbits Using Environmental Enrichment

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The 8th edition of *the Guide For The Care And Use Of Laboratory Animals* states that single housing of a social species should be the exception to housing standards. Based upon these guidelines, we have developed a process of pair housing our colony of New Zealand white rabbits. Adult nonrelated females, in addition to male and female related weanlings, have been effectively paired using our pair housing process. Previous studies have found adequate environmental enrichment may reduce anxiety and stress reactivity; therefore, we hypothesized that environmental enrichment would facilitate greater success in pair housing by decreasing aggressive behaviors that arise around the age of sexual maturity (12–17 weeks). We currently have 23 successful pairs in our program (13 female and 10 male pairs). We have been able to maintain pairs through sexual maturity with our oldest current male and female pairs being 42 and 43 weeks old, respectively. We carefully track the rabbits' ages such that we can increase enrichment around 12–17 weeks and closely observe the rabbits for visual cues of behaviors that may precede aggression. In our experience, these behaviors include excessive urine spraying, barbering, and increased chasing. All cages are provided with at least 1 toy plus food treats as approved by our environmental enrichment committee. However, if any of these aggressive behaviors are observed, extra enrichment is added to the cage from that point forward. Typically this additional enrichment includes loose hay daily in addition to novel interaction items 3 times per week (cardboard, or paper bags, or boxes stuffed with hay, or food treats). Even after male pairs were found with minor fight wounds, we were able to deescalate aggressive interactions and maintain the pairs with increased enrichment. Additionally, adding extra enrichment prevented the reoccurrence of new fighting-related lesions. Based upon these successes, we recommend institutions that pair New Zealand white rabbits use increased environmental enrichment to decrease aggressive behaviors and increase the number of pairs that are maintained successfully past sexual maturity.

P178 Efficiency of Cold Water Cagewash and Sanitation

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The Animal Welfare Act requires that hard surfaces of primary enclosures be sanitized with hot water (at least 180 °F [82.2 °C]) with soap or detergent, by means of a mechanical cagewasher. This is also comparable with the *Guide* stating to clean primary enclosures with chemicals, hot water (143-180 °F [61.7-82.2 °C]) or a combination of both. Cleaning and sanitizing animal caging and equipment is crucial to the welfare of laboratory animals. Cagewash facilities are the largest consumer of energy and water in the animal facility. Decreasing the washing and rinsing temperatures could have significant impact on reducing energy and steam requirements for research facilities without compromising cage sanitation. Using both an acid wash (product) and an alkaline wash (product), the optimal temperature for sanitation of primate cages was determined. Lower temperatures were tested to determine optimal washing and rinsing temperature while staying within optimal working range of detergents as set by the manufacture. Bioluminescent monitoring device that detects adenosine triphosphate (ATP) was used as an alternative to verify sanitation of cage units. Each cage unit was tested at 4 sites: top wall, top floor, bottom wall, and bottom floor following a template for collecting each sample. A cage had to have a score of 299 RLU (relative light units) or less to be considered properly sanitized. Cages were washed at 120 °F, 140 °F, and 160 °F; each with final 90 second rinse with 160 °F water. A total of 500 samples were collected, with cages having a mean 42.8 RLU at 120 °F compared to a mean of 142 RLU at 140 °F and 62.6 RLU at 160 °F. This study showed that primate cages can be properly sanitized with wash cycles of 120 °F. With the reduction of steam and energy requirements, facilities cleaning with lower temperature water can see significant energy savings.

P179 Maintaining Increased Cage Floor Temperature for Sick and Deficient Rodents

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Maintaining an area of heated floor for animals requiring additional warmth to supplement their own body temperature is especially important when dealing with sick and/or modified (genetically or surgically) mice. Many transgenic strains vital to research can be relatively less healthy and require additional cage warmth while also maintaining a regularly cool area for them to use for regulation. This becomes a problem as there are few cages built and designed for constant temperature variation. In order to achieve this economically and efficaciously, there are modifications that can be done to existing cage models. Using the proper heat strip placed at the proper position will increase cage floor warmth at one end of the cage while allowing the other end to remain within standard levels. A thermostat is used to safely adjust and monitor cage floor temperature. Animals monitored under these conditions survived longer, maintained higher average bodyweights, and exhibited less declining health than animals maintained regularly. Above all, this removes the timely and inefficient use of heat pads and allows isolator cages to be left on the rack for proper airflow.

P180 Demeanor Scoring System as a Tool for Evaluating Behavior in Laboratory-Housed Cats

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Objectively measuring and monitoring demeanor and behavior of laboratory housed cats is of utmost importance in regard to these cats' day-to-day quality of life. Laboratory cat stress levels can affect the animals' health status, ability to reproduce, overall demeanor, behavior, welfare, as well as many research study parameters. Many different behavior scoring systems for cats have been developed over time, but have not been altered for routine use on laboratory-housed cats. A scoring system developed for hospitalized cats was adapted to be used in multiple laboratory cat colonies on campus. Cats housed in 3 different laboratory animal facilities were scored by veterinarians, veterinary technicians, and husbandry technicians using this modified behavior scoring system. The cats scored with this system varied between inhouse bred cats used for acute or long term studies, long term housed breeding cats, vendor-supplied nonconditioned animals for acute use, and vendor-supplied conditioned cats. Cats (n = 62; 11F, 51M) ranged from 8 weeks to 10 years of age, and were housed either conventionally, under SPF conditions, or in ABSL-2 cubicles. The parameters scored included visual assessments from outside the cat's primary enclosure and the animal's response to human touch and handling. Raw scores were converted into the following categorical demeanor scores: friendly, friendly and shy, withdrawn, withdrawn and aggressive, and overtly aggressive. Results between observers were evaluated using a kappa statistic. Categorical results indicate high interobserver agreement (low interobserver variability) between veterinarians (κ=0.71, substantial), veterinarian and veterinary technician (κ=0.59, moderate), and veterinarian and husbandry technician (κ=0.80, substantial). Therefore, this scoring system may be used to measure and monitor demeanor and behavior of laboratory cats as a simple, objective tool by any member of the animal care staff. This evaluation tool can be used to monitor cats, as well as their response to many aspects of their enrichment or environment, and used to improve their quality of life.

P181 Necropsy Lab Makeover

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Management of available resources in a necropsy laboratory is an essential component of an efficient health surveillance program for an animal research facility. Lab disorganization can lead to lost productivity, depleted supplies, and excess supply wastage. This poster describes lessons learned in inventory and supply management. During initial occupancy of the lab, there were minimal standard operating procedures in place for lab maintenance and no organizational structure to coordinate resource needs. A necropsy lab makeover project was initiated in order to organize the lab, and standardize storage and supply restocking procedures. This makeover led to greater organization, efficiency, and use of innovative methods to make the lab more functional for all users. Also, the project led to more consistent communication among the staff members assigned to maintain the lab and greater customer service to cross-functional groups using the facility.

P182 A Novel Device for Locally Heating Ventilated Rodent Cages

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That the rodent's thermoneutral zone is higher than man's (~30° vs. ~22° Celsius, respectively) has been known for decades, as well as potential adverse effects on animal welfare and research data when vivarium rooms (and cages) are maintained at temperatures comfortable for personnel rather than mice and rats. But, it is neither affordable nor desirable to keep entire housing rooms at rodent thermoneutral zones. Thus, a simple device was devised to bridge that gap and provide variably heated air locally to ventilated rodent cages, if welfare or experimentation so justify, while rooms can remain comfortable for husbandry staff and investigators. This device is inexpensive, durable, and easily sanitized, operated, installed, and detached for maximum flexibility and minimum cost. Physical, biologic, and operational features of this local cage air heating device will be described, along with application options.

P183 Initial Observations on Connected Cage Communities of Mice

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A recent enhancement of IVC rack housing that permits up to 10 adjacent cages to be connected by commercially available external tunnels provides new opportunities to explore laboratory mouse behavior and resultant impacts on husbandry efficiencies. Four hundred 8-week old CD-1 mice were housed 5 males or females/cage for over 8 weeks, in accordance with space recommendations in the *Guide*, in cages either isolated or linked for an entire row so that 50 mice shared the same expanded space. Animals were individually identified via tail tattoo and observed daily for general health; social compatibility, nesting behavior, and body weights were documented weekly. Degree of cage soiling was regularly assessed, independent of the maximum interval of 2 weeks between changing as conventionally performed. Mice were observed moving freely between connected cages. At cage change, numbers ranging from 0–20 mice were found in each connected cage. Some males used connecting tunnels as perches to observe and sometimes impede other males entering the cage. Females in connected cages were more likely to create group-nesting cages, collecting commercially available paper from other cages while removing corncob bedding out of those nesting cages. Most males occupied and heavily soiled only a few connected cages in a row while other cages on the same row appeared untouched. Food consumption in connected male cages appeared to follow a pattern where several cages with empty food hoppers were adjacent to each other and cages with full hoppers were adjacent to each other. These and other details will be presented, along with consideration of this caging arrangement to improve production of fragile phenotypes via collective rearing and for use in studies with behavioral endpoints, in a space-efficient manner.

P184 A Comprehensive Temperature Monitoring and Reporting System

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Real time monitoring of temperatures and managing temperature deviations in a multisite research institution operating under HVAC systems varying in age and reporting capabilities can be quite challenging. Conventional (min/max thermometers) means of monitoring temperatures provided retrospective performance, but lacked in providing a prospective solution to mitigate out of range temperature excursions. The plan was to minimize, if not eliminate, critical temperature excursions of a given set point that could potentially lead to animal health issues or experimental alterations. The goal, in accordance with *The Guide for the Care and Use of Laboratory Animal: 8th Edition* (the *Guide*) was the implementation of a comprehensive system that included the following points:local and remote monitoring 24/7; HVAC temperature performance; identify system notification warning thresholds and critical temperatures; identify responsible individuals to receive system notifications; develop a plan for response to warning and critical temperature excursions. Veterinary resources husbandry management have since deployed a simple, user friendly and inexpensive monitoring system

that utilizes temperature sensors installed in each animal room and aquatic tank that sends its data to a departmental server. The data collected is analyzed using software tools and reported at defined frequencies. A notification system was designed and implemented that provides alerts through email for temperature conditions outside the acceptable range and a SMS text message alert for critical alarms. Different events trigger different notifications to management staff depending on the level of required response. This real-time reporting of temperatures also allows us to be notified as temperature events develop, providing time to respond to a potential temperature problem before it presents an animal welfare concern. These warning and critical notifications allow us to work closely with the campus HVAC staff to remediate a standard operating procedure for response has been developed and has become part of our disaster plan. Veterinary resources husbandry management currently monitors 122 locations spanning 9 buildings with one tool that husbandry management and veterinary medical personnel can see in real time.

P185 An Evaluation of a Laboratory Animal Research Program Using the CIPP Model

TM Thomas*

Charles River, Orlando, FL

The purpose of this poster is to provide an overview of how to conduct an evaluation of a LAR program. Most LAR programs are designed to improve the scientific objectives of the organization so that researchers can focus on their science. A program evaluation strives to improve a program where possible and ensure the organization's goals have been met. A formal evaluation will also seek to determine if the components and program support systems were implemented as designed. It will seek to determine the impact and effectiveness of the program, as well as investigate the components that should be sustained and describe any unanticipated effects. An effective LAR program evaluation can be conducted by using the Stufflebeam CIPP (context, input, process, and product) evaluation model. The author will provide an overview of the CIPP model and how it can be implemented by directors, program managers, and/or facility leaders.

P186 Biscuit Feeder Increases Foraging in Baboons and Reduces Biscuit Waste

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Working for food is one of the most frequently found species-typical activities in nonhuman primates. Providing foraging opportunities to captive primates is imperative. A low cost way to increase this activity is to provide a novel biscuit feeding device. Our baboons are fed a regular monkey chow diet twice a day in a standard metal feeder. On average an adult single-housed baboon receives 40–45 biscuits at each feeding. The baboons then forage through the feeder for their favorite biscuits resulting in about 75% of those biscuits falling to the floor, out of the animals reach. The baboons on average are only spending 10 minutes foraging for food and only consuming about 25% of the biscuits and wasting the rest. By adding a commercial suet feeder turned into a basket attached to the top of the cage, the average time spent retrieving biscuits increased to 45 minutes with only 15–20% of biscuit waste. The device requires the primates to use skillful manipulation to maneuver the biscuits through the basket, while also increasing their physical activity by requiring them to stand and reach. This device proves to be beneficial by promoting mental stimulation, increasing foraging time, and decreasing the waste of biscuits.

P187 Infection of *Corynebacterium bovis* in a Strain of Hirsute, Immunologically Altered Murine Model of Neoplasia

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Testing for *Corynebacterium bovis* was recently added to our mouse health surveillance program via PCR analysis. *C. bovis* was detected from dirty bedding sentinels in one room within a specific pathogen-free barrier facility. This room houses 8 strains of transgenic and wild type mice of varying immunologic status, all belonging to a single investigator. Another room used by the same investigator to house mice under quarantine, also tested positive for *C. bovis*. Further testing in the barrier room narrowed the source of *C. bovis* to a single rack, a mobile tablet shared between the 2 rooms, and a single strain of mouse transgenic for human papillomavirus type 16 (K14-HPV16). K14-HPV16 transgenic mice are hirsute and serve as a model for human papillomavirus-induced neoplastic progression. Skin lesions in this mouse model ultimately progress to dysplasia and papillomatosis, but are initially characterized histopathologically by acanthosis and hyperkeratosis, two hallmark lesions present in *C. bovis* infection of athymic nude mice. Infection of K14-HPV16 transgenic mice may be explained by expression of HPV16 oncoproteins in the skin resulting in a local immunosuppressive environment. This case illustrates the importance of adequate health surveillance monitoring, including pathogen testing via PCR and testing of the environment as well as animals. It also illustrates the confounding influence of *C. bovis* on a mouse model of skin disease. Furthermore, it raises questions about what factors affect the susceptibility of hirsute immunodeficient mice to *C. bovis*, as several strains of mice with altered immune function were potentially exposed to *C. bovis*, but were not persistently infected. Lastly, this case demonstrates that mice other than nude immunodeficient models may unknowingly serve as a source of *C. bovis* that may infect nude immunodeficient models in the same facility.

P188 Cross Training Laboratory Animal Care Personnel in Physically Separate Animal Facilities at a Land Grant Institution

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A land grant institution maintains physically separated laboratory animal care facilities under centralized management. Species of research animals utilized and the subsequent average daily census on campus varies dependent upon the principal investigators' research focus and availability of grant funding. Accordingly, the requirement for animal care staffing in each facility also varies widely. A system for cross training the civil service system animal care staff was developed and implemented to optimize staffing levels by demand and improve overall operations. Cross training of employees reduced overtime, allowed movement of staff from facility to facility, improved our ability to respond to researcher needs, and improved overall employee job satisfaction.

P189 Design of a Wet Lab for Surgeons Using Zebra Finches

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Researchers at our institution who are planning to perform surgeries on zebra finches (*Taeniopygia guttata*), the most popular songbirds used in biomedical research, have historically attended the same wet labs as researchers using rodents. However, as a result of their distinctive anatomy and physiology, birds present unique challenges to the researcher that mammals do not. For example, the avian respiratory system includes air sacs that take up much of the body cavity and must be avoided during intracoelomic injections, and

feathers cannot be readily removed with clippers. In addition, the neurosurgical procedures typical for zebra finches at our institution are highly intricate, carried out by a solo surgeon, and require a range of specialized instrumentation that make it impractical to drape or sterilize all equipment not directly contacting the surgical site. To address these issues, we developed a zebra finch-specific surgical training lab. After a brief didactic primer, the new teaching module provides hands-on practice with zebra finches, and covers topics such as proper restraint methods to assure normal avian respiration, giving injections safely by different routes, monitoring anesthetic depth, positioning and preparation for surgery, hemostasis and fluid replacement therapy, sterile gloving, the practice of gentle tissue handling, and other elements of proper surgical technique. We present the researcher a low-cost, readily implementable surgery plan that combines the use of sterile "instrument-tip" technique with a well-considered, organized workflow; together these allow for the maintenance of sterility at the surgical site despite the intraoperative manipulation of nonsterile surfaces (for example, microscopes and stereotaxic dials). An illustrated handout serves as a reference and reinforces all "take-home" messages.

P190 Streamlining Spaces Where Research Animals Receive Hazardous Materials

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MIT, Cambridge, MA

Onsite communication between research, veterinary, and animal care staff is critical when animals are receiving hazardous materials (hazmats): biologic, chemical, and/or radioactive. At our institution, rodents exposed to hazmats are housed within isolation cubicles in containment areas (CAs). Our environmental health and safety office assesses the risk period during and post-dosing for each particular hazmat; for a few days after each dose, the research staff is often in charge of the animals. Historically, assorted paperwork for each study (hazmat-specific safety guidelines, researcher contact information sheets, and researcher-to-provide husbandry logsheets) was displayed on the cubicle doors, while the hazmats approved for use within a given cubicle were handwritten on a central hazard sign via permanent marker. This led to confusion as to which hazmats were actually in active use, and the copious documentation obstructed visibility into the cubicles. Because of such deficiencies, an overhaul of the system was undertaken. A preeminent change was to transfer most paperwork off of the cubicle door and into an organized binder within each cubicle. Instead, only 2 signs remain posted on the cubicle doors: 1) a list of IACUC-approved protocol numbers covering animals within the cubicle, and 2) colored hazard signs to communicate what hazmats are actually being used in the cubicle. The handwritten list of hazmats has been replaced with magnetized labels put on the cubicle door by the researchers, along with special hazmat cage cards, when they start an experiment; both are removed when any risk period has ended. To monitor for compliance, veterinary and facility personnel check each cubicle at least weekly for appropriate documentation of active hazmat use and animal husbandry. The CA makeover has reduced the number of emails needing to be sent to investigators by 90%.

P191 Optimizing Husbandry Practices During Murine Pinworm Outbreaks

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Balancing treatment costs, labor resources, veterinary decisions, and researcher interests can be challenging during murine pinworm outbreaks. The university faced two challenges when *Aspicularis tetraptera* outbreaks involving 8 large, multi-investigator animal holding rooms with IVCs occurred: 1) quarterly sentinel results were positive only for a subset of animals in each room; and 2) husbandry procedures for environmental decontamination may be extensive to prevent further spread of the infection. Thus, we evaluated the effectiveness of isolating positive animals into a separate room and limiting treatment to only known positive animals, as well as the necessity for environmental decontamination. Room isolation and treatment with a 6-week course of alternating fenbendazole chow or ivermectin water was performed for rooms with less than 50% of the racks affected. The negative animals were left untreated in their original animal rooms and personnel were reminded to follow proper microisolation technique. No moratorium on research was issued in all rooms. To assess environmental contamination in 5 contaminated rooms, swabs, of 9 room objects commonly handled by personnel (for example, door handles, outlet covers, countertops, light switches, food bin, food scoop) were pooled and submitted for PCR. Swabs were taken before treatment was completed and a complete room change out with environmental disinfection performed, as well as one week after treatment and environmental decontamination procedures. Subsequent quarterly sentinel testing of rooms housing negative or treated positive animals revealed that animals were pinworm-negative. All environmental swabs were positive prior to but were negative after room change out and disinfection. In conclusion, we were able to isolate pinworm-infected animals effectively and optimize the number of change outs performed to prevent environmental decontamination, ultimately decreasing the strain on labor resources and cost, and minimizing potential research effects of pinworm infection and treatment.

P192 Nesting Boxes (Tubes) Doubled as Anesthetic Induction Chambers for Marmosets in a BSL3-AG Biocontainment Environment

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Marmosets require nesting boxes to provide the enrichment and housing conditions to meet their welfare needs. Nesting boxes are often attached inside the cage and double as transport devices. In addition, they are used to confine marmosets during routine husbandry procedures where caretakers must enter the animals' enclosure to perform their duties. For safety reasons, it was not possible to install such a box inside the animals' cage, as operating in a biocontainment environment prohibits direct contact with awake primates. The solution was to design a stainless steel tube-shaped nesting "box" and attach it to the exterior of the cage, eliminating the need to open the cage to catch the animal. By affixing the removable tube to the front of the cage, we were able to entice them to run into the box with a food treat and slight squeeze persuasion as necessary. Anesthesia ports were installed on either side of the nesting tube to create anesthesia induction chamber capability. This allowed us to anesthetize marmosets before blood collection and bacterial challenge procedures. The distal end of the tube (away from the cage) was fitted with a polycarbonate resin window so we could observe the animal while in the box. This also provided added enrichment for several marmosets who preferred to perch in the tube and observe both human staff activities and other marmosets. This multi-role nesting tube worked exactly as intended. The animals used the tubes as a required enrichment nesting box, the staff successfully captured them when it was necessary for cage cleaning and for technical procedures, and the box doubled as an induction chamber for anesthetizing the animals. This multi-purpose marmoset nesting tube proved to be critical to successful housing, management, experimentation, and welfare for this species in a biocontainment environment where direct contact with an awake animal is not permissible.

P193 Breeding Strategies for Morpholino Oligonucleotide Microinjections in Zebrafish

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The zebrafish model has rapidly become a popular animal model of human diseases in the last decade because of its high fecundity, rapid external development, and optical transparency throughout all developmental stages. We use zebrafish to investigate gene function by injecting morpholino oligonucleotides into 1–2 cell embryos, requiring about 1000 embryos per experiment over a 3 hour period. Thus, the time window to obtain synchronized 2 cell stage embryos is narrow. Therefore, timed-breeding by using dividers to separate males and females is widely used. However, if dividers are removed too late, the eggs may not be fertilized or spawning may not occur naturally. To ensure good quality embryos for each experiment thereby improving cost efficiency, we established 4 breeding strategies. 1. Age: healthy 5–12 month old fish for breeders are chosen so there is high chance to obtain good embryos. 2. Multi-pairing: 4 pairs of fish per tank are kept together for breeding and collecting eggs. In this configuration only 1 female spawns at a time thereby multiple females naturally prolonging spawning. 3. Use early clutches of eggs: the first 2 clutches of eggs in the same tank are used for injection because later laid eggs may be unfertilized. 4. Timing: dividers should be removed within 1.5 hours after lights come on. By using these strategies, large numbers of good quality embryos were obtained. Experiments can be finished in 2–2.5 hours, and about 20% less embryos need to be injected due to reduced failure rate. Furthermore, there is a decrease in the labor intensive activity required for breeding set-up and injection. This research demonstrates that good breeding strategies lead to reduction and refinement of the 3Rs.

P194 Efficacy of an Enrichment Device for Long Term Breeding of C57BL/6J Strain Mice

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C57BL/6J strain mice have been seen barbering during long term breeding. One possible cause of barbering is fighting among mice. We observed superior mice biting the hair of inferior mice. We estimated that an enrichment device might prevent barbering because such a device has a place for inferior mice to hide. Thirty C57BL/6J mice were divided into 2 groups. One group was housed with an enrichment device (E-group) and the other group was housed without a device (C-group). Each group had 4 cages. Each cage housed 3 or 4 mice. We observed mice from 4 to 46 weeks of age. Body weight, food, and water consumption were measured once a week. We checked barbering of mice once a week. At 46 weeks of age, mice were euthanized by collecting blood from the caudal vena cave under isofluralne anesthesia. Blood cells were counted and biochemical examinations (glucose, total-cholesterol, BUN, total-bilirubin, GOT, GPT) were performed. After taking pictures of the backs of the mice, the area of barbering was measured. After an autopsy, weights of main organs were measured. Statistical analysis was conducted using unpaired student's t-test. A p value less than 0.05 was considered statistically significant. There was no significant difference in either the number of cages in which barbering mice were found or the total number of barbering mice between the 2 groups. There were no significant differences of body weights and organ weights between the 2 groups. There were no significant differences of food and water consumption between the 2 groups. There were no significant differences of blood cells examination results. GOT and GPT values of the E-group were higher than those of the C-group. However these differences were within a range of normal values. The mean value of the barbering area of the E-group (0.6 cm^2) was significantly smaller than that of the C- group (5.7cm^2). In summary, the enrichment device we used could reduce barbering of C57BL/6J strain mice during long term breeding without changing any physiologic parameters. One reason for the reduction of barbering in C57BL/6J strain mice may relate to the enrichment device we used in which inferior mice can escape and hide from superior mice. These results may contribute to the welfare of laboratory animals.

P195 A Comparison of Husbandry Methods to Control Urinary Tract Infections in BTBR Male Mice

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Male BTBR (Black and Tan, BRachyury) ob/ob mice spontaneously develop diabetes at 6 weeks of age and are used to study diabetic nephropathy (DN) and end stage renal disease progression that more closely mimic the human condition. High incidence of early urinary tract infections and consequent pyelonephritis leads to premature deaths, thereby severely impacting the use of this model to effectively evaluate preclinical drug candidates. The goal of our study was to evaluate the effects of husbandry practices to minimize health concerns and improve survivability. All mice in this study were pair-housed in either IVC (individual ventilated cages) with bedding, or metabolic cages with metal support grids. Cage changing frequencies of 3 times a week versus once a week were compared for mice housed in IVC cages with bedding. This study also compared the use of acidified water versus standard reverse osmosis water. Once a week cage change resulted in a greater than 20% wet cage surface area by 9 weeks of age and had to be stopped due to welfare concerns. Surprisingly, the mice housed in metabolic cages had a higher incidence of urine scald of the mouse ventral skin surface and had to be euthanized. We believe that the metallic surface and the inability of the preputial area to dry due to constant urine production contributed to these lesions. Acidified drinking water reduced the severity of urinary tract infections but also reduced urine volume, blood glucose, and triglycerides thereby impacting the model. Standard bedding, frequent bedding changes (~ 3 per week) was found to be the best husbandry practice for preventing secondary bacterial infection. Studies with female BTBR mice are currently in progress to see if anatomic differences reduce the incidence of UTIs and pyelonephritis.

P196 Blunted Refeeding Response in C57BL/6J Background Mice Subjected to Diet-Induced Obesity

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Studies of lipid and carbohydrate metabolism often examine varying feeding conditions. While data gained from the free-fed and fasting states are useful, the dynamics of the transition from utilization of endogenous to exogenous fuel may be necessary to illuminate differences between control and experimental animals. This is commonly accomplished using a fast-refeeding paradigm; however, its application requires animals to resume voluntary food consumption when diet is reintroduced. We observed a blunted refeeding response in C57BL/6J background mice subjected to diet-induced obesity. Specifically, animals (n = 24) were fed a low-fat (LF) diet (10% kcal from fat) ad libitum from weaning onwards and at 10 weeks of age half were switched to a high-fat (HF) diet (60% kcal from fat). At 18 weeks of age there was a significant weight difference between diet

groups (LF 27.7 ± 0.27 g vs. HF 43.0 ± 1.50 g). At this time all mice were fasted for 24 hours, starting at 0800h (12 hours light: 12hours dark, lights on 0600h), with blood collected 4h and 24h after food removal. Subsequently, respective diets were reintroduced. Blood glucose and food consumed were measured at 6h post-refeeding. Food consumed between 6-24h of refeeding was also measured. LF-fed mice consumed more food in the first 6h following refeeding (6.37 ± 0.44 Kcal; 1.66 ± 0.11 g) compared to HF-fed mice (2.62 ± 0.19 Kcal; 0.50 ± 0.04 g). However, calories consumed between 6h-24h did not differ between groups. Changes in blood glucose reflected differences in food consumption. Fasting reduced blood glucose in both diet groups. But, when diet was reintroduced blood glucose levels in LF-fed mice returned to baseline while HF-fed mice maintained fasting blood glucose levels. This data shows differential refeeding responses between normal and diet-induced obese mice. Of note, HF-fed mice only exhibited impairment in food intake in the initial 6h (daylight feeding), but normal nocturnal feeding was not impaired, suggesting time of refeeding may be a factor. Fast-refeeding studies in diet-induced obese mice may be inappropriate, as differences in food consumption complicate the interpretation of lipid profiles and other physiologic parameters, or should be revised to accommodate this altered refeeding response.

P197 Normal Physiologic and Pathologic Values for the Sinclair Miniature Swine

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Miniature swine overall are increasingly recognized as a nonrodent model in regulatory toxicology and dermal toxicology. The similarities of their cardiovascular, renal, and digestive systems to those of humans make them a suitable animal to model the human counterpart. They are also amenable to all routes of compound administration. Additional attractive traits that make them a good substitute to model humans are that they are omnivorous, easy to handle, prone to obesity, and will develop atherosclerosis and dyslipidemia when fed a high-fat diet. The Sinclair miniature swine (SMS) is the oldest strain of miniature swine developed for research, and is one of the smallest as well. In an effort to generate a database of baseline information about the normal physiologic status of the SMS, information was retrospectively collected from control animals in various toxicology studies, as well as from studies designed solely to collect baseline information. Animals selected were 3 to 5 months of age and were required to be healthy and immunologically naïve. Animals used as negative or vehicle controls in systemic or dermal toxicology studies were included. Control animals that were part of wound healing or surgical studies were excluded. Physiologic data were collected from equal numbers of both male and female, and include weight and body measurements, hematology, serum chemistry, coagulation profile, urinalysis, ECG rhythm and segment intervals, and organ weights, including the brain and pituitary gland, thoracic organs, reproductive organs, and abdominal organs excluding stomach, pancreas, and intestines. Body measurements such as height, width, circumference, and tail-head length were taken of 20 SMS at 3, 4, and 5 months of age. Urinalysis samples were collected from 40 SMS via metabolic cages, Multiple-lead ECGs of 22 SMS were collected with the SMS in sternal recumbency in slings, and organ weights were collected from 12 SMS at necropsy. The resultant data from this retrospective study will benefit the SMS as one of the nonrodent species in research by providing baseline information with which to correctly interpret regulatory toxicity and other testing results.

P198 Development of a Model of Dermal Inflammation and Irritation in the Miniature Swine

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Histamine, the major mediator of mast cells, is known to induce a short-lived urticaria when applied intradermally in humans. The objective of this study is to develop a reproducible dermal urticaria model in miniature swine. Three female Hanford miniature swine, age 3 to 18 months, were used in the study. The animals were pricked on their backs with a skin test device that was loaded with vehicle or histamine in vehicle. The irritation and wheal and flare responses of the dermis were evaluated with Draize scoring and with wheal size measurement. Histamine dose dependently induced skin irritation at both 10 and 20 minutes post treatment. The most prominent erythema and edema (Draize score) responses were observed at 10 minutes post treatment; the responses were slightly diminished at 20 minutes post treatment. Histamine also caused skin wheals that ranged between 4-7 mm in diameter. Although wheal sizes increased over time following treatment, this change in wheal size appeared unrelated to histamine effect. The optimal concentration of histamine to induce urticaria appeared to be 25 mg/mL. When urticaria was induced with 10 mg/mL histamine, topical antihistamine slightly inhibited both dermal irritation and wheal and flare, whereas topical hydrocortisone inhibited the wheal and flare only. When urticaria was induced with 25 and 50 mg/mL histamine, topical antihistamine as well topical hydrocortisone inhibited both dermal irritation and wheal and flare. The inhibitory effects of antihistamine and hydrocortisone were observed at 25 and 45 minutes post dose. Histamine at 50 mg/mL was shown to induce urticaria with sustained dermal irritation and wheal and flare in Hanford miniature swine. In conclusion, a histamine-induced urticaria model has been established with the female Hanford miniature swine and can be used for the testing of topical treatments.

P199 Tape Stripping Repetitions Reduce the Stratum Corneum Inversely in Yucatan Miniature Swine

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Miniature swine are a recognized predictive model for human drug candidate dermato-pharmacology studies. Tape stripping is a simple and effective method for removing the stratum corneum (SC) and is commonly employed during in vivo studies investigating the percutaneous penetration and disposition of topically applied candidate drugs. The objective of this study was to assess the remaining thickness of the SC following 0, 10, 20, 30, 40, and 50 repetitions of tape stripping. Animals were young adult, male Yucatan miniature swine weighing 33-36kg (n = 3). Animals were maintained under general anesthesia for the entire duration of the procedures. Following clipping of the pelage over the dorsal lumbar and thoracic areas, 6 sites, approximately 5cm by 5cm, were demarcated and skin was stripped using 1.8mm clear acrylic adhesive tape applied with uniform, firm pressure. Following tape applications, the center of the each test area was punch biopsied (8mm) and samples fixed in 10% neutral buffered formalin. Samples were processed, stained by H&E, and read under light microscopy. The results showed an inverse pattern of remaining SC thickness to the number of tape stripping repetitions. Fifty passes were required to remove nearly all SC. In conclusion, data demonstrate that skin can be stripped of SC in a linear fashion based upon repetition of the technique.

P200 Determinants of Ischemic Wound Healing in Diabetes: A Bipedicle Flap Wound Model in Diabetic Yucatan Miniature Swine

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The objective of this study is to understand the relationships between wound sizes versus ischemic area dimensions in full-thickness ischemic wounds using a bipedicle flap model in diabetic miniswine.

Full-thickness paraspinous chronic ischemic wounds were created in diabetic miniswine (duration of induced diabetes onset >10 months). The wounds were comprised of A: bipedicle ischemic flap wounds (75 cm^2) with a center punch biopsy (diameter: 0.8 cm) with corresponding nonischemic control full thickness punch biopsies or B: bipedicle ischemic flap wounds (216 cm^2) with a center punch biopsy (diameter: 5 cm) with corresponding nonischemic control full thickness punch biopsies. Each of the flaps had a silicone film underlying the flap. Dressing changes were performed 3 times per week until the end of the study at 3 weeks. During dressing changes, all wounds were photographed. Time to complete healing was determined by clinical observation and photographic documentation. The 8 mm diameter punch full-thickness wounds on 75 cm^2 bipedicle flaps showed fully delayed and/or incomplete healing at day 21 and the flaps did not break down. However, the 8 mm punch biopsies were too small for measuring wound healing rates other than time to complete healing. The 5 cm diameter full-thickness wounds on 216 cm^2 bipedicle flaps did not heal due to apparent ischemic necrosis and dehiscence of some of the flaps. In summary, the bipedicle flap chronic ischemic wound model was successfully employed in our diabetic Yucatan miniswine. The 8 mm ischemic wounds showed clear evidence of impaired wound healing. The 5 cm full-thickness ischemic wound model showed evidence of impaired wound healing with associated necrosis. Our data suggest that a bipedicle flap wound model with an estimated 2:1 ratio for flap width:height produces wounds that can be useful in diabetic ischemic wound healing research.

P201 Criopreservation Methods for *Toxocara Canis* Embryoned Eggs

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Human toxocariosis is a worldwide zoonotic disease. The infective stage 2 developed (L2) larvae cause damage in the human brain, liver, lungs, and muscle. The interaction between humans and the paratenic hosts increases the possible infection in humans, especially when coexisting with paratenic hosts infected with *Toxocara* spp. When investigators want to study this parasite, they look for adult parasites, or they infect adult dogs. In this study we proved that cryopreservation of *T. canis* L2 eggs is feasible, and they remain viable by keeping their infective migratory capacity. *T. canis* eggs were cryopreserved by testing cryoprotectants agents DMSO (dimethyl sulfoxide) 5% and Glycerol at 10%, in mixture and single. Viability post vitrification was tested using typan blue and MTT assay. Infectivity and migratory ability of cryopreserved eggs was tested performing an experimental infection in BALB/C mice. Vitrificaion with glycerol 10% showed 169:500 eggs dyed with trypan blue, and 138:500 with DMSO 5%, indicating parasite death. The MTT showed 165:500 eggs preserved in glycerol at 10% and 148:500 eggs at DMSO at 5% dyed, showing metabolic activity. Infected mice tissues were tested by trichinoscopy. In the group of mice infected with cryopreserved eggs with glycerol at 10% were found to have 7 larvae in brain, 2 in lung, and 1 in liver. In the group of mice infected with cryopreserved eggs with DMSO 5%, 6 larvae were found in brain, 1 in lung, 1 in liver. Compared with control group were mice infected with no cryopreserved eggs of Toxocara canis; we found 12 larvae in brain, 3 in lungs and 2 in liver. The tissue of a mouse infected with 5% DMSO cryopreserved eggs was analyzed by PCR detecting the ITS- 2 gene, that showed the presence of *Toxocara canis* DNA. The results of this study demonstrate the feasibility of cryopreserved infective eggs of *Toxocara canis* , retaining viability, infectivity and migratory capacity. It can be very useful to have this material available for the establishment of experimental models.

P202 The Susceptibility to Intestinal Inflammation in Irgm1-Deficient Mice is Influenced by the Gut Microbiota

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Polymorphisms in the human Immunity-related GTPase M (IRGM) gene are associated with Crohn's disease. We have shown previously that mice deficient in the IRGM homologue, Irgm1, exhibit increased ileitis and colitis in response to dextran sulfate sodium (DSS)-induced intestinal injury and display altered Paneth cell (PC) morphology and function when housed in conventional (CV) conditions. In humans, the severity of Crohn's disease (CD) is impacted by the host intestinal microflora; therefore, we sought to determine if the intestinal phenotype of this murine CD model is similarly influenced. We hypothesized that mice re-derived into "clean" specific pathogen-free (SPF) housing conditions would exhibit an attenuated phenotype in regards to DSS susceptibility and PC dysmorphology. Irgm1 heterozygous mice were re-derived into SPF conditions and their subsequent WT and KO offspring (n = 8-10/group) treated with 3% DSS for 7 days before collection for intestinal analysis. Response to DSS was assessed both clinically and histologically. PCs in untreated SPF WT and KO mice were characterized by determining numbers of PCs per crypt, PC location, and ileal antimicrobial peptide mRNA expression levels via qRT-PCR. Microbiota analyses were performed on DNA extracted from fecal and ileal samples of untreated WT and KO mice (n = 11/group). This was accomplished using 16S rRNA sequencing of the V6 region. In contrast to our previous findings in CV mice, SPF Irgm1 KO mice did not exhibit increased susceptibility to DSS-induced intestinal inflammation as measured by clinical and histologic scoring. Differences in PC morphology were abrogated in the SPF conditions and expression of all major antimicrobial peptide classes was statistically similar between WT and KO groups. Sequencing analyses showed profound differences in gut bacterial communities between mice raised in SPF versus CV housing ($P < 3.4x10^{-4}$). The intestinal microflora influences both PC morphology and intestinal inflammatory response to DSS in Irgm1-deficient mice. This finding further emphasizes the potential impact of housing conditions and, subsequently, gut microflora on the phenotype of murine models of intestinal disease.

P203 A Novel In Vivo Mouse Model Of Implant-Related Spine Infection

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Implant-related infection following spine surgery is a devastating complication for patients and can potentially lead to significant neurologic compromise, disability, morbidity, and even mortality. While case series have supported new techniques of local antibiotic delivery, few animal models exist to test hypotheses in a controlled environment. Existing animal models of spinal implant infection are histology-based and in large animals, requiring euthanasia of the animal for a single data point. The purpose of this study was to establish an in vivo mouse model of spinal implant infection that evaluates postoperative bacterial burden longitudinally, humanely, efficiently, and accurately. Survival surgery was performed using genetically engineered mice that have fluorescent neutrophils (lysEGFP), in which a stainless steel implant was press fit into the L4 spinous process and placed longitudinally along the posterior elements. The mice were randomized and received an inoculation of $1x10^2$, $1x10^3$, or $1x10^4$ colony forming units (CFU) of bioluminescent *Staphylococcus aureus (S. aureus),* or sterile saline (control group). Bacterial burden and immune response were tracked longitudinally with quantitative bioluminescence and fluorescence imaging up to postoperative day (POD) 42. Ex vivo CFU counts were performed on POD 42. During all 42 PODs, infected mice had a significantly higher

bioluminescence and fluorescence signal compared to uninfected mice ($P < 0.05$). Tissue CFU counts were significantly higher in the all infected groups in comparison to uninfected groups ($P < 0.05$). Mice in the $1x10^4$ group developed skin breakdown secondary to infection. Bioluminescent bacteria can be applied to a spinal implant surgery in a genetically engineered mouse to create a novel model system that can track infection and immune response non invasively. $1x10^3$ CFU is the ideal inoculum of *S. aureus* to establish a chronic implant-related infection as higher doses cause wound breakdown and lower doses can be cleared by the immune system. This mouse model of postoperative implant-related spine infection represents a novel approach to study new therapeutic strategies such as implant novel coatings, powders, and metals in the future.

P204 Characterization of the Lateral Ventricular Volumes in the Juvenile WPK Rat Model of Hydrocephalus

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The determinations of lateral ventricular volumes in hydrocephalic individuals are clinically assessed by measurement of coronal head dimensions. This method presents challenges in rodent models of disease, where head dimensions are largely detected late in disease progression and are difficult to obtain due to size and structure. In an effort to improve this, we hypothesized that accurate determination of ventricular volumes could be attained by noninvasive MRI. Moreover, we further hypothesized that ventricular volumes would depend on genotype. Wistar Polycystic Kidney (WPK) rats are an orthologous model of Meckel Gruber Syndrome (MKS) and the homozygous mutant animals ($WPK^{-/-}$) have cystic renal disease as well as severe hydrocephalus. Fifteen animals were genotyped at postnatal (P) day 3 using a derived cleaved amplified polymorphic sequences (dCAPS) methodology. On P7/8 and P17/18, juvenile rat pups were briefly removed from their litter, anesthetized with 1-2% isoflurane, and high resolution T2-weighted (T2W) MRI images were acquired using a clinical scanner. Volumes of interest (VOI) of lateral ventricles were determined from threshold based segmented images using specialized software. Based on this analysis, noninvasive T2W MRI resulted in 3D volumes with cerebrospinal fluid contrast suitable for VOI analysis in both P7/8 and P17/18 pups, with an effective resolution of 0.2 x 0.2 x 0.2mm. Quantification of VOI for WT, WPK(+/-), and WPK(-/-) for P7/8 were 6.8±2.2, 24.4±16.4, and 67.5±10.5mm3, respectively. Similarly, VOI for WT, WPK(+/-) and WPK(-/-) for P17/18 were 8.1±2.8, 106.1±88.8, and 501.6±86.4mm3, respectively. These findings depict accurate noninvasive determination of lateral ventricular volumes could be made by T2W clinical MRI. Moreover, VOI analysis revealed a gene dose dependent change with genotype at both P7/8 and P17/18.

P205 Comparison of Route of Administration of D-Luciferin for In Vivo Bioluminescent Imaging

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In vivo bioluminescent imaging (BLI) requires administration of exogenous substrates which are metabolized by cancer cells transfected with the luciferase gene. To ensure complete absorption by the blood stream, intravenous (IV) routes of administration have been reported. However, due to the equipment and technical expertise required for mice tail vein injections, intraperitoneal (IP) and subcutaneous (SC) routes have been adopted as more common alternatives. Because absorption rates differ with each route, we hypothesize that the route of administration will impact the temporal kinetics of substrate utilization. However, we also hypothesize that substrate exposure as measured by area under the curve (AUC) will be independent of route of administration with tumor growth. To test this, 18 NOD/SCID mice were implanted over the flank with 1×10^6 SF767 glioblastoma cells transfected with a fusion protein of mCherry and Luciferase (mCh/Luc-SF767). Once tumors reach 0.2 mm^3, mice were anesthetized with 2-3% isoflurane, D-luciferin substrate was injected via IV, IP, or SC at a dose of 150 mg/kg, and then optically imaged using BLI every 2 min for 44 minutes. To follow tumor growth, animals were imaged at weekly intervals for 4 weeks. In all cases, route of administration significantly impacted the temporal kinetics for IV, IP, and SC which yielded time-courses with Cmax values of 12.9E+09±2.8E+09, 4.55E+09±1.84E+09, and 4.22E+09±1.40E+09 Ph/s.mm2 during week 1, respectively. By contrast, substrate exposure was not statistically different between routes or across time based on AUC. These results indicate that the route of administration of exogenous substrates in in vivo BLI result in kinetics which differ based on route of administration; however, the overall substrate exposure was found to be identical regardless of route and indicates SQ or IP injections offer the same efficacy when evaluating AUC.

P206 Comparison of Ketamine Anesthesia versus Low-Dose Ketamine in Combination with Dexmedetomidine on the Intraocular Pressure of Cynomolgus Macaques (*Macaca fascicularis*)

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Intraocular pressure (IOP) is commonly measured in the laboratory setting using the nonhuman primate model. Ketamine has become the preferred anesthesia method when chemical restraint is required. Ketamine has been predominately used for nonhuman primate IOP studies, as it is inexpensive, safe, has a wide therapeutic index, is short in duration, and rapid in onset and recovery. Beyond these reasons, ketamine does not substantially affect IOP. For many studies, multiple time-point/day IOP collections are required. Ketamine has numerous benefits, however it additionally has numerous side effects. When macaques are sedated with ketamine for multiple time points/day, side effects are often potentiated. A study was developed to investigate the use of an alternate anesthesia that would potentially have fewer side effects and also, have little effect on IOP for multiple time point/day IOP studies. Eight male cynomolgus macaques and 8 female cynomolgus macaques were anesthetized at 5 time points/day using ketamine anesthesia alone (protocol 1). The following week, the same animals were anesthetized at 5 time points/day using low-dose ketamine/dexmedetomidine, followed by atipamezole reversal, (protocol 2). IOP was measured in triplicates on both eyes of each nonhuman primate at all of the 5 time points during both protocols. A statistical difference was found only in IOP measurements of the right eyes of the female macaques at the 4-hour time point. With protocol 1, 4 primates vomited at various (sometimes multiple) time points and 3 primates exhibited head pressing just prior to the 8 hour or last time point. This is compared to an isolated incident of 1 animal vomiting at the 8-hour time point when protocol 2 was used. The authors understand that additional research is needed, however these findings suggest that the combination of low-dose ketamine/dexmedetomidine with atipamezole reversal may be a safe, effective, and novel alternative to ketamine for multiple time-point IOP measurements in nonhuman primates.

P207 Mesenteric Lymph Nodes in Diet-Induced Obesity

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Obesity has developed into a worldwide epidemic that is associated to cardiovascular disease, insulin resistance, and likely intestinal inflammation. Mesenteric lymph nodes (mLN) figure into the inflammatory response and acquisition of oral tolerance. However, their function in systemic inflammation after diet-induced obesity is still unknown. C57BL/6 male mice were fed a high-fat diet containing 60% of calories from fat or a control low fat (10%) diet for 16-20 weeks. Body weight and glucose tolerance were determined. Mesenteric lymph nodes were removed and real-time PCR, as well as flow cytometry, were performed. Furthermore, an antigen-specific ELISA of gut lavage samples were carried out. After 9 weeks feeding, animals were sensitized on their depilated abdominal skin against the haptens 2,4-dinitrofluorobenzene (DNFB). Allergic dermatitis was challenged by topical application of the haptens on the ear skin after 16 weeks and ear thickness was measured 24 hours later. The study displayed that in obese mice the cell subset composition in mLN changed within 20 weeks. Studying the inflammatory response exhibited the induction of antigen-specific immunoglobulins and an increase of pro-inflammatory cytokines such as IL-2 and IL-6 whereas anti-inflammatory cytokines were reduced. Although DNFB treatment as a model of allergic contact dermatitis showed no significant increased ear swelling in obese mice, mLN immune cells were strongly activated. This was characterized by increased homing on all lymphocyte populations. In summary, diet-induced obesity resulted in mobilization of inflammatory cell subsets, an elevated level of pro-inflammatory cytokines, and activated immune cells into mLN. These findings indicate a pivotal function of the mLN activating immune cells after diet-induced obesity and provide new insights into the immunologic mechanisms of obesity related systemic inflammation.

P208 Substrain Dependent Metabolic Balance in Diet-Induced Obesity

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Obesity has emerged into a worldwide epidemic that is associated with several diseases, such as cardiovascular disease, insulin resistance, and probably intestinal inflammation. Genome wide association studies revealed various genes increasing the risk for obesity. It is well known that C57BL/6 mice substrains showed genetically and phenotypically differences. Here we want to reveal the differences in a diet-induced obesity model. Therefore, male C57BL/6NCrl (B6N), C57BL/6J (B6J), and C57BL/6JHanZtm (B6JZtm) mice were fed a high-fat diet containing 60% of calories from fat or a matched control low fat (10%) diet for 10 weeks. Body mass was measured weekly and glucose tolerance was determined at day 0 and after 10 weeks. Serum was collected and blood parameters, such as HDL and LDL, were defined. PCR and real-time PCR were performed to identify obesity-induced genes. Here we show that in a diet-induced obesity mouse model the substrain is important. Analyses of the body mass showed major differences between B6N and B6J or B6JZtm mice. Glucose tolerance was measured and found to be increased in BL6N mice. Blood parameter such as HDL and LDL varied between the substrains. Genes connected to obesity were found to be differently expressed between Bl6N and Bl6J. In summary, C57BL/6 substrains showed phenotypic differences in the diet induced obesity mouse model.

P209 Cd14-Deficiency Increases Disease Susceptibility for Experimental Colitis by Impairing the Intestinal Barrier Function

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Inflammatory bowel disease (IBD) is characterized by relapsing inflammation of the gut. Though its pathogenesis still remains unknown, disturbance of the intestinal homeostasis through intestinal barrier disruption likely plays a key role in IBD development. Genetic analyses in the interleukin-10 (Il10) deficient mouse model of IBD revealed Cd14 as a major candidate gene with likely protective properties. To determine the role of Cd14 for colitis development its influence was investigated in a chronic model of intestinal inflammation based on Il10-deficiency, as well as in a DSS-induced model of acute colitis. Therefore, barrier function was analysed in B6.129S1P2-*Il10*^{tm1Cgn}*Cd14*^{tm1Smg} (B6-*Il10*$^{-/-}$*Cd14*$^{-/-}$) mice and in DSS-treated C57BL/6J.129S1-*Cd14*^{tm1Smg} (B6-*Cd14*$^{-/-}$) mice. Intestinal permeability was investigated in vivo by intestinal FITC-dextran uptake and in vitro by qRT-PCR of Tight-Junction-Proteins (TJ) and immunohistological staining of Occludin, Ki67, and TUNEL apoptosis assay. Severity of intestinal inflammation was evaluated histologically and TNFα and IFNγ gene expressions were quantified by qRT-PCR. Untreated B6-Cd14$^{-/-}$ mice showed no differences in this experimental setup compared to wildtype controls. However, FITC-dextran uptake was increased and TJ expression was decreased in B6-*Il10*$^{-/-}$*Cd14*$^{-/-}$mice compared to *Il10*-deficient mice. Likewise immunohistological stainings indicated a barrier disruption in B6-*Il10*$^{-/-}$*Cd14*$^{-/-}$ mice. Histology and inflammatory cytokine expression revealed increased intestinal inflammation in B6-*Il10*$^{-/-}$*Cd14*$^{-/-}$mice. In addition, DSS-treated Cd14-deficient mice showed an increased intestinal barrier disruption in the acute DSS colitis model compared to the wildtype controls referring to FITC Dextran uptake, mRNA expression of TJ proteins and immunohistology. Histologic scores and inflammatory cytokine expression were likewise higher in the DSS-treated Cd14-deficient mice compared to the wildtype controls. Cd14 deficiency has no influence on epithelial tightness under steady state conditions but it untightens the intestinal barrier under inflammatory conditions. Thus Cd14 plays a protective role in IBD development by enhancing the intestinal barrier function.

P210 Severity Assessment in a Mouse DSS-Colitis Model: Utilization of the Time to Integrate to Nest Test

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Severity assessment in laboratory animals is important for the implementation of the 3R concepts and is an integral aspect in current regulations of the EU. Usually, severity assessment in colitis mouse models takes place by clinical observation, especially by monitoring reduction of body weight. In an acute colitis model induced by dextran sulphate sodium (DSS) over 7 days it is necessary to observe each mouse on a daily basis, which is time consuming, and because of the handling, stressful for the animal. The time to integrate to nest test (TINT) is a simple way to detect postoperative pain in laboratory mice, by measuring the time mice need to integrate nesting material to their already existing nest. Subject of the present study was the evaluation of TINT in a mouse colitis model to detect severity in DSS treated mice in a dose and strain dependent manner. To define experimental housing conditions we first analyzed if composition of group housing influences the TINT results. TIN test with 1,2,3,4, or 5 inhoused mice per cage revealed that most consistent time durations were reached in groups of 4 to 5 mice. For the evaluation of TINT in a colitis model, we induced intestinal inflammation with 1% or 1.5% of DSS in group-housed mice who are deficient for the TLR4 co-receptor CD14 (CD14$^{-/-}$) and in corresponding wild type (WT) mice. Clinical observation of both mouse strains treated with 1% or 1.5% DSS revealed higher clinical scores and pronounced loss of body weight in 1.5% DSS treated mice compared to the 1% DSS treated mice. TINT time durations showed no dose dependent differences. When analyzing strain related differences we found increased clinical scores and body weight reductions as well as increased TINT time durations in CD14$^{-/-}$ mice compared to WT mice. TINT is a simple and practicable method for severity assessment in a mouse colitis model detecting mouse strain related

differences, but not dose dependent differences in this study. As TINT revealed most consistent results in group-housed mice we recommend its utilization as an additional method substituting clinical monitoring of the individual mouse.

P211 The Effects of Chronic Ultra Small Paramagnetic Iron Oxide (USPIO) Dosing in the Rat (*Rattus norvegicus*)

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Ultra small paramagnetic iron oxide (USPIO) compounds are often administered intravenously (IV) to laboratory animal species to increase temporal resolution and decrease distortions during short sequence functional magnetic resonance imaging (fMRI). Chronic IV administration of USPIO compounds has been shown to cause iron accumulation in liver, spleen, and other tissues. Anecdotally, chronic administration of USPIO compounds in nonhuman primates has been reported to result in progressive brain MRI signal dropout and inability to acquire meaningful fMRI data. We hypothesized that rats chronically administered USPIO compounds would experience MRI signal dropout due to tissue iron accumulation within the brain. To test our hypothesis, 3 male, 8-week-old Sprague–Dawley rats (crl:SD) were administered 1 of 3 different USPIO compounds IV once daily for a period of 8 weeks. Rats were imaged once weekly using MRI pulse sequences (T1, EPI, and R2*/QSM) to evaluate for signal dropout and to quantify tissue iron levels. At 9 weeks, serum biochemistry, serum iron levels, complete blood count, and complete necropsy were performed to evaluate iron accumulation and histopathologic changes. Preliminary T1 MRI pulse sequences showed progressive brain signal dropout while R2*/QSM sequences were consistent with tissue iron accumulation. Changes in T1, and R2*/QSM images correlated with elevated serum iron levels and histologic iron accumulation noted within the brain, liver, spleen, heart, lungs, kidneys, lymph nodes, intestines, testes, and gastrointestinal tract. While mild to moderate cardiac fibrosis was seen histologically, no clinical signs were observed on study. Preliminary findings show correlation between chronic USPIO administration and iron accumulation in brain and other tissues. Additional studies are underway that will explore the relationship between USPIO compounds and brain iron accumulation with the goal of reducing MRI signal dropout during long term imaging studies.

P212 Development of a Chronic Pulmonary Arterial Pressure (PAP) Model in the Beagle Dog

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The measurement of pulmonary arterial pressure is critical for the efficacy assessment of pharmaceuticals that may have the potential to reduce pulmonary arterial pressure. Many techniques are described in the literature, however most involve an acute assessment or the placement of a Swan Ganz catheter, introduced through the jugular vein and passed through the right atrium and ventricle to the pulmonary artery. However, a permanent catheter through the tricuspid and pulmonary valves has a high risk of causing cardiac insufficiencies, infection, and endocarditis. This method would not be suitable as a chronic model for screening compounds designed to alter pulmonary arterial pressure. We developed an alternative method to assess pulmonary arterial pressure via measurement of right ventricular pressure. This approach required the placement of the pressure catheter directly into the right ventricle to measure right ventricular systolic pressure as an index of PAP. This surgical procedure was performed in 2 beagle dogs. They were implanted with telemetry transmitters and after a 4-week surgical recovery the model was tested for magnitude and consistency of response by hypoxic challenge via inhalation of 10% O_2. Pulmonary arterial resistance is known to be increased by hypoxia. A clear and immediate response was noted during the hypoxic challenges. The challenges were performed in 2-3 challenges per session for a total of 3 sessions. The sessions were designed to demonstrate consistency between sessions, challenges, time of day, and comparison across animals. This model was in fact very consistent and showed little variation in response between sessions, challenges, and animals. Although this approach is not a direct method to measure pulmonary arterial pressure, it allowed for the indirect assessment of pulmonary arterial pressure without compromising right ventricular function. This model demonstrated a predictable and reproducible response that can be used as a screening model to assess efficacy of compounds designed to reduce or prevent elevations in pulmonary arterial pressure.

P213 Genetic Analyses of Newly Isolated Simian Betaretrovirus from Cynomolgus Macaques in Cambodia

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Simian betaretrovirus D (SRV) is one of the most important nonhuman primate (NHP) pathogens to be controlled. In many cases, SRV-infected animals stay asymptomatic or become latent, and disseminate the infection unnoticed. Reactivation of SRV during biomedical and preclinical studies can cause many problems, such as loss of experimental objects and influence on experimental data. To this end, it is important to know the existing serotypes of the virus in a particular colony to carry out appropriate diagnostics. From our intensive NHP screening for SRV infection by PCR in a Cambodian captive colony, we have suspected that the colony harbors a new serotype that is genetically distinct from SRV1-5. In this study, we have identified a SRV-infected animal that yields a positive PCR diagnostic result with universal SRV primers but negative results with serotype 1-5 specific primers and probes. Then, we have isolated virus from the asymptomatically infected cynomolgus macaque by co-culturing peripheral blood mononuclear cells (PBMC) of the infected animal and Raji cells. The provirus DNA was extracted from infected Raji cells, and its DNA sequence was determined by PCR with primers that target conserved regions and by sequencing the PCR products. Phylpgenetic analyses of *env* and *gag* genes of the newly determined sequence and known sequences of SRV1-5 were performed. Infected Raji cells have shown cytophathic effect, showing that PBMC contained infectious virus particles. Phylogenetic analyses have revealed that this newly isolated SRV is genetically distinct from the 5 SRV serotypes that are currently known, which urges the reevaluation of diagnostic methods based on nucleic acids detection.

P214 Microsampling: The Smallest Things Add the Greatest Value

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During cancer drug discovery the process of translation from in vitro activity to proof of in vivo pharmacodynamic response and, ultimately, efficacy is key. Typically this will be investigated using xenograft bearing mice, which are dosed over the course of several days or weeks. Prior to running a long-term dosing study a chronic

tolerated dose study will be performed. In the unfortunate circumstance that the compound proves to be poorly tolerated over time or that the efficacy study is found to have a negative outcome, questions may be asked of the pharmacokinetics of the test compound. We describe how microsampling has been introduced to the drug discovery unit and used as a reduction and refinement strategy in such studies. By taking serial blood samples from the tail vein during these initial safety studies, it is possible to gain a limited understanding of the pharmacokinetics of the test compound during a chronic dosing regimen. Samples of 25µl are typically collected once weekly, or up to 5 samples per mouse within a 24-hour period depending on the study purpose and duration. By collecting limited exposure data earlier in the cascade, greater confidence can be gained in the test compound prior to the commencement of more intensive studies. Early identification of drug metabolism and pharmacokinetic issues may allow potentially unnecessary efficacy or pharmacodynamic studies to be avoided. Examples of this within the drug discovery unit have guided further work with lead optimization compound series and in some cases avoided progression to acute pharmacodynamic studies that would have required up to 60 mice per compound. These instances represent a substantial reduction in experimental animal usage.

P215 Effect of Nutritional Supplement Administered at Varying Time Points on Body Weight of Mice Treated with Docetaxel

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Providing a nutritionally balanced lab diet does not guarantee that mice treated with chemotherapeutics will maintain body weight. We were interested in validating the use of nutritional supplements to minimize body weight loss of mice treated with Docetaxel, a chemotherapeutic known to cause body weight loss. In this study, two types of nutritional supplements were used: a nutrient fortified water gel and a nutritionally complete diet targeting both hydration and nutritional needs. One hundred and five 6-week old female NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG) mice were randomized into 7 groups of 15 mice based upon initial body weights. The first group received no nutritional support. Groups 2-4 received the nutritional supplement aimed at treating hydration beginning on days -7, day 0, and at 10% body weight loss, respectively. Groups 5-7 received the supplement targeted at treating both hydration and nutritional needs beginning on days -7, day 0, and at 10% body weight loss, respectively. Docetaxel (20mg/kg) was intravenously administered once a week for 2 weeks to all 7 groups beginning on day 0. Body weights and clinical observations were monitored twice weekly over 27 days. The nutritional supplements were changed twice weekly. Less than 1 week following the first dose of Docetaxel and for the remainder of the study, mice that received either nutritional supplement weighed significantly more than mice only provided a standard lab diet ($P = 0.002$). Surprisingly, Docetaxel did not cause a dramatic loss in body weight as it has in previous studies and mice that were to receive nutritional supplementation at 10% body weight loss did not lose enough body weight to warrant nutritional supplementation. On average, nutritionally supplemented mice weighed 4% more than mice that did not receive supplementation overall. There were no significant differences in body weight between the 2 types of nutritional supplementation provided nor the time of administration at day -7 or day 0. Therefore, administration of nutritional support at the time of dosing or prior to dosing is recommended to minimize body weight loss when dosing with chemotherapeutics.

P216 Induction of Cre Recombinase by Tamoxifen in Neonatal Rats

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Tamoxifen administration in mice has been used extensively to induce Cre recombinase expression and knock out genes at specific time points to generate genetically engineered disease models. With recent advances, it is now possible to make similar genetically engineered rats. In situations where gene knock out may lead to embryonic lethality, the flexibility to knock out genes at any age, including immediately after birth, has great utility to biomedical researchers. However, reports involving inducible Cre-mediated recombination in neonatal rats are limited. Based on our preliminary data from previous experiments examining tamoxifen dose and route of administration regimens in neonatal rats, our current hypothesis is that tamoxifen can be safely and effectively administered to transgenic neonatal rats in order to induce Cre recombinase expression. Initial in vitro experiments were performed using a rat kidney fibroblast cell line (NRK) double transfected with a tamoxifen-inducible human *PKD1* promoter-driven Cre recombinase construct and a floxed Green Fluorescent Protein (GFP) construct. Cells were incubated with a range of tamoxifen concentrations for 24 hours, then examined for GFP fluorescence at 48, 56, and 72 hours post transfection. To demonstrate tamoxifen induced Cre-recombination in vivo, neonatal rats carrying transgenes derived from the contructs tested in vitro were dosed with 3.72mg/kg body weight tamoxifen subcutaneously (SQ) at approximately 24 and 48 hours after birth. The pups were sacrificed at 5 days post injection and Cre recombinase induction was assessed via live imaging for GFP and immunohistochemistry (IHC) staining of relevant tissues. Demonstration of successful induction of Cre recombinase with tamoxifen administration will help establish protocols for its use in genetically engineered neonatal rats.

P217 Gene Expression in Liver and Adipose Tissue from Long-Term Compared to Short-Term Methionie Restriction in Fisher344 Rats

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Methionine restriction (MR) extends the lifespan in rats by 30-40%. This is attributed to increased insulin sensitivity and decreased adiposity, effects proposed to be driven by the upregulation of fibroblast growth factor-21 (FGF21) and stress response genes in liver. Despite the long-term lifespan effects, reported MR gene expression studies were conducted in 3-month MR fed rats. To examine long-term MR effects on gene expression, Fisher 344 rats were fed control (0.86% methionine) or MR (0.17% methionine) diets for 18 months (n = 6 per treatment group). RNA from liver and adipose tissue (AT) was analyzed in Affymetrix arrays using a 1.5-fold and $P < 0.05$ filter to identify significantly expressed genes between the two treatment groups. A total of 3990 and 2768 genes were differentially expressed in liver and AT from the 18 month-fed rats, respectively. Of these, 573 and 390 genes fell under the 1.5-fold and $P < 0.05$ filter in liver and AT, respectively. In liver, 69% of significantly expressed genes followed similar gene expression trends as those reported for 3-month MR studies, 14% were newly expressed genes, 12% were not significantly changed at 3 months, and 5% showed the opposite gene expression trends. FGF21 and stress response genes were not significantly upregulated in livers from the 18-month MR fed rats. In AT, only 21% of significantly expressed genes followed similar gene expression trends as those reported for 3-month MR studies, 14% were newly expressed genes, 49% were not significantly expressed at 3-months, and 16% showed opposite gene expression trends. Among the significantly changed genes in AT from 18-month MR-fed rats were IL18 BP and NUPR1, which inhibit inflammation and provide resistance to stress, respectively. In conclusion, a large number of gene expression changes observed in long-term MR feeding are distinct from those observed in short-term MR studies, and FGF21 and stress response genes in liver do not seem to play a role in the favorable long-term metabolic and lifespan effects of MR. This study could provide insight about potential genes contributing to health span in humans subjected to diets similar to the MR regimen such as vegan diets or caloric restriction.

P218 The Role of Adaptive Immunity in the Control of *Myocoptes musculinus* Populations in Mice (*Mus musculus*)

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Myocoptes musculinus-infested weanling mice harbor high mite loads, yet mite burdens decrease in an age-dependent manner. It is likely that the development of adaptive immunity, specifically the Th2-type response, plays a role in regulating murine fur mite burdens over the course of infestation. In this study, we followed *Myocoptes musculinus* burdens in infested mice deficient in adaptive immunity (*Rag1* -/-) and infested, immune-competent controls (C57BL6/J) from weaning to 36 weeks of age. Mite burdens were assessed by microscopic exam of fur plucks. Finally, we compared clinical scores, body weights, and histologic findings from noninfested and infested mice of both strains over time. Alopecia, excoriations, pruritus, and visible mite debris were assessed and tallied to yield the clinical score. Our results confirmed the finding that mite burdens decrease in B6 mice with age, with young B6 mice having higher mite burdens than did older mice. *Rag 1* -/- showed an initial decrease in mite burdens, but mite numbers were not significantly different at weaning and 36 weeks of age. Additionally, *Rag 1* -/- mice had higher mite burdens than B6 mice at 4, 16, 20, 24, 30, and 36 weeks of age (n = 12 per strain per time point). Higher mite burdens resulted in a higher clinical score. Infested mice (both B6 and *Rag 1* -/-) exhibited clinical signs of fur mite infestation, which included abdominal alopecia, poor weight gain, mite debris, and pruritus when compared with noninfested controls. These results suggest that adaptive immunity plays a role in the reduction of mite populations on the murine host over the course of infestation, and that high mite burdens result in increased likelihood of clinical acariasis.

P219 Lidocaine Hydrochloride versus MS222 for the Euthanasia of Adult Zebrafish (*Danio rerio*)

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MS222 is the most commonly used chemical agent for euthanasia of zebrafish despite its disadvantages. Fish exposed to it demonstrate aversive behavior. In powder form it is hazardous to personnel, and stock solutions must be stored appropriately to maintain effectiveness. Lidocaine hydrochloride was recently evaluated as a chemical anesthetic for zebrafish. It elicits less aversive behavior, is not hazardous to personnel, and does not require special storage. Although lidocaine hydrochloride has several advantages over MS222, its effectiveness as a euthanasia agent for zebrafish is unknown. To examine this question, lidocaine hydrochloride was compared to MS222 for the euthanasia of adult zebrafish. Fish (n = 10/group) were exposed to 250 mg/L of MS222 and 400, 500, and 600 mg/L of lidocaine hydrochloride. Once opercular movement ceased, fish remained in the euthanasia solution for 10 min prior to placement in a recovery tank for a 30-min observation period. Time to loss of righting reflex, time to cessation of opercular movement, aversive behavior, and recovery were evaluated. Time until loss of righting reflex was similar between the MS222 group and all lidocaine hydrochloride groups. Opercular movement ceased faster in all lidocaine hydrochloride groups compared with the MS222 group. Fewer fish in the lidocaine hydrochloride groups displayed aversive behavior such as piping or erratic swimming compared with the MS222 group. No fish in the lidocaine hydrochloride groups recovered from euthanasia, whereas one fish in the MS222 group recovered. Our results suggest lidocaine hydrochloride may be an effective alternative chemical euthanasia agent to MS222 for laboratory zebrafish.

P220 PCR Screening of Barrier and Conventionally Reared Hamsters for Polyomavirus

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Hamster polyomavirus (HaPV) is a naturally occurring pathogen of Syrian hamsters (*Mesocricetus auratus*) that has been reported in colonies within the United States and Europe. Endemically infected hamsters typically develop epitheliomas, and may develop lymphomas, which can significantly impact research. In this study, a HaPV polymerase chain reaction (PCR) assay was used to screen conventionally and barrier-reared hamsters for infection. The PCR assay employed degenerate primer sets to target a region of the VP1 gene that is partially conserved among polyomaviruses, including those found in mice and hamsters. This assay detected HaPV in a broad range of specimens, including feces, mesenteric lymph node, spleen, kidney, skin, salivary gland, mammary gland, urine, body swabs, and oral swabs, from the conventionally housed hamsters of various ages. The percentage of PCR-positive specimens was higher in 6-10-week-old animals (58%) than in 10-14- week-old (44%) animals. Corresponding specimens from the barrier-reared hamsters of several ages were PCR negative. The specificity of the PCR amplification was confirmed by DNA sequencing. According to BLAST analysis, the PCR products amplified from the conventionally housed hamster specimens exhibited 99% homology with the HaPV sequence published in GenBank (accession AF073287). These results demonstrate that PCR of a variety of noninvasive specimens, which may be collected antemortem, would be a sensitive and specific approach for HaPV colony surveillance.

P221 A Minimally Invasive Electroencephalographic Procedure for Rats

GLAS: Yes

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Electroencephalography (EEG) is a procedure routinely used in sleep research to measure sleep-wake behavior. Noninvasive techniques are available for both humans and larger mammals. However, in rodents, long-lasting, invasive surgical procedures are required to implant screw electrodes for EEG recording purposes. This method uses a novel procedure (using surface electrodes). In order to successfully develop this procedure, 2 experiments were conducted. For the first, the habituation time frame was assessed for tethered rat jackets by measuring weight, food intake, fecal boli, and locomotor activity of adult Sprague–Dawley rats (n = 7). The results suggest that rats habituated to the jackets in approximately 1 day following anesthesia (isoflurane) and being fitted with a spandex jacket and cable tether. For a second experiment, adult Sprague–Dawley rats (n = 12) were divided into 2 groups. For the minimally invasive procedure, 6 customized electrodes were pasted on the scalp of each rat with a protective cap glued on top of the electrodes in order to prevent tampering. Electrodes were connected to a tethering cable affixed to the back of a rat jacket. For the second group, a standard surgery was performed that attached 3 screw electrodes to the skull and 3 hook electrodes in the neck muscle. Electrodes were attached to an acrylic pedestal and tethered to the recording system. The sleep/wake behavioral states of each rat were recorded for 48 hours and scored using recordings of EEG and electromyography (EMG). After comparing data recorded using the 2 procedures, the results suggest that there was no significant difference in the percentage of wake REM (rapid eye movement) sleep, or non-REM sleep found between both procedures over the initial 24-hour period. However, there was a large increase in unscorable epochs with the new procedure, especially in the second 24-hour period during wake. This new procedure is therefore preferable for acute sleep/wake recording of 24 hours or less. This procedure fills in the gap of available literature that offers a procedure for the study of sleep/wake behavior in rodents without the need of surgery, thereby applying the 3R principles.

P222 A New Mouse Model to Study Dengue Virus Infection

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Dengue fever (DF) is a subtropical-tropical, mosquito-borne disease caused by a member of the *Flaviviridae*. Five serotypes of dengue virus have been identified. The consequences of dengue infection range from asymptomatic to severe joint/muscle pain (breakbone fever). However, some patients develop dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) which is characterized by hemorrhage, vascular permeability, circulatory failure, and in severe cases, death. There are no vaccines or specific treatments for these diseases. We are interested in using mouse models of dengue infection to investigate the role of host factors, such as long noncoding RNAs and the components of the cellular autophagy pathway to investigate new mechanisms of viral control. A frequently used mouse model for dengue infection is the AG129 mouse strain (129/SvEv-*Ifnabr*^{tm1Agt} *Ifngr*^{tm1Agt}), which carries homozygous deletions of the receptors for both Type I (IFN-α, IFN-β) and Type 2 (IFN-γ) interferons. The deletion of these genes is required to render mice susceptible to several pathogens, including dengue virus. To perform dengue infections in the more genetically tractable background of C57BL/6 mice, we generated the AG B6 strain (C57BL/6J-*Ifnabr*^{tm1Agt} *Ifngr*^{tm1Agt}), the ultimate goal being to intercross it with conditional knockouts of key autophagy genes and other host determinants of viral infection and pathogenesis. Mice with only one IFN receptor knockout, A B6 (B6.129S2-*Ifnar1*^{tm1Agt}, N10) and G B6 (B6.129.S7-*Ifngr1*^{tm1Agt}, N11), showed no clinical signs following dengue virus inoculation although RT-PCR showed some viral replication. However, in the double knockout AG B6, dengue virus titers were 10-fold higher than in AG129 mice and high viral titers lasted longer. The enlarged stomachs seen in dengue-infected AG129 mice at 72-96 hours postinfection, a symptom not observed in humans, were absent in AG B6. However, as in the AG129 mice and in humans, vascular leakage was observed. We conclude that the AG B6 strain represents a powerful new tool to study dengue virus growth, inhibition, and pathogenesis in the mouse.

P223 Refining Intraperitoneal Injection of Sodium Pentobarbital for Euthanasia in Rats

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The AVMA and CCAC euthanasia guidelines classify intraperitoneal (IP) pentobarbital as an acceptable euthanasia method in rats but do not specify dose or volume. The aims of this study were to assess and improve efficacy and consistency of this euthanasia method. In a randomized, blinded study, healthy adult female Sprague–Dawley rats (170, 495 g) received 1 of 3 pentobarbital (240 mg/ml) IP euthanasia protocols in the right caudal abdominal quadrant: low-dose low-volume (LL, n = 11, 200 mg/kg pentobarbital), low-dose high-volume (LH, n = 12, 200 mg/kg pentobarbital diluted 1:3 with saline) and high-dose high-volume (HH, n = 11, 800 mg/kg pentobarbital). Times to cessation of breathing (CB) and heartbeat (CHB) were recorded. Video-recordings of LL and HH were analyzed for pain-associated behaviors (writhing and back arching). Between-group comparisons were performed with 1-way ANOVA and Games-Howell post hoc tests. Variability for CHB was assessed by coefficient of variation (CV) calculation. Data are mean ± SD. The fastest euthanasia method (time to CHB) was HH (283.7 ± 38 s), compared with LL (485.8 ± 140.7s, $p = 0.002$) and LH (347.7 ± 72.0s, $p = 0.039$). Consistency of the euthanasia technique was greatest in the HH group (CV, 13%), followed by LH (21%) and LL (29%). The CB time was fastest in HH (177.5 ± 40.8 s), compared with LL (393 ± 125s, $p < 0.001$) and LH (246.3 ± 74.9s, $p = 0.03$). Pain-associated behavior incidence ranged from 36% (LL) to 45% (HH). These data illustrate refinement of this euthanasia technique. Both dose and volume contribute to speed of death with IP pentobarbital. The shortest euthanasia time and greatest consistency was achieved with a combination of high dose and high volume.

P224 Assessment of a Novel Mechanical Sensory Threshold Testing Device

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Mechanical allodynia is commonly assessed with nylon monofilament application in preclinical pain models. However, this method of testing has been called a "tin standard" due to inconsistencies in testing methods, filament properties, and non-linearity. A novel electronic von Frey device (RM) was tested against traditional von Frey filaments (vF) in a fully independent evaluation of a commercial device in development. We hypothesised that the RM device would identify a significant change in withdrawal threshold and that the RM device would provide consistent withdrawal threshold data with less frequent testing. Male Wistar rats (8 weeks old) were randomised to assessment of mechanical allodynia following intraplantar carrageenan injection with either vF filaments (n = 10) or the RM device (with different probe diameters capable of continuous measurement [RM0.09, n = 11; RM0.5, n = 11; RM0.9, n = 15]). Withdrawal thresholds were identified with a standardised testing protocol. Within group comparisons were made with 1-way repeated measures ANOVA, and between group comparisons with 2-way ANOVA. The RM device was able to identify changes in withdrawal threshold following carrageenan injection (construct validation). All RM probes (and vF) identified a reduction in withdrawal threshold following treatment ($p < 0.05$). RM0.09 did not differ from vF ($p > 0.05$) but showed a lesser percentage decrease at the withdrawal nadir (RM0.09, -28%; vF, -79%). The ability to identify changes in withdrawal threshold was maintained with reduced testing frequencies. Withdrawal threshold testing results were consistent when a single test was compared to either the average of 3 or 5 tests (all $p > 0.05$). These data indicate that the RM device produces data comparable with vF with the advantage of a shortened testing period without sacrificing accuracy.

P225 The Influence of Rater Training on Reliability and Accuracy of the Rat Grimace Scale

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The Rat Grimace Scale (RGS) is a coding scale developed to assess facial expressions reflective of pain and has been shown to accurately and reliably quantify spontaneous pain behavior through 4 facial action units (orbital tightening, nose/cheek flattening, ear position, and whisker position). The role of training in RGS scoring has not been assessed. Aims of this study were to assess the effect of training on accuracy and reliability of RGS scoring with multiple raters. Four raters with no experience using the RGS underwent structured training. Training images were blinded, and a standard score for each image was set by a fifth rater, who had discussed image scoring with the developer. The multi-step training process was as follows. A set of 42 images (S1) were scored individually without any preceding discussion, with the aid of a training manual. Following S1 scoring, scores were reviewed, and areas of inconsistency discussed. Then, a new set of 150 images (S2a) was scored by each individual. Following review and discussion, the S2 image set was re-scored (S2b).

Inter-rater reliability was assessed with an intraclass correlation coefficient (ICC) for average RGS scores and for individual action unit scores. Scoring accuracy was assessed by comparing each rater's scores with that of the experienced rater. The ICC results were classified as: "very good" (0.81–1.0), "good" (0.61–0.80), "moderate" (0.41–0.60), "fair" (0.21–0.40), or "poor" (< 0.20). Training was effective at improving both reliability and accuracy of RGS scoring. Group ICC scores improved from "moderate," with wide 95% CI for S1 (0.58 [0.43-0.72]), to "good" for S2a (0.68 [0.58-0.76]), and "very good" for S2b (0.85 [0.81-0.88]). After S2b scoring, all individual action units were in the "good" or "very good" range. Orbital tightening had the highest ICC (0.85 [0.81-0.88]), and whiskers had the lowest ICC (0.63 [0.57-0.70]), consistent with published data. These data suggest a substantial training effect when learning to apply the RGS and highlights the critical role of training in successfully adopting this novel pain assessment tool.

P226 Physical and Chemical Changes in Autoclaved Rodent Feed

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Sterilization of rodent feed by steam autoclave is a common practice in many research institutions. Most often, we only focus on the beneficial effects of this process, that being the reduction of microbial contamination. We often forget that the high temperatures and pressures used in steam autoclaves can have negative effects on the diet quality, and subsequently the animals that eat this feed, including the production of acrylamide, a known neurotoxin. The purpose of our study was to assess both the physical and chemical changes to a standard rodent diet at different sterilization temperatures. Standard NIH-31 rodent feed was autoclaved at 4 different sterilization temperatures (230^0F, 250^0F, 260^0F, and 270^0F). Feed pellet hardness and acrylamide (AA) concentrations of the diets were tested and compared to irradiated NIH-31 feed. Study diets were fed to male, C57BL/7NCrl mice (n = 10/feed group) for 28 days, after which samples were collected for analysis of AA and glycidamide (GA - active metabolite of AA) and possible genotoxicity. Autoclaving the feed causes an increase in the hardness of pelleted NIH-31 and produces acrylamide (AA), a known toxin. Both hardness and AA levels increased with increasing sterilization temperatures. While pellet hardness can affect feed intake and growth in mice, there were no significant differences in feed intake and body weight gain in all study mice. Analysis of the in vivo AA and GA levels and genotoxicity are pending; however, the levels of acrylamide produced by autoclaving the diets were similar to those previously found to generate genotoxicity in mice.

P227 Harness versus Button Device with Automated Blood Sampler

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Improving animal welfare and reducing device incidents during pharmacokinetic (PK) studies required reevaluating the blood collection system devices. Issues seen with the harnesses included adjusting it over time to allow for animal growth, animals chewing on improperly placed/adjusted harness, harnesses stuck in animals' mouths, limbs stuck in the harness impeding movement, and infections resulting from exposed surgical sites. The new button device provides an effective solution to the above issues. Two groups, 40 of the harnessed animals and 40 of the buttoned animals, (each group consisted of n = 4) were evaluated over 6 months, with a 4-week observation period for each smaller group. It was noted that >50% of the animals in the harness group had issues requiring intervention and extra care. Buttoned animals exhibited normal behavior and did not display any discomfort or require additional care to maintain the device. Additionally, in 25% of the animals, the harness caused abrasion-related skin reactions, which did not recover by the end of the study. Button groups were not observed with any skin irritations, recovering to near naïve state. The button is made from medical-grade polyester felt with a septum to create a closed loop system. It includes polarized magnets to provide a strong keyed connection with the tether and the button can be capped to permit group housing when not in a study. It is subcutaneously implanted in the animal and does not require a harness to keep it in place. Implanted at the time of catheterization, this secures the device to the animal and completely closes the surgical site. The pin ports at the top of the device allow access to the CAC/JVC connections. In conclusion, transition from the harness to the button device is as simple as swapping out the tether and correcting for the tether tube volume in the ABS system software. Thus, the button device alleviates the common harnesses related issues while allowing the animals to return to behavior as close to that of a naive animal as possible.

P228 Labor and Time Savings Associated with the use of Nail Trimming to Treat Ulcerative Dermatitis in Mice (*Mus musculus*)

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In 2012, the University of Colorado at Denver reported that trimming the nails of mice at the time of initial identification of ulcerative skin lesions lessened healing time. We sought to determine if this was true for cases of ulcerative dermatitis (UD) at our institution, and if so, if treating in this manner resulted in a labor and time savings. Cases of mild UD at our institution are traditionally treated with a commercially available antihistamine in the drinking water. Cases of moderate UD are additionally treated with topical triple antibiotic ointment (TAO). Mild UD is defined as a dry lesion between 5-10mm in size. Moderate UD is any moist or pruritic lesion, or lesion over 10mm. It was estimated that preparing a medicated water bottle took staff ~3mins, and that TAO application took ~1min. Staff members were trained and timed on nail trimming, with an average task completion time of 60 seconds. To determine the efficacy of treating mild and moderate cases of UD via nail trim, mice with mild UD treated with antihistamine in the drinking water (N = 5) were compared to mice with mild UD treated with weekly nail trims (N = 6). Moderate UD mice (N = 4) treated with antihistamine and TAO were compared to moderate UD mice treated with weekly nail trims (N = 4). Results gathered from these comparisons allowed us to calculate time and labor savings using an estimate of 3 week resolution for mild UD when nail trims were preformed versus more than 4 weeks with traditional treatments, and 2 weeks for resolution of moderate UD with nail trims versus 4 weeks with the traditional treatments. Consequently, we determined that treating with nail trims only could result in a savings of 10 minutes or more per mild UD case and at least 20 minutes for moderate cases. Our investigation further supports the use of nail trimmings in the treatment of UD, not only in lessening time to resolution for such cases, but also providing a labor and time savings benefit for staff.

P229 Evaluation of Transdermal Buprenorphine Delivery in Domestic Pigs

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The use of transdermal, or patch, drug delivery mechanisms have offered great advancement in continuous analgesic administration for many animals. Fentanyl patches have been used in a variety of species, including dogs, rabbits and nonhuman primates, and more recently, buprenorphine patches in dogs and cats. In swine, a commonly used large animal surgery model, the reported results of Fentanyl use have been inconsistent but, promising results with the

use of buprenorphine patches in mini-pigs have recently been shared. This study examined the use of buprenorphine patches in 45 kg domestic pigs. The patches used have been reported to provide 5-day opioid analgesia in humans. Plasma buprenorphine levels were compared in centrally catheterized animals that received a buprenorphine patch for 5 days (n = 3) versus controls that received bupernorphine via traditional IV or IM routes (n = 3) at doses previously proven to produce analgesia. Twenty-one blood samples were taken per animal at time points between 0 and 120 hours after drug administration. Similar to Fentanyl, individual animal-to-animal variation was noted in the swine that were administered buprenorphine transdermally. Overall, plasma drug levels were consistent for multiple days, but did not reach the same concentrations as IV or IM dosing peaks, although levels did coincide with reported serum buprenorphine levels in other species in which buprenorphine patches have been used. No changes in behavior, appetite or health were noted in pigs that received patches. Application and removal of the patches was simple and patches stayed secure for 5 days. Buprenorphine patches may be a viable analgesic option for large breeds of pigs, but, similar to Fentanyl, their use as a sole means of pain relief might not always be adequate.

P230 The Feasibility of Using Rice Hulls as Bedding for Laboratory Mice (*Mus musculus*)

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Factors considered when selecting laboratory mouse bedding include animal health and comfort, cost, impact on equipment and personnel, and bioactive properties. Corncob, our facility standard, is economical and facilitates low intracage ammonia, but influences outcomes of some endocrine studies. Rice hulls have been used as bedding by rodent fanciers and some international animal research facilities. As a side product of rice agriculture, they are an economical, environmentally friendly material. They have not been well characterized as a bedding substrate, though a previous study indicated that they moderately induce 1 hepatic p450 cytochrome. In this pilot study, various aspects of bedding performance were compared for rice hulls and other materials. Absorbency of materials was determined by weighing bedding samples before and after they soaked for 1 hour in saline. A novel method for assessing moisture at the bedding surface was developed, in which moisture-detection paper was held in contact with the bedding surface hourly for 10 hours after application of water aliquots to the bedding. Rice hulls were significantly less absorbent on a per-volume basis than was corncob or recycled wood pulp (RWP) (group means: rice hulls 0.327 mL/cm^3, corncob 0.413 mL/cm^3, RWP 0.433 mL/cm^3). Rice hulls had significantly higher odds than corncob (7,402:1) or RWP (35:1) of having moisture present at the bedding surface. The results of the absorbency test coupled with the results of preliminary monitoring of intracage ammonia raised concern about the ability of rice hulls to adequately control ammonia in some cages with high occupancy. Relative expression of 3 cytochrome p450 genes was compared among mice housed on rice hulls, corncob, RWP, or pine shavings, using real-time reverse-transcription quantitative PCR of liver tissue. Expression of Cyp 1a2 was 1.7x higher in the rice hulls group than in the pine shavings group, but other comparisons were insignificant, so further research is needed to explore the relevance of this finding. This study provides guidance for future investigation on the merits of rice hulls as laboratory mouse bedding, though its relatively poor moisture control is a major disadvantage that might preclude its widespread use.

P231 Vitamin A Dietary Source and Ulcerative Dermatitis in C57BL/6J Stearoyl Co-A Desaturase Mutant Mice

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Ulcerative dermatitis (UD) describes a clinical pathology observed in laboratory mice (particularly C57BL/6 strain) and reported in the literature for over 5 decades. Despite this awareness, the etiology of UD is currently unknown. Contributing risk factors include age, diet composition and amounts consumed, and genetics. Recent studies have reported that Stearoyl Co-A Desaturase (*SCD1*) homozygous mutant mice have an increased susceptibility to UD, modified by diet (a purified diet formulation induced UD [100% incidence] relative to chow diet [0%] in 4 weeks). As *SCD1*$^{-/-}$ have defects in lipid storage in white adipose tissue, hyper-activated brown adipose tissue and alterations in skin vitamin A metabolism, we tested whether the dietary vitamin A source (which is usually different between chow and purified diets) was inducing UD in *SCD1*$^{-/-}$ mice. Male and female *SCD1*$^{+/-}$ and $^{-/-}$ mice (~7 weeks of age) were singly housed and fed an NIH-31 chow for one week, then randomized to 1 of 4 diet groups (n≥4/sex/group) and fed [*ad libitum*]: 1) NIH-31 based chow - control, 2) AIN-76A based purified, 3) NIH-31 chow with retinyl palmitate or 4) AIN-76A purified with retinyl acetate. Mice were housed in standard polycarbonate cages with normal conditions (~21°C, 12:12 light dark cycle) for 4 weeks to monitor the onset of UD, along with weekly measures of body weight and food intake. All 4 diets were consumed by both genotypes and sexes, with significantly greater gram and energy intake of diet #3 (chow with retinyl palmitate) than the chow or purified diets ($P < 0.001$, ~30-80% increase versus chow). Despite this hyperphagia, *SCD1*$^{+/-}$ mice gained weight on the diets with no significant difference between vitamin A sources within diet type. While no *SCD1*$^{+/-}$ mice developed UD during the study, all diets tested induced UD (93% total incidence, combined sexes) in *SCD1*$^{-/-}$ mice. Switching vitamin A sources in a chow (NIH-31 based) diet from retinyl acetate to retinyl palmitate resulted in a significant hyperphagic response with no significant change in body weight relative to the control chow. In *SCD1*$^{-/-}$ mice, all diets induced UD, obfuscating the potential to determine the influence of the vitamin A source on UD incidence in this cohort.

P232 Comparison of Injectable IL23 and Topical Imiquimod for Inducing Psoriasis in the Mouse Ear Model

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Psoriasis is a common, chronic, relapsing/remitting immune-mediated skin disease. The skin lesions seen in psoriasis vary in severity from minor localized patches to complete body coverage and affects 1-3% of the general population. Among animal models for psoriasis there are two mouse models that directly affect the Interleukin 23 (IL23) pathway. One is the direct intradermal injection of IL23 and the other is the topical application of Imiquimod 5%. IL23 is a heterodimeric cytokine that is a key tissue-specific effector cytokine that amplifies the inflammatory response and is also found to be highly expressed in psoriatic skin lesions. IL23 drives epidermal hyperplasia and infiltration of immunocytes (primarily T-cells) in mice. IL23 is delivered via intradermal injection into the ears of an anesthetized mouse using a 30ga needle and 100ul glass syringe. IL23 is typically administered to 40 mice 3 times a week for 2 weeks (6 doses). Imiquimod is a TLR7/8 ligand and potent immune activator and can induce and exacerbate psoriasis by inducing epidermal expression of IL-23, increasing keratinocyte proliferation and increasing splenic Th17 cells. The Imiquimod is applied with a saturated cotton swab to both sides of both ears of 40 mice daily, typically for 7 days. We used both models to determine the efficacy of candidate therapeutic compounds or biologics. Each model uses caliper measurements of ear thickness and a scoring system to confirm and monitor disease progression. These measurements and scores are completed at study start for baseline measurements. These measurements and clinical scores are then conducted 3 times a week.

In instances where dosing is scheduled the same day as the measurements, measurements are taken before dosing occurs. Both models use 44 mice and have a high success rate, with 100% of subjects developing disease. We found that the imiquimod model shows an increased level of keratinocyte proliferation and hyperkeratosis and a decreased level of immunocyte infiltrate relative to the IL23-injection model, while the IL23 model shows increased infiltrate that can be assessed by flow cytometry. Both t both models capture features of human psoriasis, and together they provide excellent tools for evaluation of therapies for human psoriasis.

P233 The Effects of Polyphenol Rich Peanut Skin Diets on Weight Gain, Lipid Profile, and Blood Chemistry in Rats

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A diet rich in polyphenols plays an important role in disease prevention. Peanut skin is a polyphenol rich byproduct of peanut processing with little economic value. However, peanut skin extract has exhibited potent antioxidant activity in food models. This study investigated the impacts of dietary peanut skin on weight, blood cholesterol profile, hematology, and blood chemistry of laboratory rats. Twenty five female Sprague–Dawley rats, age 7 weeks were randomly assigned to 5 groups (G1, G2, G3, G4 and G5). Rats in G1 were fed basal diet; rats in G2-G5 were fed 1% cholesterol diets containing 0, 2.5, 5, and 10% peanut skin, corresponding to 0, 0.18, 0.36, and 0.73% of peanut skin polyphenols. Feed consumption and weight of rats were monitored every other day. Blood samples were taken weekly for 8 weeks. Blood cholesterols, biomarkers for liver function (alanine aminotransferase and urea nitrogen), and kidney and pancreas functions (creatine and glucose) were measured. At the end of feeding period, rats were euthanized and their organs (brain, liver, heart, lung, kidney, and spleen) were harvested, weighted, and visually observed for abnormality. Results showed that rats in G2-G5 consumed more feed and gained more weight than rats in G1. Rats in G4 and G5 had lower triglycerides and total cholesterol than rats in G2. During the first a few weeks, rats fed with the peanut skin diets had higher LDL and lower HDL than rats in G2. However, this trend reversed after 5 weeks. Compared to G2, rats in G4 and G5 showed lower AAase, BUN, and glucose levels, particularly at later feeding stages. Organs of rats in G2 were significantly larger than the organs in other groups. This study suggests that long-term consumption of diet containing ≥ 5% peanut skin may significantly lower the health risk of consuming high cholesterol diet.

P234 Protective Cell-Mediated Immunity Generated with Adjuvanted Nonreplicating Antigen

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Vaccines can be classified by whether or not the antigen is able to actively replicate. Replicating antigens, such as attenuated viruses, elicit antibody and cell-mediated immune responses, but carry significant risks of adverse events. Nonreplicating antigens such as proteins are safer, but only elicit antibody responses. Nonreplicating vaccines that induce both types of immunity are desirable for targeting diseases such as influenza, tuberculosis, HIV, and malaria. Preliminary data suggested that in mice a novel commercial adjuvant could elicit both cell-mediated and antibody responses to nonreplicating antigens. To evaluate this, groups of mice were vaccinated once or twice at 3-week intervals by subcutaneous or intranasal administration of the model protein antigen ovalbumin or inactivated H3N2 influenza viruses, with and without the adjuvant. To assess the generation of cell-mediated immune responses and the establishment of memory T-cells, the frequency of antigen-specific CD8+ T cells was evaluated at multiple time points following vaccination. Some mice vaccinated with ovalbumin were challenged by infection with H1N1 influenza viruses expressing a CD8-specific ovalbumin peptide. Some mice vaccinated with H3N2 viruses were similarly challenged with an H1N1 influenza infection. Six days after infection, antigen-specific CD8+ T cell frequency in spleen and lung, and viral titers were evaluated. Primary CD8+ T cell responses were negligible at all time points in mice vaccinated without adjuvant. Mice vaccinated once with adjuvant had moderate primary responses in spleen and lung, and those vaccinated twice had strong primary and memory responses. Following challenge, mice vaccinated twice intranasally exhibited lung viral titers significantly lower than unvaccinated mice, and often undetectable in H3N2-vaccinated mice. These results clearly demonstrate that this adjuvant is capable of eliciting primary and memory CD8 + T cell responses to nonreplicating antigens. Further, intranasal vaccination can confer protection against viral challenge. The findings of this study are significant as they enhance the knowledge of how a commercially available adjuvant works, which in turn could lead to the development of new vaccines of global significance.

P235 Advantages and Limitations of Hair Corticosterone as a Biomarker of Chronic Stress in Rats

GLAS: Yes
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The potential use of hair glucocorticoids as a noninvasive, retrospective, biomarker of long term hypothalamic-pituitary-adrenal (HPA) axis activity is of great interest and it is gaining acceptance in humans and animals. Our research group developed and validated a method for measuring hair corticosterone concentration (HC) in rats. Our preliminary data demonstrated that HC measurement in rats appears advantageous to evaluate integrated chronic changes in circulating corticosterone. From these results we believe that this new tool needs to be applied to better characterize the overall impact in rats of common laboratory routines and to investigate potential confounding variables of this technique such as sex, hair color, and genetics. In the first experiment, 20, 8-week-old male Long Evans (LE) and Sprague–Dawley (SD) rats were subjected to external noise, room cleaning, handling, or injecting other rats in the room. In the second experiment, 25, 7-week-old male and female SD rats were subjected to social instability procedure, with alternating phases of isolation and crowding. In both experiments, during a 4-week period, body weight was recorded and blood sampling was performed weekly in the morning and in the afternoon for control and stress groups. All animals were shaved at the beginning and at the end of each experiment, and thymus and adrenal glands were collected. In both experiments no significant differences were detected in HC concentration and for body, adrenal, and thymus weight in both groups. However, HC concentration was higher in female than male rats (71%); LE rats showed higher HC concentration than SD rats (47%), and higher HC concentration in black than white hair (35%). The present findings indicate that this tool may not be able to detect low and transient changes in the HPA axis, that HC concentration in rats may differ in sex and strain, and that color may affect coricosterone's incorporation into and retention in the hair matrix.

P236 Is Activity Anorexia Really Anorexia?

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A number of studies have examined the phenomenon of activity anorexia (AA) in rats. AA is a paradoxical self-starvation emerging within days of a diet restricted to a 1-hr/day serving of food with

access to a running wheel during all but the feeding hour. It is thought that the loss of interest in food (anorexia) is a product of increased interest in running. We questioned whether the phenomenon is truly an anorexic one or simply an inability to consume enough food to support the energy expenditure of excessive running. We hypothesized that rats should reveal a decrease in food consumption as excessive running emerges within days of starting the protocol if anorexia is the key process. Twenty Sprague–Dawley rats were run in a 4-day baseline with unlimited running wheel access and ad libitum rat chow. Following baseline, rats were fed during a 1-hour time period after 23 hours deprivation each day for 9 consecutive days. Access to the running wheel was prevented during eating. Food consumption and wheel revolutions were recorded daily. Repeated-measures ANOVA confirmed a statistically significant increase in food consumption from 3.35 gms/day to an asymptote of 7.5 gms/day across days of deprivation ($P < 0.05$) after stabilizing at 13-14 gms/day of food during baseline. Activity in the running wheel increased linearly across the nine days ($P < 0.05$) following stabilization during baseline. Activity levels during the fourth baseline day correlated significantly ($P < 0.05$) with activity during the 9 protocol days. These data indicate that the critical phenomenon in AA is not anorexia because rats are eating as much as they can in a 1-hr feeding period but increasing activity beyond the energy consumption limits created by the restricted eating period.

P237 Anatomic and Functional Characterization of Parvalbumin Striatal Interneuron in Cre Transgenic Mice

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The striatum is the input nuclei of the basal ganglia circuit that is implicated in voluntary movement, procedural learning, and some other cognitive functions. There are 5 kind of different neurons, 2 of them are projection neurons called medium spiny neurons (MSN), and represent the 95% of the total population in the striatum. The remaining 5% are interneurons, including "fast spiking" (FSIs) striatal interneuron, tonic activated neuron (TAN), and low threshold spike neuron (LTS). The present work has been focused on (FSIs) that play critical role in the regulation of the physiologic activity of motor behavior. Due to relative small number of these cells, their anatomic and electrophysiological properties have been poorly studied. For these reasons, we used mouse transgenic tools to permit the direct manipulation of FSI interneuron in the study of cortico-striatal motor networks. In order to describe the anatomic and electrophysiological properties of the specific population (FSIs), we used the homozygous transgenic mouse line B6;129P2-*Pvalb*$^{tm1(cre)Arbr}$/J, (PV-Cre) combined with stereotaxic surgery to deliver in dorsal striatum the Cre-dependent adenovirus CAG.FlexTdtomato, to express the TdTomatoe fluorescence protein only in (FSIs). We also made patch clamp recordings in vitro using the animals transfected (n = 20) to describe the electrophysiological properties. In this work we showed the firing pattern that characterize these neurons and the synaptic integration of the corticostriatal pathway stimulation, as well as the anatomic cellular reconstruction of the (FSIs). Histologic analyses revealed prominent tdTomato expression in the FSIs striatal interneurons and anatomic complexity of dendritic and axonic arborizations over principal neurons. The relevance of these studies are fundamental for understanding normal cortical motor commands and their role in severe motor disorders such as Parkinsonism, dystonia, and Tourette syndrome. In conclusion, we are showing the enormous advantage of using specific transgenic tools to understand the striatal complex microcircuits.

P238 Characterization of the Gut Microbiota in Different Inbred Mouse Strains

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The gut microbiota (GM) has been recognized as a variable in animal experimentation. Previous studies have shown that the host genotype has a high impact on the GM composition. However, only a few studies have attempted to systematically survey the GM of laboratory mice. This study characterized and compared the GM profile in inbred mice of various genetic backgrounds, ages, sex, and room health status using 454 pyrosequencing of 16S ribosomal RNA gene. Fresh fecal samples were collected from 6 inbred mouse strains, including C57BL/6J (n = 60), BALB/cJ (n = 29), A/J (n =16), CBA/J(n =11) , NSG (n =15), and CByB6F1/J (n =8) at the age of 3, 5, 8, and 12 weeks, among which CByB6F1/J were housed in specific-pathogen-free (SPF) room, and other strains were housed in rooms at different barrier levels commonly defined by commercial vendors. Clustering analysis of microbial relative abundances observed significant strain-related differences even at the phylum level. With Bacteroidetes and Firmicutes being the most abundant phyla in all samples, Firmicutes was dominant in CByB6F1/J, A/J, and CBA/J, and Bacteriodetes was dominant in NSG, C57BL/6/J, and BALB/cJ. A similar pattern was seen at the species level with 2 major clusters by strain, but not by age, sex, or room health status. Microbial richness by OTU rarefaction revealed individual variability between samples, with the unsaturated rarefaction curves suggesting that larger numbers of samples and additional sequencing coverage are needed for better understanding of the microbial richness. Comparison of the rarefaction curves within strain C57BL/6J revealed that 12-week-old mice had higher richness than 3, 5, and 8-week-old mice; and interestingly, the microbial richness was positively correlated with the room barrier levels. Inter-sample dissimilarity by PCoA of weighted uniFrac distances indicated that 36% of variance was driven by strain difference, while 14% and 13% of variance reflected a mixture of age and mouse room differences. Our finding represents an important step in understanding the complexity of the mouse GM and can have implications with respect to the design of animal research and development of animal models.

P239 Estrus Detection and Endoscope-Guided Transcervical Artificial Insemination in Guinea Pigs (*Cavia porcellus*)

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Gestation in guinea pigs shares similarities with gestation in humans, making them good models for reproductive disorders such as dystocia. Additionally, guinea pigs are sensitive to teratogenic effects induced by many environmental toxins and are used as models for developmental and reproductive toxicology studies. Disadvantages to using guinea pigs include the relative difficulty in synchronizing and detecting estrus necessary to obtain a large cohort of timed pregnant females. Our goals for this study were to optimize estrus detection and to develop an efficient method to artificially inseminate (AI) guinea pigs. Altrenogest (0.22 mg) was administered orally to 29 young adult Hartley guinea pigs for 15 days to synchronize estrus. Estrus was determined by behavioral signs and vaginal cytology. Video recording was set up for detection of behavioral estrus (lordosis, receptivity to vasectomized male) during the dark cycle. Vaginal smears were taken daily for 3-4 days starting 3 days after the last altrenogest dose. Vaginal swabs from a subset of animals were submitted for bacterial culture. Females demonstrating signs of estrus were anesthetized, and endoscopy was used to guide placement of a tomcat catheter through the cervical opening. Sperm collected from proven breeders was delivered into the uterus transcervically. Pregnancy was determined by palpation and by either ultrasound or radiographs. Nineteen of 29 (65.5%) females were in behavioral estrus 4-5 days after the last dose of altrenogest. Three additional animals were determined to be in estrus based on vaginal cytology alone and were also inseminated. Of 22 animals inseminated, 20 (91%) became pregnant. We conclude that 1) behavioral estrus is moderately consistent with vaginal cytology, but that using both methods simultaneously is best for determining when to breed or AI females; 2) collection of daily vaginal smears

may promote vaginitis; 3) radiographs depicting fetal calcification around day 40 were best for pregnancy detection; and 4) endoscope-guided AI is a reliable method for obtaining timed pregnant guinea pigs.

P240 Use of Physical, Environmental, and Reproductive Outcomes to Define a Satisfactory Performance Standard of Animal Welfare in Individually Ventilated Cages

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A 28-day study was conducted with 3 different stocks and strains of mice (C57BL/6, CD-1, CD1-Foxn1nu) comparing an array of environmental, physical, hematologic, clinical chemistry, and reproductive parameters for individually ventilated cages (IVC) versus static microisolation system cages (MI). We used over 100 mice in both static cages and IVC for this comparative study. There are metabolic data, food and water intake data, hematology, clinical chemistry, and reproductive outcomes data included. Cages were changed every 7 days.These endpoints were selected to provide objective measures of animal wellbeing and welfare to optimize housing type selection for research studies. Statistically significant differences were observed for most of the environmental parameters for all three mouse lines. MI cages contained elevated levels of carbon dioxide, ammonia and humidity and lower oxygen levels. Significantly elevated food and water intake and lowered body weights were detected in IVC housed C57BL/6 mice compared to those housed in MI cages. Elevated thyroxin and/or triglycerides were present in sera from all 3 mouse lines housed in the IVC, suggesting differences due to the 2 cage types. These alterations may indicate a metabolic shift due to a thermoregulatory response in IVC housed mice. Corticosterone levels were elevated in the MI caged C57BL/6 and CD-1 mice while neutrophil: lymphocyte ratios and neutrophil counts were depressed in MI housed nude mice. Overall, these data suggest that MI cages present a more stressful environment compared to the IVC. Reproductive data from timed pregnant females and litters revealed few differences for pup survival, average pup weight, or litter sizes. Establishment of a welfare-based performance standard for IVC or MI must include an array of both biologic and physical/environmental measurements and that no single stock or strain should be used to extrapolate a performance standard for another stock or strain. The value in this study is that it evaluated 3 stocks or strains simultaneously and clearly indicates a single stock or strain is not a good indicator to extrapolate to all stocks or strains when selecting a cage and housing system. These data support that conclusion. We believe the comprehensive nature of the study and data are what make it different from other studies reported in the literature. Overall, the results obtained from this study reveal that IVC have many beneficial effects over MI on animal welfare.

P241 Influence of Cdcs1 on the Development of a Colitogenic T Cell Population

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The interleukin-10-deficient mouse (Il10$^{-/-}$) is well suited to model human inflammatory bowel disease. The colitis severity in this model depends on the background strain. A quantitative trait loci (QTL) analysis between B6.129P2-Il10^{tm1Cgn} (B6-Il10$^{-/-}$) and C3Bir.129P2-Il10^{tm1Cgn} (C3Bir-Il10$^{-/-}$) mice revealed 10 QTL called Cdcs1 to 10. Animals congenic for Cdcs1 revealed that this C3Bir derived locus has the strongest influence on colitis susceptibility. The aim of this study was to determine the influence of Cdcs1 on adaptive immune responses that trigger colitis development. Naive T cell subsets were isolated from B6-Il10$^{-/-}$ mice and from two Cdcs1-congenic strains (BC-R2 and BC-R3) and transferred to B6.129S7-Rag^{1tm1Mom} (B6-Rag1$^{-/-}$) mice to induce colitis. Magnetic resonance imaging (MRI) and histologic scoring were used to determine typhlo-colitis development in vivo and ex vivo, respectively. A microarray analysis was performed with a distinct T cell subset. Gene expression was compared between the resistant B6-Il10$^{-/-}$ and the susceptible strains C3Bir-Il10$^{-/-}$, BC-R2, BC-R3, results were confirmed by qRT-PCR. We have observed that results obtained by MRI and histologic scoring showed a good correlation. The severity of colitis induced by naive CD4^{+} T cell subsets depends on the Cdcs1 region. Different subsets of naïve T cells, characterized by different markers, induced a more severe colitis when they carried the Cdcs1 haplotype derived from C3Bir-Il10$^{-/-}$ compared to those carrying the B6-Il10$^{-/-}$ haplotype. The colitogenic properties of the different subsets obtained from each strain were similar. An impact of IL-10 producing regulatory T cells was excluded. Furthermore, the onset of colitis depended on the number of transferred cells. A microarray analysis of naive T cells revealed 47 probes differently expressed between resistant and susceptible strains. The development of a colitogenic T cell population in the transfercolitis model is attributed to the Cdcs1 locus derived from C3Bir. MRI is a suitable in vivo tool to detect and grade colitis in this model.

P242 Efficacy and Drying Times of Various Disinfectants Applied to Environmental Surfaces Spiked with *Staphylococcus aureus* or *Klebsiella oxytoca*

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Criteria for selecting the optimum disinfectant, exposure time, and Replicate Organism Detection and Counting (CONTACT) plate type used to enumerate bacteria vary in animal research facilities. The objectives of this report are to determine efficacy and required drying time of different disinfectants applied to environmental surfaces containing known concentrations of *Staphylococcus aureus* or *Klebsiella oxytoca*. CONTACT plates (Tryptic Soy Agar with or without 5% sheep blood or with Lecithin and Polysorbate 80 or D/E neutralizing agar) were used to determine the number of viable bacteria. A known concentration of *Staphylococcus aureus* or *Klebsiella oxytoca* (1.0 McFarland suspension or 3.0 x 10^8 cfu/ml) was applied on sterile polycarbonate rat cages, autoclaved stainless steel tables, and sealed epoxy floors and allowed to dry for approximately 1 hour. Designated surfaces were sprayed until wet with the appropriate disinfectant and sampled at 0, 1, 5, 10, and 20 minutes and 24 hours using different CONTACT plates. Total viable bacteria were determined from CONTACT plates after a 24-hour incubation period. The drying times for the different disinfectants varied from 7 to 10 minutes [70% Ethyl Alcohol and Accel TB (.5% hydrogen peroxide)] to more than 20 minutes (Clidox, MB-10, Virkon-S, Vimoba 128, Quatricide PV and Sani-plex 128M). Factors affecting the selected disinfectant include required drying time on different environmental surfaces and type of media used to enumerate viable bacteria. A greater reduction in the number of viable bacteria was observed from samples collected from dry surfaces exposed for ≥ 20 minutes. It was concluded that despite the fact that CONTACT plates should be collected from dry surfaces, all disinfectants reduced the number of viable bacteria from all samples collected from wet surfaces and Accel TB (.5% hydrogen peroxide), Virkon-S, and 70% Ethyl Alcohol demonstrated the greatest reduction in viable bacteria after 20 minutes.

P243 Expression-Targeted Gene Therapy for the Treatment of Murine Bladder Cancer

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Cancer of the urinary bladder is the second most common malignancy of the urinary tract (after prostate cancer). Novel treatments

are needed to specifically target cancer cells to minimize side effects due to bystander effects upon healthy cells. Expression-targeted gene therapy is a novel approach to the treatment of bladder carcinoma. Expression-targeted gene therapy refers to the use of transcriptional control elements that are active in specific cell types, to target expression of delivered transgenes to cells of interest at the transcriptional level. In this study, promoter sequences from the genes genes cox2, ran, and tnxl1 were tested for their specificity and ability to direct an efficacious response in an orthotopic murine model of transitional cell carcinoma of the bladder. Previous work has demonstrated that targeted expression of inducible caspases, under the direction of the cox2 promoter, was an efficacious means of bringing about targeted apoptosis in cancer cells. We hypothesized that expression-targeted gene therapy with the promotors for ran or tnxl1 (or the cox2 promoter, a positive control) can be used to selectively induce apoptosis in carcinoma cells via the expression of a constitutively active form of caspase 3. The mice used for this experiment were strain C57Bl6J mice divided into 8 treatment groups. Two of the 8 treatment groups are negative controls containing either sterile saline (Sham, n = 8) or a transfection solution containing a plasmid (pUC19, n = 6), which is not upregulated in cancer cells. In this model, mouse bladders infused with cells from the transitional carcinoma cell line MB49 develop tumors within 6 days. Transfection solutions containing engineered plasmids complexed with the polycation poly(ethylenimine) were administered intravesically every 72 hours for 3 treatments following the 6-day tumor incubation period. Six of the 8 treatments contained either 1x or 2x concentrations of 1 of the 3 engineered plasmids (cox2, n = 8; ran, n = 10; tnxl1, n = 10; 2x cox2, n = 6; 2x ran, n = 6; 2x tnxl1, n = 6). On the third day following the final treatment, animals were sacrificed and their bladders assessed for tumor mass and morphology. Bladder tumor size was assessed via bladder weight, and disease progression was monitored by animal weight and the presence of hematuria. Results indicate the targeting specificity and therapeutic efficacy of using cox2, ran, and tnxl1, restrict the growth of bladder tumors via induction of apoptosis. This work will serve as a stepping-stone toward the treatment of patients with transitional cell carcinoma of the bladder.

P244 Validation of a Cage-Side Glycosylated Hemoglobin Test (HbA1C) for Cynomolgus Macaques (*Macaca fascicularis*)

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Recent outbreaks of encephalomyelitis caused by arthropod-borne alphaviruses reveal their importance as an emerging cause of significant human disease and disability. An outbreak of Venezuelan equine encephalitis in the mid 1990s in Venezuela and Columbia affected an estimated 75,000 to 100,000 people, and the number of human cases of eastern equine encephalitis in the northeastern United States has markedly increased in the last decade. Patients that recover from clinical disease, especially infants and children, are often left with life-long debilitating neurologic defects, such as intellectual disability, impaired motor control, and emotional and behavioral disturbances. Sindbis virus (SINV), the prototypic alphavirus, provides a valuable model for studying alphavirus-induced encephalomyelitis. Previous studies have shown that infectious virus is cleared within 7-8 days, but viral RNA is cleared more slowly and persists in neurons at low levels for the life of the animal. We hypothesized that mice infected with a nonfatal strain of SINV would develop neurologic deficits measurable by behavior tests, and these deficits would persist beyond the period of active virus infection. Five-week-old C57BL/6 mice were intranasally inoculated with SINV or PBS control and underwent a battery of behavioral tests to assess neurocognitive function at different phases of infection. Following behavioral tests, brains were collected, and infectious virus titers, SINV RNA levels, and tissue pathology were assessed. At the height of active virus infection, characterized by peak infectious virus titers, SINV-infected mice demonstrated increased locomotor activity ($P < 0.01$) and decreased anxiety ($P < 0.01$) in open field testing and markedly impaired hippocampal-dependent memory in contextual and cued fear conditioning ($P < 0.0001$). Following recovery from clinical disease, SINV-infected mice continued to show memory deficits in contextual fear conditioning ($P < 0.05$) when only viral RNA persisted in the brain. These findings show that SINV induces long-term neurologic sequelae in mice that persist beyond active virus infection and correlate with viral RNA presence.

P245 Transcription Activator–Like Effector Nuclease Mediated Targeting of Nkx3.1 Induces Preneoplastic Prostate Cancer in Rat

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Nkx3.1 is expressed at high levels in prostate epithelial cell and regulates prostate epithelial cell proliferation. In addition, it is frequently down regulated or completely lost in high-grade prostate cancer. Therefore, It is thought that Nkx3.1 functions as potential role in prostate carcinogenesis. However, there is not a rat model for studying biofunction of Nkx3.1 in a prostate cancer environment. In this study, we generated a Nkx3.1 gene disrupted rat using transcription activator–like effector nuclease(TALEN) in rat prostate. TALEN technologies are powerful strategies for the genome engineering of laboratory animals. We have used the efficient and convenient TALEN system to generate rat strains that carry mutations in Nkx3.1 genes through microinjection of TALEN mRNA into 1-cell embryos. The embryos were transplanted in surrogate mother rat and we obtained gene modified offspring after 3 weeks.The Nkx3.1-knock out rat displayed a decrease of Nkx3.1 mRNA expression and an increase of oncogne in prostate. Furthermore, the rats present prostatic epithelial hyperplasia and dysplasia, modeling a preneoplastic condition. The results from our study suggest that the TALEN mediated Nkx3.1 targeting rat is a outstanding model for the investigation of prostate hyperplasia and prostate cancer.

P246 Tumor-Initiating Stem Cells Are Regulated by α-CaMKII-Induced VEGF in Human Osteosarcoma

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Osteosarcoma (OS) is among the most frequently occurring primary bone tumors, primarily affecting adolescents and young adults. Chemoresistance and disease recurrence are major challenges in the clinical management of OS and are thought to be caused by a small subpopulation of tumor-initiating stem cells (TISCs). Human OS TISCs are characterized by their expression of surface antigens $CD117^+$ and $Stro\text{-}1^+$, and the stem cell regulating transcription factors Sox2, Nanog, and Oct4. These OS TISCs are known to express high levels of receptors for vascular endothelial growth factor (VEGF). We have previously demonstrated that alpha-Ca^{2+}/Calmodulin kinase two (α-CaMKII) regulates VEGF and its autocrine signaling functions in human OS. Here, we examine whether OS TISCs are regulated by α-CaMKII-induced VEGF. Using fluorescence-activated cell sorting, we discovered that the pharmacologic inhibition of α-CaMKII or VEGF in 143B OS cells by tamoxifen (1 μM) or bevacizumab (1 μM), respectively, decreases the population of $CD117^+$ and $Stro\text{-}1^+$ TISCs, and the gene expression (60%) and protein levels (80%) of Sox2, NANOG, and Oct4. Additionally, we developed a novel preclinical xenograft mouse model to examine the recurrence and metastasis of human OS. 143B OS cells were intratibially injected into mice, and tumors were allowed to grow for 2 weeks. Hind limbs-containing tumors were then amputated, and mice were confirmed to be tumor free by bioluminescent imaging 7 days post surgery. Mice were

randomized into 4 treatment groups: saline, tamoxifen (500µg/kg/day), and/or bevacizumab (5µg/kg twice weekly) and monitored monthly by bioluminescent imaging for the development of metastasis. The incidence of pulmonary metastasis/recurrence in saline treated mice was 100% 2 months after amputation. However, the incidence decreased to 38% in bevacizumab-treated mice, 12% in tamoxifen-treated mice, and 0% when both drugs were used. The levels of the TISCs subpopulation in pulmonary metastasis were determined by immunohistochemistry for Sox2, NANOG, and Oct4. We show that the number of TISCs were significantly increased in the recurrent metastatic pulmonary tumors when compared to primary amputated tumors. Furthermore, we show that treatment with tamoxifen and/or bevacizumab significantly decreases the number of TISCs when compared with saline treated mice. Taken together, our results demonstrate that α-CaMKII-induced VEGF controls the levels of TISCs both in vitro and in vivo.

P247 Correlation Between Surface Temperature and Core Body Temperature in Long Evans Rats

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There is need for body temperature measurement in rats used for research, including as an overall marker of animal health, a prognostic indicator, and as an experimental endpoint. Temperature is traditionally measured in rats either by placing a thermometer into the large intestine of the test subject or implanting telemetry equipment. Both methods can be problematic, however. Surface temperature has shown promise as an alternative method of temperature measurement in mice, but little data exists in rats. We hypothesized that surface temperature of rats would correlate to core body temperature. To test this hypothesis, we anesthetized 24 female adult Long-Evans rats with isoflurane gas and took measurements both traditionally (wire thermistor inserted through the anus) and using a handheld, noncontact infrared recording device. Additionally, animals were placed either on a warm (37^0C) or room temperature (20-22^0C) table to quantify the effect of exogenous warming on rat body temperature during anesthesia and determine the robustness of the infrared surface measurements. We demonstrated that surface temperature, measured at the xiphoid process of the sternum, correlates to core body temperature in rats on a nonheated surface (97.4%), and a to a lesser extent on a heated surface (81.0%). We also determined that there was no significant difference in core temperature in female Long-Evans rats for the first 20 minutes on heated versus unheated surfaces, which could indicate that exogenous heat is not required for short term procedures in these animals.

P248 Tumor Growth Progression in Mice Xenografts: Comparison Between Cell Lines of Canine And Human Inflammatory Breast Cancer

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Human and canine inflammatory breast cancer (IBC and IMC) are the most aggressive mammary neoplasia that affect women and female dogs. There are few cell lines to study IBC, such as SUM149. Thus, recently IPC-366 has been established as good model for studying the human disease. Xenografts can grow in immunosuppressed hosts, such as SCID mice, and tumor material can be injected into the host either ectopically or orthotopically. Molecular bioluminescent imaging techniques have been developed to monitor tumor pogression and possible metastasis. The aim of this study was to report the differences between ectopic and orthotopic xenografts in tumor growth and biodistribution by imaging techniques in IMC cell line (IPC-366) and IBC cell line (SUM149). Forty 6-8-week0old female mice (Fox Chase SCID® Beige CB17.Cg-PrkdcscidLystbg-J/Crl) were used. Cells lines were transduced with the lentiviral vector for bioluminescent imaging. A suspension of 10^6 cells were implanted subcutaneously into the fourth inguinal mammary gland or orthotopically into the fourth mammary fat pad. If tumors were detected, they were weekly monitored by palpation and measured by calipers. Optical imaging was performed weekly using a bioluminescence/fluorescence optical imaging system. Results revealed that in IPC-366 ectopic xenografts, 100% reproduced a tumor in 2 weeks p.i. compared to the 70% of orthotopic xenografts that reproduced a tumor 1 week later. SUM149 xenografts did not show significant differences in frequence of tumor appearance (80% ectopic and 70% orthotopic xenografts) and in time of tumor emergence (3 weeks). Imaging techniques showed that IPC-366 and SUM149 grew rapidly in vivo, as nodes invading skin denoting the aggressiveness of these cell lines and signal from ectopic xenografts was more dispersed than orthotopic. Both cell lines were capable to produce metastasis in lungs; the metastatic rates were higher in ectopic models. We demonstrated that the ectopic model can be validated as a good and useful model of tumor development in addition to the orthotopic model, and techniques such as bioluminescent imaging are a successful tool to monitor tumor progression and the apparition of metastasis in vivo in mice.

P249 Can Isoflavones Affect the Onset of Puberty in Male Wistar Rats?

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Isoflavones are found in many vegetal products, such as soy, which has the highest concentration. Additionally, isoflavones have a diphenolic structure similar to the estrogen 17β estradiol; therefore, they could affect hormonal secretion. The aim of this study was to measure serum testosterone in male prepuberal Wistar rats after exposure to high and low doses of genistein, daidzein, or a mixture of both isoflavones and their effects on epididymal histology and the epididymal sperm count. One hundred seventy-five prepuberal male Wistar rats (RjHan:WI) were allocated into 7 groups: 1 control group and 6 experimental groups that were orally administered a high or low dose of genistein, daidzein or a mixture of both. Testosterone determination were assayed by EIA. The testes and body weights were measured, and the histology of the epididymis with the spermatozoa content and epididymal sperm count were evaluated. The control group showed an increase in the serum testosterone levels at the week 3 (52 days), which corresponded to the onset of puberty in these rats. The same increase in serum testosterone levels was observed at week 4 in rats that received low doses of isoflavones. However, semi-quantitative (histologic number of epididymal spermatozoa) and quantitative (epididymal sperm count) differences in the count of spermatozoa were found. In week 2, control and low dose groups had a fewer number of epididymal spermatozoa. The other groups' sperm count showed a lower quantity. Finally, at week 4, the low-dose groups presented a considerable increase in the content of spermatozoa, demonstrating a delay (1 week) in the production of spermatozoa with respect to the onset of puberty in the control group. In the high-dose groups, the spermatozoa content had not reached normal levels by week 5 of the experiment. We concluded that the onset of puberty was delayed. At high doses, there was no significant increase in testosterone levels, which could be related to the fact that these male rats did not reach puberty. These findings were supported by the results obtained from the analysis of the epididymal content as well as the testes/body weight ratio.

P250 Early Detection of Autoimmune Colitis in Mice Using In Vivo Imaging System

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Inflammatory bowel disease (IBD) is a common, chronic, and progressive inflammatory intestinal disease in the western world. IBD includes two major types: ulcerative colitis and Crohn's disease (CD). CD affects the entire gastrointestinal tract, especially the terminal ileum and colon. The characteristics of CD are hyperplasia of the intestinal epithelium and formation of granuloma from mucosa to submucosa with a large amount of $CD4^+$ T cells infiltrating the intestinal lesions. Passive transfer of purified $CD45RB^{hi}$ $CD4^+$ T cells from CD mice into immunodeficiency mice can induce autoimmune colitis with pathologic lesions similar to those of CD. The progressive readouts of this model include weight loss and severe diarrhea 4 weeks later. However, these 2 indicators are low sensitivity and sometimes do not match the pathologic findings. Here we transferred luciferase-expressed $CD45RB^{hi}$ $CD4^+$ T cells into Rag1-KO host mice followed by the induction of autoimmune colitis. The host mice developed colitis with progressive luciferase responses as detected by an in vivo imaging system (IVIS) 2 weeks after transfer. However, only weak luciferase responses were detected in $CD45RB^{hi}$ $CD4^+$ T cell-transferred mice co-transferred with $CD25^+CD4^+$ regulatory T cells or treated with dexamethasone. All IVIS results were parallel to the pathologic abnormalities of the gut. In summary, IVIS not only provides a more sensitive, corrective, and objective mean to monitor the $CD45RB^{hi}$ $CD4^+$ T cells disease course of CD than previous phenotyping methods, but also the 3R principles of animal welfare.

P251 Disruption of Folliculin Interacting Protein-1 Inhibits B Lymphoma Growth and Sensitizes B Cell Lymphomas to Cell Death

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Folliculin interacting protein-1 (Fnip1) is a cytoplasmic protein originally discovered through its interaction with Folliculin (Flcn), a tumor suppressor mutated in the human disorder Birt-Hogg Dubé syndrome. Fnip1 and Flcn interact directly with the master metabolic regulator AMP kinase (AMPK), an enzyme that stimulates energy/nutrient production and inhibits energy/nutrient consumption in response to low ATP. Using Fnip1 deficient mice, we previously determined that Fnip1 is required for the development of B-lymphocytes, and for B lymphocyte transformation induced by the Myc oncogene in a murine model of human Burkitt's lymphoma). In this study, we used the Cre-LoxP system to conditionally disrupt Fnip1 in primary murine B cell lymphomas to determine the potential clinical efficacy of inhibiting Fnip1 in cancer. Groups of 8-9 C57BL/6J syngeneic recipient mice were injected intravenously with spontaneously occuring B cell lymphomas from Fnip1floxedEμ-MycMx1-Cre mice, where the floxed Fnip1 gene can be inducibly deleted by intraperitoneal injection of polyinosinic-polycytidylic acid (PolyIC;10 mg/kg) to induce Cre expression from the Mx1-Cre (interferon-inducible Cre) transgene. We found that conditional knockdown of Fnip1 significantly delayed lymphoma onset and prolonged survival following transplantation. Fnip1 depletion increased metabolic stress in primary murine B cells by significantly increasing both oxidative phosphorylation and glycolysis (and thus metabolic demand), as measured using the Seahorse metabolic analyzer. Mass spectrometric analysis revealed that inhibition of Fnip1 significantly altered the representation of metabolites involved in glutaminolysis, glycolysis, antioxidant, and serine biosynthesis pathways. Using flow cytometry, we found that disruption of Fnip1 increased apoptosis of primary murine Eμ-Myc cells in vitro, and sensitized primary Em-Myc lymphoma cells to apoptosis in vivo. These results suggest that inhibition of Fnip1 may provide a novel strategy to increase death of B lymphoma cells, in part by sensitizing tumor cells to cytotoxic and metabolic stress.

P252 Evaluation of Growth Kinetics and Cancer Therapeutic Standard of Care of Patient-Derived Xenograft Models Using Humanized NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ Mice

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Patient-derived xenograft (PDX) models of a wide array of cancer types have been engrafted into mice and widely retain their original histologic characteristics. The development of such mouse models aided in FDA-approved drug evaluation, as well as in preclinical compound testing. However, PDX mouse models do not have the functional immunity of a human and these models may not always accurately predict how the compound will interact in a human host. By engrafting PDX models in mice with a reconstituted human immune system we may more closely encapsulate the environment of the original tumor in the patient. We compared the growth kinetics of sarcoma, lung, and breast PDX tumor fragments by subcutaneously implanting each into 10 NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice and 10 humanized NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (hNSG) mice. Tumors were measured twice a week for up to 70 days. Analysis demonstrated that there was no statistically significant difference between the growth rates of each tumor in the hNSG and NSG models ($P < 0.05$). This concludes that the hNSG is a reliable platform for PDX engraftment studies despite a lack of human leukocyte antigen matching between the PDX tumor and the CD34+ T cells for humanization. We further conducted a standard of care (SOC) study using a PDX colon model and the therapeutic regimen as applied to the human patient to determine if hNSG mice are a functional platform for evaluating drug efficacy against patient tumors. Forty hNSG mice were engrafted with colon tumor fragments, and tumor volumes and body weight measurements were performed once a week. Once tumor volumes reached 70-300mm^3, 27 mice were randomized into 3 treatment groups—a control group with intravenous (IV) saline administration, a second with IV 5-Fluorouracil (5-FU) administration, and a third with intraperitoneal (IP) administration of bevacuizumab. Body weight and tumor measurements were performed twice weekly. Both 5-FU and bevacuizumab demonstrated a statistically significant inhibition of tumor growth ($P < 0.05$), confirming the viability of using hNSG mice as a model for PDX efficacy studies.

P253 Characterization of a Rag1 Deficient Rat: A Potential Animal Model for Omenn Syndrome

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The LEW/Ztm-Rag1^{em1Ztm} was generated by Rag1 specific zinkfinger-nuclease microinjection into the cytoplasm of zygotes. The recombination activating genes Rag1 and Rag2 code for proteins are essential for the V(D)J-recombination. As a result of this Rag1 mutation with an autosomal recessive inheritance, the disturbed maturation of B- and T-lymphocytes leads to a highly restricted immune response and to clinical symptoms. Almost all organs were

evaluated histologically, focused on the infiltration with immune cells. Additionally, the different T cell subsets in the peripheral blood and lymphoid organs were analyzed by flow cytometry. ELISA was performed to determine the serum immunoglobulin levels. Finally we investigated the lymphocyte proliferation activity in vitro by use of mixed leucocyte cultures. Although the Rag1 deficient rat is kept under germfree and specific pathogen-free conditions, it develops clinical symptoms, including alopecia, reddened or scabby skin, wet and sticky fur, failure to thrive, enlarged or small lymph nodes with a decreased number of lymphocytes, pathologic changes in lung tissue, and an increased number of inflammatory cells in tissues. These symptoms manifest mostly on day 49 to 91. By characterizing the white blood cells, we noted a complete lack of B cells, a large amount of $CD4^+/CD8^+$ double positive cells, and significantly lower values of T-lymphocytes, showing also an altered proliferative response against allo- and xenoantigen stimulation. The IgG serum levels of this rat were decreased, while the IgE levels are located in normal range. This rat model is the first described inbred rat strain with a Rag1 deficiency. It is a potential model for other immune deficiencies than SCIDs and its potential for being a model for Omenn syndrome (OS) is investigated at the moment. Most complete Rag1 KO mouse models do not display residual levels of mature T cells, but the LEW-Rag1 does. And our rat model shows a lot more clinical symptoms than the other rats and mice do, although we keep them under germ-free conditions. Furthermore, rats are more suitable for clinical studies, which include handling and surgery (such as transplantation studies) because of their bigger size. OS is a severe combined immunodeficiency, which is caused in 90% of cases by a defect of the Rag1 or Rag2 gene.

P254 Development and Progression of Nephropathy in Lean and Obese ZSF1 Rats

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We sought to characterize renal disease progression in the ZSF1 rat, a model of obesity, hyperglycemia, hypertension, and dyslipidemia, with a particular focus on renal histologic endpoints and the time course of urinary biomarkers of renal damage. Male ZSF1 obese rats (ZSF1 RatZSF1-LeprfaLeprcp/Crl) and their age-matched lean controls (n = 10 per group) were studied from 10 through 41 weeks of age for the development and progression of diabetic nephropathy (DN). Urine was collected weekly for determination of urinary protein and biomarker analysis. Histologic quantification of glomerular and interstitial lesions was performed on subsets of animals at 11, 25, and 41 weeks of age. Biomarker quantification of urinary β-2 microglobulin, calbindin, clusterin, cystatin C, KIM-1, MCP-1, NGAL, and osteopontin was performed at 12, 17, 25, 30, 36, and 41 weeks of age. Proteinuria developed by 16 weeks of age (lean: 25±1; obese 61±6, mg/day) and continued to rise in obese animals with age. Incidence of glomerular (Lean: 0.9±0.1, 1.2±0.1, and 0.9±0.2; obese: 2.3±0.3, 19.7±0.7, and 82.9±1.7 percent at 11, 25, and 41 weeks of age, respectively) and interstitial (lean: 0, 0, and 0.6±0.4; obese: 0.9±0.3, 76.9±3.6, and 167.6±9.2 total number of foci at 11, 25, and 41 weeks of age, respectively) lesions increased over time in obese ZSF1 animals compared to their aged-matched lean counterparts. In parallel, urinary β-2 microglobulin, clusterin, cystatin C, KIM-1, MCP-1, NGAL, and osteopontin all increased with age in obese animals while leans remained unchanged over time. Importantly, these urinary biomarker increases correlated positively with renal damage. The obese ZSF1 rat model displays key hallmarks of early DN and progression toward overt diabetic nephropathy including increased proteinuria and renal structural damage concomitantly with elevations in urinary biomarkers of nephropathy.

P255 Investigation of Retinal Degeneration in Albino Rabbit Eyes Associated with Environmental Lighting

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New cage height requirements were instituted for rabbits in the eighth edition of the *Guide for the Care and Use of Laboratory Animals.* This resulted in new cage racks that are 6 inches taller than previous cages plus having nonsolid, wire-covered, open tops. Since New Zealand white rabbits are used at our facility for ocular irritation and toxicity studies, there was concern that light exposure would be increased. Light intensity levels were measured and found to be similar for the bottom and middle rows to previous caging, but the top row of the new cages had light levels approximately twice that of the previously used cages (525 lux as compared to 228 lux). It is well documented that albino rodents are susceptible to light-induced retinal degeneration; however, there is little information about albino rabbits. To investigate whether this finding would be applicable to rabbits, we conducted a study to evaluate the potential of light-induced retinal degeneration from the increased light exposure. The study was conducted with 6 New Zealand White rabbits. Three rabbits were housed in the top row of the new, taller cages with nonsolid, open cage tops and 3 rabbits were housed in the bottom row. At study initiation, and at 90 and 180 days, rabbits had ophthalmic assessments which included slit-lamp exams, intraocular pressure measurements, and funduscopic exams. The rabbits were euthanized at 180 days followed by enucleation and histologic processing of eyes. All ocular assessments were within normal limits and no differences, including histologic differences, were noted between animals based on cage location. Caging albino rabbits on a top cage close to room light source for 180 days resulted in no apparent light-induced retinal degeneration.

P256 Regulation of Lymphocyte Accumulation in Tumor-Draining Lymph Nodes

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Overcoming cancer-regulated suppression of an antitumor immune response remains a major problem in tumor immunology. Little is known about the cellular and molecular mechanisms that allow tumor immune tolerance and eventual metastasis. Grossly, tumor-draining lymph nodes are enlarged in many human cancer patients, as well as murine tumor models. Our lab has previously shown that murine tumor-draining lymph nodes develop extensive lymphocyte accumulation, lymphangiogenesis, and increased lymph flow. These changes are dependent on the presence of B-lymphocytes, which predict and promote tumor spread to draining lymph nodes and distant organs. In this study, we addressed the hypothesis that lymphocyte accumulation in tumor-draining lymph nodes is due to increased lymphocyte entry into reactive lymph node environments. Using a syngeneic murine model of cancer, we implanted B16-F10 melanoma cells into the left rear footpad of C57Bl/6 mice. After 3 weeks of tumor growth, we analyzed the accumulation of immune cell populations, lymphocyte entry and exit, and chemokine expression in tumor-draining (left rear popliteal) and contralateral (right rear popliteal) nondraining lymph nodes (n = 6 to 11 mice per group). Flow cytometry and immunofluorescent microscopy were used to phenotype immune cells based on the presence of characteristic cell surface markers. To assess lymphocyte entry and exit, fluorescent-labeled splenocytes were injected intravenously, and at timed endpoints popliteal lymph nodes were harvested and labeled cell populations measured using flow cytometry. Statistical differences were determined using Wilcoxon signed rank tests. We found that the lymphocytes of tumor-draining lymph nodes do not develop an activated phenotype, suggesting that they are suppressed. In addition, the kinetics of B and T lymphocyte entry and exit were different in tumor-draining and nondraining lymph nodes, and were regulated by distinct mechanisms. These insights could provide new therapeutic targets to limit lymphocyte trafficking and accumulation

within tumor-draining lymph nodes, and to activate antitumor immune responses.

P257 Looking for Rare Antibodies: Development of an In-Vivo Plasmablast Expansion Model

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Finding antibodies with rare specificities is challenging at the best of times, but is increasingly difficult when using transgenic animals that produce only human monoclonal antibodies. Often large numbers of mice must be immunized to increase the incidence of finding these antibodies. Recently, a novel in vivo plasmablast enrichment technique was described to produce large numbers of antigen specific plasmablasts. We replicated the model inhouse by injecting in vitro antigen stimulated cells into spleens of immunodeficient mice, hoping to see >100-fold expansion in antigen-specific plasmablasts. We sought to further expand our ability to shape the immune response ex vivo and tailor it to enrich for rare antibodies that would not be captured using standard antibody generation methods. Successfully employing this model would significantly reduce the number of animals used in an immunization project. We first sought to repeat the study in mice with a normal immune system (C57BL/6), using our test antigen, human Epidermal Growth Factor Receptor (EGFR). Donor plasmablast cells from these mice were grafted to the spleens of irradiated NOD scid gamma mice. Animal procedures were optimized for the process, including splenic isolation, wound closure, analgesia, and antibiotic therapies. This study provided reliable data collection, with all animals developing titers to the desired antigen, human EGFR, following intrasplenic transplantation of cells stimulated with soluble EGFR. The animals injected with cells stimulated with an irrelevant protein, did not produce titers to human EGFR, as expected. Surgical survival rates were 100%, and postsurgery survival was greater than 97%. An experimental model has now been validated that will allow us to expand, enrich, and rescue antibodies with rare specificities, while reducing the number of animals and timeline required per immunization project.

P258 Investigation of Decreasing Anxiety Levels in Rats: A Comparison of Standard and Bi-Level Cage Configurations

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The welfare of all research animals is a priority. It is known that stressors causing increased anxiety in animals can have negative impacts on research. Therefore, research into enriched environments that can create lower anxiety for rats is warranted as it would benefit both animal welfare and research alike. Post-weaning, animals that have a more complex caging environment have exhibited behavior changes indicative of improved welfare; however, increasing cage complexity in the form of a bi-level cage design has not yet been studied. To determine if bi-level caging would decrease anxiety in post-weaning rats, 3 individually ventilated racks were examined: one rack with standard "green-line" rat cages (140 square inches), one with larger "blue-line" cages (232 square inches) but on a single level, and one with "double-decker" cages (232 square inches) designed into a bi-level environment. Twelve breeding rat pairs were placed into each caging type (4 pairs per type). The pairs were allowed to breed and the male pups weaned at day 21-23 into the same caging type in which they were born. These offspring were tested at approximately day 45 and again at approximately day 100 in both a social interaction test and an open field/locomotion test. The above methods will be completed for 3 litters per breeding pair. Data for the first litters has been analyzed. The offspring from the blue-line cages (n = 13) had significantly lower social interaction at 45 days than that of the green-line cages (n = 26); however, the green-line (n = 26) and double-decker cage (n = 23) offspring did not show a significant difference in social interaction. The offspring from the blue-line cages had a significantly lower locomotion score than offspring of the double-decker cages; and the offspring of the blue-line cages and green-line cages had a significantly lower central locomotion score (line crosses in the center of the open field grid) than the double-decker cages. Adding complexity and vertical space to the environment (Double-decker caging) results in lower behavioral anxiety levels than standard or horizontally larger (blue-line) cages.

P259 Pathogen Contamination of Human Tumors and Patient-Derived Xenografts

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Murine models implanted with human tumor cells and patient-derived xenografts (PDX) are being increasingly used for preclinical evaluation of experimental cancer therapeutics. Like other human tissues, tumors and PDX samples may contain human pathogens. Handling of contaminated human tumors and mice implanted with xenografts may pose a health hazard to laboratory and animal care personnel. To assess the potential risk, human tumor and PDX samples submitted to our laboratory were evaluated by real-time PCR assays for the presence of the following human pathogens: 2 strains of human immunodeficiency virus (HIV1, HIV2), 3 hepatitis viruses (Hepatitis A, B, C), 2 strains of human T-lymphotrophic virus (HTLV1, HTLV2), Epstein-Barr virus, 3 hantaviruses (Hantaan, Seoul, Sin Nombre), 2 herpes simplex viruses (HSV1, HSV2), human cytomegalovirus, 2 human herpes viruses (HHV6, HHV8), human adenoviruses, Varicella zoster virus, and lymphocytic choriomeningitis virus. A minimum of 1,413 samples were evaluated. Results indicated the presence of viral genomic sequences for Epstein virus (2.0%), hepatitis B (0.8%), HHV6 (0.4%), HHV 8 (0.1%), HIV 1 (0.8%), and HTLV1 (0.1%). A more limited number of samples were evaluated for the presence of 2 human papillomaviruses (HPV16, HPV18). Of the 190 samples tested, 4.2% were positive for HPV16 and 0.5% were positive for HPV18. The results of this study provide data indicating the potential prevalence of human pathogens in PDX samples and the need for vigilance by laboratory and animal care personnel in handling human-derived samples or animals implanted with human tumors.

P260 An Alternative Cage Design for Rat Hindlimb Unloading by Tail Suspension

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Rat hindlimb unloading by tail suspension is a widely used animal model for examining the effects of microgravity, inactivity, and disuse. Previous studies have described highly detailed technical instructions for hindlimb suspension cage construction and animal preparation for tail suspension, commonly referred to as the NASA method. Although the NASA method has been widely cited for its efficacy at inducing bilateral hindlimb muscle atrophy, the construction of the cage is complex and expensive. Here, we present instructions for a hindlimb unloading cage that is cost effective and simple to construct with common building materials. Using a clear polycarbonate storage box and a stainless steel shafting as the primary framework for our tail suspension method, we investigated the safety and efficacy of inducing hindlimb muscle atrophy in Sprague–Dawley rats. Comparable to the NASA method, 7 days (n = 17) and 14 days (n = 23) of hindlimb suspension led to significant muscle atrophy and decreases in muscle function relative to control (n = 16) with minimal side effects to the animals. On average, decreases in body mass were between 2% to 6%. After 7 and 14 days, decreases in plantaris and soleus muscle mass were 7% to 13% and

12% to 40%, respectively. In summary, we demonstrate a safe, efficacious, and cost-effective cage design for hindlimb unloading by tail suspension.

P261 Large Nests Do Not Interfere with the Ability to Identify Sick or Dead Mice

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Nesting material (NM) is an important means of environmental enrichment that offers a positive impact on the health and wellbeing of mice used in research. Previously, we found that 2-4 grams (g) of NM did not hinder the ability of husbandry technicians (HT) to identify mice in need of veterinary intervention nor did it affect the ability to identify mice that died in the cage. However, published literature suggests larger amounts of NM are needed to effectively reduce cold stress. In effort to provide a more effective amount of NM to our mice, we evaluated the impact of a new commercial product, a tea bag filled with 6 grams of crinkled paper, and its effect on the ability of HT to identify sick or dead mice. We hypothesized increased NM would not interfere with the HT ability to identify health concerns or death. The pilot study (PS) was performed in the same rooms, over the same 3-month period, as the historical study (HS) the year prior. The amount of mice reported for veterinary attention in the no-nesting HS and the 6g NM PS were approximately the same, 183 and 177 respectively, with no difference in the number of mice identified that had severe illness or required euthanasia. However, in cages without NM there were significantly more cages flagged by HT as requiring veterinary attention that upon vet staff exams were determined to be healthy. During the PS, the significant majority of mice flagged for veterinary exam (42%) were identified when the animal's condition was minor, requiring monitoring but no treatment. Additionally, compared to the no-nesting HS, nearly the same number of mice were found dead during the NM PS (78 vs 71); therefore large nests did not interfere with the HT ability to identify dead mice. Finally, roughly the same number of cases were identified in cages with NM at cage-change as during routine cage-side health check exams, 58 and 67 respectively, with nearly an equal distribution of clinical condition severities, with the 1 exception of more mice being identified during routine cage-side exam that required immediate euthanasia. In conclusion, the increased amount of NM did not interfere and may have even assisted with early and accurate identification of the mice requiring veterinary attention.

P262 Characterization of Monkeypox Infection in Cynomolgus Macaques Using 4 Routes of Exposure

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Smallpox, caused by the variola virus, killed millions of people before it was considered to be eradicated worldwide in 1980. A renewed interest in smallpox vaccine development is due, in part, to zoonotic poxvirus infections; the possibility of a reemergence of smallpox; and relatively rare, but severe, adverse reactions associated with first-generation smallpox vaccines. Since smallpox vaccines in development cannot be tested for clinical efficacy in humans, the animal rule provides a regulatory mechanism for approval based on evaluation of the efficacy of new-generation smallpox vaccines in suitable animal models, per 21 CFR 601.90. One animal model of poxvirus infection employs an orthopoxvirus, monkeypox (MPXV), as the challenge agent in the cynomolgus macaque. The goal here was to characterize the dose response, reproducibility, disease progression, and pathogenesis of monkeypox following exposure by the intravenous, intratracheal, intranasal, and aerosol routes in cynomolgus macaques. Range-finding studies were conducted to assess disease progression via telemetry, clinical observations, and other parameters. The dose range-finding studies (n = 21-24) were conducted to identify challenge doses that resulted in severe disease in 90% of animals (SD90). These studies collected information on clinical signs, clinical pathology, lesion counts, and gross and microscopic pathology used to design the pathogenesis studies. Serial sacrifice pathogenesis studies were then conducted for each route once an appropriate challenge dose had been determined. After challenge, groups of 3 or 4 animals were terminated every 2 days (IT, IN, aerosol) or 3 days (IV). Clinical observations were performed at least twice daily and animals had telemetry implants to monitor body temperature, heart rate, blood pressure, and respiratory rate. Additional endpoints included clinical pathology, viral load of tissue and blood, (plaque assay and PCR), throat swabs, immunohistochemistry on tissue, flow cytometry for T-cell proliferation, intracellular cytokine levels, and macroscopic and microscopic pathology. Each respiratory route was evaluated at a different laboratory, but all laboratories conducted an intravenous challenge study to evaluate reproducibility across the facilities. The data demonstrate that challenge by both the intravenous and aerosol routes of exposure are reproducible across studies and laboratories when a single source of virus stock is used and all challenge routes result in disease progression accompanied by wide virus dissemination.

P263 Reliability of Soiled Bedding Sentinels and Exhaust Air Dust PCR for the Detection of Mouse Norovirus

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Soiled bedding sentinel (SBS) is the gold standard for health monitoring of rodent colonies. However, many infectious agents do not transmit successfully through soiled bedding. Several have taken advantage of the exquisite sensitivity of PCR to detect pathogens on exhaust air dust (EAD) of IVC rack plenums. Multiple discrepancies have emerged with this methodology that may be attributed to the infectious agents or the type of rack, caging, bedding, and dust being tested. Mouse norovirus (MNV) is the most prevalent viral pathogen in research rodents. The virus is transmitted by fecal-oral route, is shed consistently, and is effectively transmitted to SBS although failures may occur. Reviewing historical SBS data, we identified IVC racks in our facilities that were consistently negative or showing high or low MNV prevalence. Using this system, we compared the reliability of SBS and EAD PCR to detect MNV under these 3 conditions. We first tested EAD by PCR in parallel with SBS at quarterly sentinel rounds and found that SBS and EAD PCR consistently detected MNV on high prevalent racks while SBS was more reliable on low prevalent racks. Unfortunately, these results could not differentiate a true or historical contamination of the plenums. We decided to perform a controlled time course study. Racks were power washed and autoclaved to eliminate any residual viral particles or nucleic acid. Naive sentinels were assigned and racks populated with high and low prevalent, as well as negative rodent colonies. SBS were tested by serology and plenums by PCR before housing and then every 2 weeks for 3 months. SBS detected MNV 2 and 4 weeks after assignment in high and low prevalent colonies, respectively. In high prevalent racks, EAD PCR was positive at 2 weeks and alternated between positive and negative thereafter. The inconsistencies of EAD PCR in detecting MNV could reflect intermittent shedding, variable persistence of the virus in the environment, or dilution of dust samples that could affect the sensitivity of the assay. These results reveal some limitations of this new methodology and the need for more systematic studies on various pathogens under different prevalence and housing conditions to further validate EAD PCR as a potential alternative to health surveillance.

P264 Health Monitoring for Unusual Rodent Species

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Unusual rodent species hold an important niche in biomedical research by providing valuable models in behavioral, infectious disease, and cancer research programs, among others. No less true than for laboratory mice (*Mus musculus*) and rats (*Rattus norvegicus*), that account for the vast majority of rodents used in biomedical research, timely and accurate diagnosis of infectious disease in unusual rodent colonies is critical to assuring the integrity of research data and preventing the spread of pathogens to other rodent colonies. The lack of commercially available serological reagents for detection of unusual rodent pathogen-specific antibody has necessitated the use of laboratory mouse and rat dirty bedding sentinels for serological health monitoring of unusual rodent colonies. Unfortunately, this approach may fail to detect pathogens that are not efficiently transmitted by dirty bedding. To address this concern, reagents were developed to allow direct serological evaluation of unusual rodent species. Serum samples from a variety of unusual rodent species such as: cotton rat (*Sigmodon hispidus*, n = 50), sand rat (*Psammomys obesus*, n = 6), kangaroo rat (*Dipodomys* sp., n = 12), African grass rat (*Arvicanthis niloticus*, n = 4), and deer mice (*Peromyscus* spp., n = 50) were evaluated by Multiplex Fluorescent Immunoassay (MFI) against a comprehensive panel of serological assays, including, *Clostridium piliforme*, *Encephalitozoon cuniculi*, *Mycoplasma pulmonis*, adenoviruses, coronaviruses, hantaviruses, parainfluenza viruses, parvoviruses, picornaviruses, rotaviruses, lymphocytic choriomeningitis virus, pneumonia virus of mice, and reovirus. Antibodies to various pathogens were detected by MFI in multiple samples. The results suggest that direct serological testing may provide a reliable method for unusual rodent species health monitoring.

P265 Gold Nanorod Accumulation within Splenic Macrophages in Sprague–Dawley Rats

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The spleen is responsible for filtering blood and providing an immune response in many disease conditions. In certain disease states, this immune response becomes pathogenic and prevents a fast recovery. Recently, a rod-shaped gold nanoparticle coated with polyethylene glycol (GNR-PEG) was constructed that selectively localizes in splenic macrophages after IV injection in mouse models. These nanoparticles may serve as an agent for "silencing" the spleen. However, rat models are commonly used for studying a number of diseases (such as spinal cord injuries) due to their larger size and the significant amount of physiologic data available. We hypothesize that the GNR-PEG will similarly localize in splenic macrophages after IV injection in a rat model. To test this hypothesis, 24 Sprague–Dawley rats were divided into 2 groups, each consisting of 6 males and 6 females. The experimental rats received a 60 mg/kg tail vein injection of GNR-PEG. The control rats received a tail vein injection of 0.6-1 ml of saline. After 24 hours, all rats from both groups were euthanized using CO_2 gas and cervical dislocation. Spleens were collected for flow cytometry, histology, dark field microscopy, and bright field microscopy. Results demonstrated all experimental rats accumulated GNR-PEG within splenic macrophages using all methods of detection (histology and dark/bright field microscopy). The control rats showed no evidence of GNR-PEG. In conclusion, GNR-PEG localizes within splenic macrophages of both mouse and rat models. Therefore, this agent could be used in rat models in which experiments aim to "silence" the spleen in diseases where macrophage release from the spleen is pathogenic.

P266 Evaluation of Safety, Efficacy, and Analgesic Requirements Following Intratesticular Injection of Zinc Gluconate for Chemical Sterilization of Crl:CD1(ICR) Mice

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Vasectomized mice are used to generate pseudopregnant female recipient mice before embryo transfer in the production of genetically modified mice. Previous research suggested that intratesticular zinc gluconate (Zng) injection provided a refinement to surgical vasectomy in generating sterile BL6CBAF1/J male mice. We sought to evaluate the safety, efficacy, and optimum analgesic requirements following use of intratesticular Zng in outbred mice. While not considered necessary by the manufacturer, analgesics are commonly used following Zng treatment of dogs in private practice. Limited data is available on the duration and magnitude of pain following chemical sterilization. Seven week-old, male Crl:CD1(ICR) mice were anesthetized with isoflurane prior to intratesticular injection of Zng (n = 6), sterile saline (n = 4), or sham injection (n = 4). All mice received 2mg/kg meloxicam SQ post-manipulation. Mice were monitored daily for 7 days following injection to evaluate scrotal appearance, body weight, body condition score, and time-to-integrate-nest test (TINT) scores. No postinjection complications were noted, with all mice maintaining body weight and restoring TINT score by 2 days post injection. Zng-injected mice had significantly decreased testicular weight ($P < 0.0001$) and sperm counts ($P < 0.02$) at 3 weeks post injection compared to controls. At 7 weeks post injection, samples from the vas deferens of Zng-injected mice contained only 2 live sperm. Marked fibrosis, inflammation, and mineralization of the seminiferous tubules was seen on histopathology of Zng-injected mice. The second study compared mice treated with an intratesticular injection of saline (n = 12) or Zng (n = 23) under 4 analgesic protocols (2 mg/kg, 10 mg/kg, and 20mg/kg meloxicam and 0.1 mg/kg buprenorphine). Zng-injected mice were significantly ($P = 0.009$) more likely to fail TINT 1 day post injection than saline controls; however, there was no difference in TINT failure among analgesic groups. Epididymal sperm analysis (n = 4 saline, n = 6 Zng) conducted 4 weeks post injection using an automated sperm analyzer found that Zng-injected testes had significantly lower sperm counts ($P = 0.0003$), a lower percentage of progressively motile sperm ($P = 0.03$) and a higher percentage of static sperm ($P < 0.002$) compared to saline-injected testes. Test matings performed 4 weeks post injection found that Zng-injected males were able to plug 12/15 females over a 5-day period. Of the 12 plugged females, 7 females failed to become pregnant and 5 females were pregnant at necropsy. Overall, our results suggest that intratesticular Zng is safe to use in Crl:CD1(ICR) mice, but further evaluation of efficacy is warranted.

P267 Development of an Ex Vivo Model of Canine Corneal Fibrosis

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Corneal scarring (fibrosis) is a leading cause of blindness in humans and companion-animal species worldwide as a result of the normal corneal wound healing response. Species-specific in vitro models are routinely used to evaluate the efficacy and safety of potential antifibrotic therapies aimed at mitigating corneal fibrosis. The majority of current in vitro models of corneal fibrosis rely on monolayer monocultures of representative corneal cell populations and cannot evaluate potential changes to corneal anatomy following treatment administration. This project aimed to develop an ex vivo model of canine corneal fibrosis that maintains normal canine corneal anatomy allowing for histologic assessment of the whole cornea following antifibrotic treatment. Canine corneas, harvested from

dogs euthanized for reasons unrelated to corneal pathology, were cultured ex vivo at the air-liquid interface in minimal essential media supplemented with 10% fetal bovine serum. Cultured corneas were wounded with 1 N NaOH and exposed to 10 ng/ml transforming growth factor beta (TGFβ1) to promote the transdifferentiation of corneal fibroblasts to corneal myofibroblasts, producing an ex vivo model of corneal fibrosis. The efficacy of TGFβ1 application to induce corneal fibrosis in naïve and wounded corneas cultured ex vivo was evaluated longitudinally through apoptosis assays, photography, histology, immunohistochemistry, and quantification of biochemical markers of fibrosis with immunoblotting and RT-PCR. Treatment of corneas cultured ex vivo at the air-liquid interface with TGFβ1 significantly increased alpha smooth muscle actin (αSMA) expression in NaOH-wounded corneas ($P < 0.01$). Corneal anatomy was not adversely affected by ex vivo culture conditions or the presence of TGFβ1. Canine corneas cultured ex vivo at the air-liquid interface are susceptible to TGFβ1-induced fibrotic change. TGFβ1 application induces myofibroblast transdifferentiation in wounded canine corneas to yield an ex vivo model of canine corneal fibrosis free of significant artifactual change. Cultured canine corneas can be maintained ex vivo at the air-liquid interface following NaOH-wounding for at least 2 weeks.

P268 Refining the PDX Mouse Model: Development of a Less Invasive and Disposable Tumor Engraftment Process

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Patient-derived xenograft (PDX) models are created using fragments of human tumors subcutaneously implanted into immunodeficient mice. These valuable models are important for chemotherapeutic efficacy screening. Engraftment in mice is commonly performed with a trocar needle under anesthesia. Three different engraftment methods were compared in NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG) mice to evaluate the tumor growth, tumor symmetry, and procedural efficiency. Method 1 uses a larger stainless steel, 13 gauge trocar needle and plunger and is considered the standard method. Methods 2 and 3 use a disposable 1cc syringe and smaller 14 gauge disposable needle. A PDX lung tumor was finely minced and evenly divided among the 3 methods and engrafted into 60 mice (n = 20). Approximately 30-40mm^3 of material was loaded into the bevel of the trocar needle in method 1 and engrafted into each mouse. Using a tumor filled, disposable syringe with methods 2 and 3, a specific volume of material (20 and 40µL, respectively) was engrafted into each mouse. Tumors were measured weekly once palpable. In 21 days post engraftment, 100% of tumors in method 3 and 90% in method 2 were palpable compared to only 60% of tumors in method 1. By day 28, 100% of tumors in all groups were palpable. Mice engrafted with method 3 had a faster growth curve and significantly larger tumors ($P < 0.0001$) as early as day 35 compared to methods 1 and 2. Tumors engrafted with method 2 were significantly more symmetrical (smaller length to width ratio) than tumors with method 1 ($P = 0.03$). While not significant, tumors engrafted with method 3 also had a smaller length to width ratio compared to method 1 and was trending towards significance. The disposable system in methods 2 and 3 provided approximately 35% time savings in performing the procedure and time spent in cleanup. Overall, the less invasive and disposable method of tumor engraftment was a success. The smaller gauge needle, with a reliably sharp tip, offers a less traumatic procedure for mice compared to the standard method. The speed of the disposable method also offers a time savings for labor. In addition, the increased tumor growth curve with method 3 may allow studies to be performed in a shorter amount of time.

P269 Impact of Western Diet on Intestinal Size in a Mouse Model of Diet-Induced Obesity

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Mice with diet-induced obesity are a frequently used model in research related to metabolic disease and diabetes, with a well-characterized metabolic phenotype. However, a systematic examination of the size of the small and large intestine in mice with diet-induced obesity is lacking. This study is relevant in the research of the relation between obesity and size of the intestine, both with regards to development of antiobesity drugs and safety issues. We fed groups of female and male C57BL/6J mice the energy-dense Western diet (n = 27 and 28 for 4 and 12 weeks) or the standard rodent diet Teklad 2018 (n = 28 and 30 for 4 and 12 weeks). Development in bodyweight was monitored, and after either 4 or 12 weeks exposure to the different diets mice were euthanized. On the day of euthanasia, an Echo MRI scanning was done on each mouse to determine lean body mass and fat mass. After euthanasia, the intestinal tract was dissected out and divided into duodenum, jejunum plus ileum, caecum, and colon plus rectum, and the length and mass of each segment was assessed. As expected, mice fed the Western diet gradually developed diet-induced obesity and after 12 weeks on Western diet, bodyweight and fat mass had increased with ≈18% and ≈300%, respectively, compared to mice fed Teklad diet. In the intestine, we observed changes of similar type in female and male mice fed Western diet, but the magnitude of the changes varied slightly, with the largest changes generally occurring in male mice. Length of the intestinal segments in mice fed Western diet was decreased with ≈5-10% compared to Teklad diet mice, except for caecum, where the length had decreased with ≈30-35%. Mass of the intestinal segments varied more. Weight of duodenum was increased by ≈13%, whereas weight of jejunum and ileum was decreased by ≈15% in mice fed Western diet. However, the largest change was observed in the large intestine; mass of caecum and colon was decreased with ≈45-50% and ≈25-30%, respectively, in mice fed Western diet. Furthermore, the changes in intestinal length and weight at the 4 and 12 week time-point were similar, which suggest the observed changes in intestinal size occur as an early adaptation to the energy-dense Western diet. In conclusion, the size of the intestine is altered when mice are fed a Western diet, and the effect of Western diet differs between the different anatomic parts of the intestine.

P270 Morbidity And Mortality Classification: A Novel Approach To Reduce, Refine, And Replace in a Laboratory Animal Facility

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To maintain healthy animal colonies, this institution established a "no tolerance" health standard using a novel 7 phase approach comprised of 1) standard establishment, 2) agent detection/identification, 3) agent containment/elimination, 4) health standard maintenance, 5) standard extension, 6) morbidity/mortality classification, and 7) mortality reduction, animal loss replacement, and experiment refinement. Here we describe phase 6 methodology and results. All animals exhibiting clinical symptoms, abnormal behavior, or gross lesions are reported and a care plan is implemented (observation, diagnostic testing, treatment, experiment termination, euthanasia, necropsy). Necropsies are performed on all animals found dead and significant lesions are evaluated by histology/pathologist review. Using a unique system, morbidity, mortality, and lesions are classified into 7 categories: undetermined, husbandry, infectious, neoplastic, trauma, specific system, and experimental. Of the 4,500 necropsies performed over a 5-year period, the highest to lowest incidence findings were undetermined (63%) no significant lesions, cause of death unknown; husbandry related (20%) malocclusion, cannibalization, fighting; neoplastic (5%) lymphoma and tumors; system related (4%) GI, reproductive, dystocia ; trauma (2%) wounds; experimental (2%) procedural, technical; and infectious (4%). Using this data to address highest-incidence problems, overall morbidity and mortality, husbandry issues, and pup loss declined by 25% and traumas decreased by 50%. Infectious incidence increased due to *Helicobacter* and MPV outbreaks. Although "no tolerance" is a high health standard to achieve

in a laboratory animal facility, this institution has successfully maintained this standard using the novel 7 phase approach. Morbidity and mortality classification data (Phase 6) can be used to reduce deaths, replace animal loss, and refine experiments (Phase 7) in compliance with animal care standards and regulations.

P271 Effect of Autologous Cumulus Cell-Enriched Culture Media on Live Pup Development after in Laser-Assisted in Vitro Fertilization in Mice

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Laser-assisted in vitro fertilization (LA-IVF) has been shown to be a useful assisted reproductive technology for rescue of cryogenically preserved mouse strains. However, live pup production rate (the number of live pups born/embryos transferred) from LA-IVF is low when compared to that of conventional IVF. There is a need to improve IVF and embryo culture conditions to increase live pup production rate during LA-IVF. This would represent a refinement of LA-IVF, since the number of donor and recipient female mice used during the procedure could be reduced. Cumulus cells (CCs) surround oocytes as part of the cumulus-oocyte-complex (COC). CCs produce growth factors that aid in the development of oocytes, consume harmful reactive oxygen species, and can be cultured in vitro. The aim of this study was to determine if CC-enriched IVF and embryo culture media could increase live pup production rate during LA-IVF. COCs were collected from superovulated C57BL/6NTac female donor mice, CCs were collected and washed, and perforations in the zonae pellucidae of the collected oocytes were created using a laser. Motile sperm from a C57BL/6NTAc male donor mouse were added to multiple microdrops containing HTF culture media and zona-perforated oocytes for IVF. After 4 hours of IVF, one-cell embryos were transferred to embryo culture dishes containing multiple KSOM culture microdrops. A high concentration of CCs were added to half of the IVF microdrops, as well as half of the embryo culture microdrops ((+) CC study group). No CCs were added to the remaining IVF and embryo culture microdrops ((-) CC study group). Embryos were cultured overnight, and surgically transferred via oviduct transfer into pseudopregnant Crl:CD1(ICR) elite female mice (n = 6/group; 12 total). At 19 days gestation, terminal cesarean section surgeries were performed on all recipient females, and the total number of live pups was compared to the total number of embryos transferred. There were a significantly greater number of live pups in the (+) CC group than the (-) CC group (P = 0.03). Co-culture with autologous cumulus cells is a simple, inexpensive procedure that may increase live pup production rate during LA-IVF procedures.

P272 *Helicobacter pylori* Infection and Iron Metabolism: Behavioral and Gene Expression Outcomes in C57bl/6 Female Mice

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Helicobacter pylori (Hp) is a bacterial pathogen that infects > 50% of the world's population. It persists without antibiotic treatment, is the causative agent of peptic ulcer disease, and is a strong risk factor for development of gastric cancer. Environmental conditions, such as iron deficiency anemia (IDA), enhance *Hp* virulence and increase risk for carcinogenesis. IDA affects billions of people worldwide, and has overlapping prevalence in geographic regions with high *Hp* prevalence. Iron is essential for neuronal energy metabolism, neurotransmitter production, and myelination, and children that experience iron deficiency during growth stages of life suffer from negative cognitive and behavioral sequelae throughout life. The primary aims of our study were to evaluate both the effect of *Hp* on iron metabolism, and behavioral and gene expression outcomes following comorbid *Hp* infection and iron deficiency in a mouse model. C57BL/6 female mice (n = 40) were used; half were started on an iron deficient (ID) diet immediately post weaning, and the other half were maintained on an iron replete (IR) diet. Half were dosed with *Hp* SS1 at 5 weeks of age, and the remaining mice were sham-dosed. There were 4 study groups: a control group (-*Hp*/IR diet) as well as 3 experimental groups (-*Hp*/ID diet; +*Hp*/IR diet; +*Hp*/ID diet). All mice underwent a behavioral testing paradigm at 8-12 weeks of age, including anxiety tasks (open field, elevated zero maze) and learning/memory tasks (novel object recognition, fear conditioning). At 8 months postinfection, hematocrit (Hct) and hemoglobin (Hgb) concentration were significantly lower in +*Hp*/ID diet mice compared to all other study groups. Analysis of behavioral data revealed significantly less vertical activity in mice in the +*Hp*/IR diet group (P = 0.016). Infection with *Hp* exacerbated a decrease in Hct and Hgb in mice on an ID diet, suggesting an alteration of iron metabolism in *Hp*-infected mice. Infection with *Hp* alone resulted in behavioral alterations. In summary, the mouse model developed in this study represents a useful tool to study the neurologic and behavioral impact of the common human co-morbidity of *H. pylori* infection and IDA.

P273 Oral Transmucosal Detomidine Gel in New Zealand White Rabbits (*Oryctolagus cuniculus*)

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Handling and restraining rabbits for routine procedures may be impossible without prior sedation, or may result in unnecessary stress or injury to rabbits or handler and may lead to increased experimental variability. Traditional sedation protocols for rabbits have used parenteral administration of sedative or anesthetic agents that can cause localized pain and tissue damage at the site of administration, especially in fractious subjects. Detomidine, an alpha-2 adrenoreceptor agonist sedative/analgesic, is commercially available in an oral transmucosal (OTM) gel formulation approved for use in horses. This study investigated the efficacy of OTM detomidine gel as a rabbit sedative, and evaluated its potential for myocardial toxicity. Eight adult male New Zealand White rabbits were used in a dose escalation study design, and randomly assigned to receive a total dose of 0.6, 1.2, or 1.8 mg/kg OTM detomidine. Heart rate, peripheral capillary oxygen saturation (SpO2), and sedation scores were assessed at 10-minute intervals from baseline to 90 minutes. Sedation scores were based on 5 reflex responses on a 0 to 3 scale; a total score of 10 was considered effective sedation for minor procedures and 15 represented heaviest sedation possible. Animals were euthanized 1-12 days after receiving OTM detomidine and gross necropsies performed; histopathology of cardiac tissue was compared with 2 control rabbits. Sedation scores increased in all rabbits, but only 3/8 rabbits reached sedation scores of 10. Sedation scores did not differ among dosage groups and the time/dose interaction was not statistically significant. Heart rate decreased rapidly in all rabbits to minimum values of 100-130 beats/min, with no difference among dosage groups. There was no effect of time or OTM detomidine dosage on SpO2. Minimal to mild degenerative changes were seen in the myocardium of treated rabbits, but there was no myocyte necrosis, inflammation, fibrosis, or mural thrombi, as reported previously in rabbits that received parenteral detomidine. OTM detomidine gel was safely and easily administered, but duration and level of sedation were unpredictable at doses examined. Therefore, its use as a sole option to facilitate handling and restraint of rabbits does not offer advantages over existing parenteral regimens.

P274 Quantification of Interstitial Insulin in Rat Adipose Tissue by Means of Direct Interstitial Access

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The biologic action of insulin is thought to be dependent on its appearance in the interstitial fluid (ISF) rather than in the plasma. Direct access to interstitial fluid can be achieved by means of open flow microperfusion (OFM). It is relevant to know the distribution of insulin between the blood and a peripheral tissue. By using OFM and the no net flux (NNF) method, an absolute quantification of insulin in an insulin-responsive tissue like subcutaneous adipose tissue can be determined. The principle of the NNF method is to perfuse a tissue with different concentrations of the substance of interest and assess the equilibrium concentration where no gain or loss of the perfusate from or to the tissue occurs. The aim of this study was to determine the absolute concentration of human insulin (HI) in the ISF of rats using the OFM technique during a hyperinsulinemic-euglycemic clamp and to investigate if the recovery of HI in the ISF was constant from probe over time. Three OFM probes were inserted into the subcutaneous adipose tissue of anaesthetized rats and HI was infused intravenously at a constant rate (80 pmol/kg/min). When steady state levels of HI was reached in the ISF the OFM probes were perfused consecutively with HI in 5 different concentrations (n = 3-4). The effluent ISF samples were collected every 40 minutes in duplicate for each concentration. A linear regression was plotted for the perfusate HI concentration (C_{in}) versus the corresponding difference between the effluent (C_{out}-C_{in}) and the perfusate concentrations. Based on the regression analysis the absolute ISF concentration was 1327pM corresponding to 42% of the plasma HI level and the relative recovery was 17%. Comparison of duplicate samples for each HI concentration showed that the recovery of HI was constant over time. In conclusion, the present data is the first to demonstrate that by using this method we were able to quantify the absolute concentration of human insulin in the subcutaneous adipose tissue of rats. In addition, the data showed that the recovery of HI was constant over time.

P275 Analysis of Mitochondrial Proteome in a Mouse Model of Dilated Cardiomyopathy

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Energy production by mitochondria is essential for normal heart beats. Here I compared protein profiles in heart mitochondria of a mouse model of dilated cardiomyopathy (4C30 strain) with those of normal control mice (C57BL/6NCr) to examine mitochondrial functions of cardiomyopathic hearts. Hearts were collected from 15-week-old males of cardiomyopathic 4C30 and C57BL/6NCr mice. Mitochondria proteins were extracted from these hearts using a mitochondria isolation kit for tissue. Approximately 25 µg of the proteins were separated by isoelectric focusing and then SDS-PAGE. After SyproRuby staining, 2-dimension electropherograms of mitochondrial proteins were compared between two strains. Some protein spots were punched out and their protein identities were determined by mass spectrometry. Of proteins referentially present in the C57BL/6NCr heart mitochondria, 3 spots were picked up and examined by mass spectrometry. Two of them (pI = ~7.5 and ~8 with the same molecular weight = ~50 KDa) were identified to be fumarate hydratase (FH), which is an enzyme working in the tricarboxylic acid cycle. The other spot (pI = ~8.5 and molecular weight = ~15 KDa) was acyl-coenzyme A thioesterase 13 (ACOT13, or THEM2), which plays a role in controlling adaptive thermogenesis. Quantitative Western blots confirmed significant decrease in the mitochondrial FH content, standardized by voltage-dependent anion channels (VDAC), in 4C30 hearts in comparison with that in C57BL/6NCr hearts (P = 0.008 by ANOVA), but not in the ACOT13 content. These results suggest that low contractive force of cardiomyopathic hearts might be due to the decrease in mitochondrial proteins critical for energy production.

P276 Angiotensin Receptor Blocker (Losartan) or CXCR4 Inhibitor (AMA3100) Enhances the Efficacy of Radiotherapy in a Metastatic Osteosacoma Model

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Osteosarcoma (OS) is the most common primary malignant bone tumor. Most OS are high grade and tend to rapidly develop distant metastases. Correlative studies have shown that tumor hypoxia and CXCR4 expression are poor prognostic factors in metastatic OS. Thus, reducing hypoxia and inhibiting CXCR4 expression may inhibit metastatic OS progression. In this study, we tested OS response to radiotherapy combined either with losartan (an angiotensin receptor blocker that reduces fibrosis and hypoxia in tumors) or AMD3100 (an inhibitor of the hypoxia-induced CXCR4 receptor). Losartan and AMD3100 are clinically available. An established highly metastatic OS model (Os-P0107) in C3H mice was used in our study. The Os-P0107 tumors showed higher spontaneous metastases (to lungs, or liver, lymph nodes, kidneys, and bones) in C3H mice within 3 months after removal of primary tumors at a size of 15 x 15 mm, and higher CXCR4 expression in primary and metastatic tumor tissues (detected by qPCR assay and immunohistochemistry staining). When Os-P0107 isografts reached 6 mm in diameter after their subcutaneous implantations, we treated them with losartan (40 mg/kg BW, gavaged) or AMD3100 (5 mg/kg BW, i.p.) daily for 7 days followed by 20 Gy of single dose local radiation (LR) at day7 (L+LR or AMD+LR). A total 80 mice were used in two separate experiments (L or AMD + LR), 10 mice in each group. We found that both of L+LR or AMD+LR treatments significantly reduced the tumor volume when compared to the control group (P = 0.01, and P = 0.05), but LR alone did not reach a significant difference when compared to the control group (P = 0.26 or P = 0.07). In summary, we have established a highly metastatic and CXCR4 expressing OS model. We used it to show the efficacy of losartan or AMD3100 combining with radiotherapy in metastatic OS treatment. Our preliminary results show that while these combinations improved local control in Os-P0107 model, they did not significantly affect distant dissemination. To address it, we are currently evaluating the safety and efficacy of a triple combination (L+AMD+LR) for both local and distant disease control in the model.

P277 High-Efficiency Gains from a Comparative Microwave Decalcification Study in Mice Bone Marrow Cells for Immunohistochemistry Staining

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Microwave technology can be used in histology laboratories for more rapid bone decalcification; however, bone processing protocols must be optimized to ensure the preservation of cellular morphology and tissue antigenicity. In an effort to determine optimal processing with efficient decalcification of bone for immunohistochemical staining of bone marrow cells, a comparative study was conducted with 3 decalcification fluids (Ethylenediamine Tetraacetate Acid (EDTA), Cal-Rite, and Immunocal) with or without the use of microwaving. Six mouse sternums were collected at necropsy and preserved in 10%

neutral buffered formalin (NBF) for 24 hours at room temperature. The sternums were decalcified with (n = 3) or without (n = 3) microwaving. Next, the sternums were processed, embedded, sectioned, and stained with antibodies cross-reactive with mouse human Ki-67, Cleaved Caspase 3, Phospho-Histone H3, and Phospho-Histone H2AX. Antigen preservation and cell morphology were maintained in the microwaved sections. When compared to Immunocal, the Cal-Rite and EDTA decalcification fluids generally helped maintain bone marrow cell morphology and appropriate immunohistochemical (IHC) staining patterns. Additionally, by employing microwave decalcification with either Cal-Rite or EDTA, processing was approximately 24 to 48 times faster when compared to routine decalcification using the same solutions without microwaving. This study demonstrates that a more rapid (24 to 48X) retrieval may be accomplished using microwave heating during decalcification, and Cal-Rite or EDTA solutions may facilitate appropriate cell morphology for histologic assessment.

P278 A Novel In Vivo Imaging Modality to Evaluate Insulin-Stimulated Glucose Uptake in Skeletal Muscle of CD-1 Mice

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GLUT-4 is a glucose transporter that is largely responsible for glucose uptake and transport in skeletal muscle and adipose tissue. Upon insulin stimulation, GLUT-4 translocates from the cytoplasm to the plasma membrane, where it binds glucose and transports the molecule intracellularly for metabolism. Several molecular techniques are available to evaluate GLUT-4, including in vitro (cell culture), microscopy (after sample harvest), and ex vivo methods. To the authors' knowledge, there are currently no in vivo techniques to evaluate the GLUT-4 pathway in the live animal. In this study, we describe a novel in vivo method and hypothesize that fluorescent glucose analogues can be combined with optical imaging to evaluate GLUT-4 mediated glucose uptake in skeletal muscle of mice. We intravenously delivered into CD-1 male mice 2-deoxyglucose, a glucose analogue conjugated to a near infrared dye (IRDye CW800 2-DG) in combination with insulin or PBS (as a control) (6 per group). We imaged the mice with an in vivo imaging system, an optical imaging unit for small animals, to observe the insulin-mediated GLUT-4 response. We observed marked 2-DG signal at 1 minute, 5 minutes, 10 minutes, and 30 minutes post injections in both groups. By 60 minutes and 24 hours, there was a statistically significant increase in 2-DG signal in the hind limbs of insulin-treated mice compared to PBS-treated animals. We conclude that this combination method of optical imaging and IRDye CW800 2-DG can be used to monitor therapeutic efficacy and pathophysiological processes in research animals for type II diabetes, obesity, and other conditions involving GLUT-4.

P279 Evaluation of Cage Filter Top PCR Testing as a Method for Detecting Pinworms and Fur Mites in Laboratory Mice

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PCR testing for rodent pathogens has been expanded to include testing of individually ventilated caging (IVC) system components. In particular, PCR testing of exhaust manifolds for fur mites have resulted in high detection rates. However, this site would likely be ineffective for IVC systems in which the exhaust air is filtered at the cage level. Pilot study results for filter top testing of cages (n = 5) housing mice infected with *Syphacia obvelata and Aspiculuris tetraptera* (n = 1), *Myocoptes musculinus* and *Myobia musculi* (n = 2), and *M. musculi* and *Radfordia affinis* (n = 2) maintained for ~30 days were positive by PCR for all agents except *A. tetraptera*. An expanded study was subsequently conducted to determine if PCR testing of filter tops in sentinel cages exposed to soiled bedding from fur mite and pinworm infected mice is a reliable detection method as compared to PCR of samples collected directly from the animals and traditional testing methods. Two bedding types, which differ in dust generation, hardwood (aspen chip bedding) and alpha cellulose (ALPHA-Dri) were used. For each bedding, 18 IVCs fitted with new filter tops, each containing 4 naïve Swiss Webster (Tac:SW) sentinels, received ~10% dirty bedding weekly (~60 ml) from infected source cages from each of 4 groups housing mice infected with *S. obvelata, A. tetraptera*, *M. musculinus*, and *M. musculi* and *R. affinis.* The remaining bedding was comprised of dirty bedding from cages housing SPF mice. Filter tops were tested prior to placement and on days 30 (n = 6), 60 (n = 6), and 90 (n = 6). At each time point, in addition to filter top PCR, the following tests were performed on each sentinel: anal tape, pooled fecal flotation, skin scrape, pooled direct PCR (fur swabs and feces), and examination of intestinal contents. This new information augments biosecurity testing programs by providing a validated alternative environmental PCR test method for institutions with IVC racks that are filtered at the cage level.

P280 Recombinant Polyoma Virus (POLY) and K Virus (K) VP1 Structural Proteins for Serological Detection of Respective Antibodies in Mice Sera

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Polyoma virus (POLY) and K virus (K) both belong to family *Polyomaviridae.* Although both of them are rare in lab animals, they are still prevalent in wild mice. An outbreak due to wild rodents biosecurity breach is possible. Therefore, both POLY and K virus continue to be members of the routine serology monitoring panels of lab mice colonies. Recombinant antigens compared to conventional antigens using whole virus cultures are better purified, more specific, safer to produce, and cheaper to manufacture. Recombinant 6X-His tagged VP1 structural proteins (45KD and 46KD, respectively) for both viruses were produced using a baculovirus expression vector system in SF+ insect cells. Purified proteins were used to develop ELISA and MFIA immunoassays for routine detection of antibodies in mice sera. Insect cells were disrupted with detergent and freeze/thaw to release expressed protein aggregates which were then purified by ultracentrifugation and cesium chloride gradient techniques. Potency of purified proteins was determined by ELISA, MFIA®, SDS-PAGE gels, and western immunoblot (WIB). Analytical sensitivity and specificity of the antigens was assessed by screening them against hyper-immunized homologous and heterologous monovalent positive sera against these agents. Antigens showed high degree of selectivity because they didn't pick up antibodies against unrelated viruses. A low-level cross reactivity was found between POLY and K virus antigens. Early detection of outbreaks with these infectious agents is paramount for routine serosurveillance of mouse SPF colonies. To assess the ELISA plates and beads coated with purified VP1, proteins were screened against sequentially collected sera from mice experimentally inoculated with POLY and K viruses. Immunoassays for both agents picked up seroconversion in animals at day 21 and remained positive thereafter. Diagnostic specificity of purified antigens was tested by screening more than 300 mouse sera from historically known negative mouse colonies. Only 1-2 samples in each case gave a positive reaction by MFIA® which were then found to be negative by subsequent IFA and WIB confirmation, such as specificity of the immunoassays using VP1-proteins is >98%. Data from these field studies suggests that serologic testing by ELISA and MFIA® using POLY and K virus VP1-proteins as antigens are highly sensitive and specific in detecting antibodies in mice sera against respective agents. In addition, low cross reactivity between the 2 immunoassays for POLY and K viruses emphasizes the importance of using virus specific antigens for screening of laboratory mice.

P281 A Novel Mouse Model of Autosomal-Dominant Polycystic Kidney Disease

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Autosomal-dominant polycystic kidney disease (ADPKD) is the fourth leading cause of end stage kidney disease in adults, and is caused by mutations in the *PKD1* or *PKD2* genes encoding polycystins. This disease is characterized by the development of cysts throughout the renal parenchyma of both kidneys. Compression of functional renal tissue by the growing cysts leads to loss of function, necessitating renal replacement therapy or renal transplantation in about 50% of affected patients. Although a number of therapies targeting diverse intracellular signaling pathways have been successful in treating existing animal models of ADPKD, none of these potential therapies have yet been adopted in human ADPKD. Our overall hypothesis of these studies is that loss of the tuberous sclerosis complex-1 gene (*TSC1*) in kidney tissue results in excessive mammalian target of rapamycin (mTOR) mediated cell growth, which in turn promotes polycystic kidney disease. The TSC1 protein inhibits activation of mTOR, a major driver of protein synthesis and cell growth. Using the Cre-LoxP tissue specific gene targeting system in mice, we generated a new model of PKD by deleting the *TSC1* gene in renal tissue. We found that tissue specific *TSC1* null mice developed slowly progressive PKD around 150 to 200 days of age characterized by increased kidney/brain weight ratio and eventually reduced renal function in 100% of mice (N = 17 conditional *TSC1* null mice; $P < 0.0001$, students t-test). Using histology and immunoblotting, we found that loss of *TSC1* in renal tissue resulted in hyperactivated mTOR and Wnt signaling pathways, which are features of ADPKD disease in humans. Dietary treatment of *TSC*1-null mice from birth (n = 5 mice per group) with the mTOR inhibitor rapamycin, a drug used for immunosuppression in transplant recipients, completely prevented the development of polycystic kidneys and resultant mortality ($P < 0.0001$). These results suggest that this new mouse model recapitulates the development of polycystic kidneys in ADPKD, and represents an important new resource for testing potential therapies for this life-threatening disease.

P282 Comparison of Rolled Paper to Crinkle Paper as Enrichment for C57BL/6J Mice

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The *Guide* emphasizes the importance of providing environmental enrichment to laboratory animals. In mice, good nesting material assists in the animals' thermoregulation, increases breeding performance, and reduces their stress by fostering natural behavior. We tested the effectiveness of rolled paper, a novel nesting material, as compared to crinkle paper, a relatively commonly used material, by determining mice preference to either material and nest quality. We hypothesized that mice would prefer crinkle paper over rolled paper. Mice were placed in cages (n = 20) with nesting material they were reared in and nest quality was visually scored using a published scoring system and 3D imaging. To determine preference, breeding pairs (n = 15) were used; each pair had a mouse reared solely in either crinkle paper or rolled paper to negate the potential effects of preweaning nesting material exposure. The pairs were placed in a static microisolation housing system of 2 adjacent cages connected by a passage so mice were able to move freely between cages; each cage contained food, water bottle, and assigned nesting material. Animal location and material use were monitored up to 7 days. Preference testing was duplicated with cages rotated to account for cage orientation/location preference. Results revealed that nest-making ability was evident in both materials but the crinkle nest scored higher on the quality scale. There was also overall preference for crinkle paper with integration of rolled paper with partial to complete integration by day 7. Although mice preferred to spend most of their time in the crinkle paper nest, there may be benefits to using rolled paper in cages as mice carried and foraged (a manipulative behavior) the rolled paper to the crinkle paper nest. However, the use of rolled paper alone as a nesting material may not be sufficient because of the poorer nest quality. 3D imaging was also successfully used to evaluate provisions to animal welfare.

P283 Secondary Analysis of Hematologic Data from Human and Nonhman Primate Malaria Vaccine Research Trials and Applicability to Safe Volume LImits

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Guidelines on safe volume limits for blood collection in research participants in humans and laboratory animals can vary widely by institution and are often based on little data. The main adverse event encountered in large blood volume withdrawal is iron-deficiency anemia, which can be a public health concern in vulnerable populations worldwide, and has an unknown prevalence in research populations. It may be possible to monitor the changes of certain parameters in a standard blood panel to prevent this outcome. Data analysis of hemoglobin (HGB) and mean corpuscular volume (MCV) values compiled from the laboratory data of 43 humans and 46 macaques was performed. Spearman correlation coefficients were used to infer trends over time, and independent sample and paired t-tests were used when comparing blood loss responses to current guidelines on withdrawal maximums. For serial blood collections of large and small volumes in humans and macaques, the change from baseline MCV was analyzed over several months of each study. An overall increase in macaque MCV indicated an ability to respond appropriately to serial volume withdrawals (Spearman's ρ 0.28, $P = 0.00$). Humans had a consistent declining trend in MCV (Spearman's ρ -0.27, $P = 0.00$) from beginning to end despite being under recommended volume limits. HGB was also tested for both groups but Spearman's ρ values were less strong ($\rho = 0.08$, $p = 0.01$ for macaques; $\rho = -0.14$, $p = 0.00$ for humans). For large volume collections, decreased MCV at 4 weeks post collection was used to analyze percent-volume-removed limits for macaques. Macaque volume limits seemed sufficient at the 12.5% blood volume and below, though at 14% and above individuals tended to fail to respond ($P = 0.00$) to losses. The overall positive erythropoietic response in the macaques was likely due to the feeding of a controlled, iron-fortified diet. The lack of response in the humans may warrant consideration of iron supplementation or reconsideration of current withdrawal guidelines. For large volume blood withdrawals, the 12.5% and below blood volume limit may be appropriate for most macaques, and levels of 14% and above should be approached with caution. Individual changes from baseline laboratory values should be a focus for safety monitoring regardless of species.

P284 Glutathione Peroxidase 3 Inhibits Prostate Carcinogenesis in TRAMP Mice

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Glutathione peroxidase 3 (GPx3) is involved in protecting cells from oxidative damage, and downregulated levels of expression have been found in prostate cancer samples. However, the biologic function of GPx3 in prostate cancer still remains to be elucidated. To test the hypothesis that loss of the GPx3 increases the rate of prostate

carcinogenesis, GPx3 expression was disrupted in the TRAMP model by crossbreeding GPx3 knockout mice with TRAMP mice in order to generate the TRAMP / GPx3(+/–) HET and TRAMP / GPx3(–/–) KO line. At 8, 16, and 20 weeks of age, genito-urinary (GU) tract weights were determined and a pathologic evaluation of the prostates was completed in each group. The incidence of macroscopic prostate tumors was also determined. By conducting immunohistochemistry of Ki67 and active caspase-3, proliferation and apoptosis in prostates were evaluated, respectively. GPx3 expression was decreased in TRAMP mice and not detected in GPx3 KO mice both in mRNA and protein levels. Disruption of GPx3 expression in TRAMP mice increased the GU tract weights and the histopathological scores in each lobes. Moreover, inactivation of one (+/–) or both (–/–) alleles of GPx3 resulted in increase in prostate tumor burden with increased proliferation and decreased apoptosis. Our current findings provide the first in vivo molecular genetic evidence that GPx3 does indeed function as a tumor suppressor during prostate carcinogenesis.

P285 Lactobacillus Probiotic Protects Intestinal Epithelium from *Citrobacter Rodentium*-Induced Colitis in a TLR 2 and 4 Dependent Manner

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Citrobacter rodentium is a naturally occurring murine pathogen that causes colitis, transmissible colonic epithelial cell hyperplasia, and disrupts the colonic mucosa. *C. rodentium* is the only known murine attaching and effacing pathogen and serves as a murine model for *Escherichia coli* and enterohemorhhagic *E.coli.* It remains a significant threat to human and animal health. A previous study reported that pretreatment with *Lactobacillus rhamnosus* (LGG) attenuates the effects of *C. rodentium* infection in mice. However, the mechanism of these beneficial effects are still not completely understood. In this study, toll-like receptor (TLR)-2 knockout (KO), TLR4 KO, and C57BL/6J mice were divided into 2 groups (n = 10). Each group was pretreated with LGG or sterile phosphate buffer saline (PBS) and infected with *C. rodentium* by orogastric gavage to determine the effects and mode of actions of LGG on colitis induced by *C. rodentium.* Mice were euthanized 10 days after the infection and disease severity was assessed. Pretreatment with LGG was effective at preventing weight loss and mortality in C57BL/6J mice. Crypt lengths of colon presented pretreatment with LGG ameliorated *C. rodentium*-induced mucosal epithelial hyperplasia. In addition, LGG-fed C57BL/6J mice had longer colons than the sterile PBS-fed C57BL/6J mice and up-regulation of TNF-α, IFN-γ, IL-17 mRNA expressions were observed in sterile PBS-fed C57BL/6J mice compared to LGG-fed C57BL/6J mice. However, in TLR2 KO and TLR4 KO mice, continuous body weight loss, low survival rate, and mucosal epithelial hyperplasia were observed regardless of pretreatment with LGG or sterile PBS. These results suggest that LGG reduce *C. rodentium*-induced colitis in C57BL/6J mice, but LGG-mediated protection is not observed in TLR2 KO, TLR4 KO mice. Therefore, LGG-mediated protection is dependent on TLR-2 and TLR-4 in *C. rodentium*-induced colitis.

P286 Impact of Environmental Enrichment on Mouse Studies

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The goal of this study was to determine whether the use of nesting material as a method of enrichment would have an impact on selected parameters commonly measured in mice during the conduct of the National Toxicology Program (NTP) 13-week studies designed to evaluate the toxicity of xenobiotics. The study design mimicked the NTP 13-week toxicity studies. Groups of 40 male and 40 female B6C3F1/N mice were assigned to control (nonenriched) and enriched (nesting material) experimental groups. The parameters evaluated included food and water consumption, body weight gain, clinical observations, mortality, gross pathology, clinical pathology, and histopathology. Enriched male mice exhibited decreased feed intake without a subsequent decrease in body weight gain compared to the nonenriched male mice. It is speculated that the use of nesting material may have lessened the effect of chronic cold stress thereby allowing for more efficient use of feed. Water consumption was not affected by the use of enrichment. There were no significant differences in the clinical pathology parameters evaluated between the enriched and nonenriched mice. There were no significant enrichment-related gross pathology or histopathological changes observed. Histopathological evaluations revealed only incidental spontaneous background changes commonly observed in B6C3F1/N mice. The use of the nesting material was frequent and consistent. In general, the use of nesting material as a source of enrichment appears to have had a positive impact on the study animals allowing them to display species typical behavior (nest building). There was no significant impact on parameters commonly measured during the conduct of NTP toxicity studies in B6C3F1/N mice.

P287 Impact of a Phytanic Acid Diet on Lipid Metabolism in SCP-2, SCP-X, and L-FABP Triple Gene-Ablated Female Mice

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Cellular accumulation of high levels of phytanic acid causes toxicity in both humans and animals. Phytanic acid, the most abundantly found dietary branched-chain fatty acid, is enriched in dairy products as a metabolite of phytol, an acyclic diterpene alcohol cleaved from chlorophyll by ruminal bacteria. Fibrates and statins, which are treatments for diabetes mellitus and cardiovascular disease, respectively, are functionally related to phytanic acid and may cause side effects similar to phytanic acid toxicity including hepatoxicity and rhabdomyolysis. The lipid binding proteins L-FABP, SCP-2 and SCP-x facilitate transport, uptake and oxidation of fatty acids and cholesterol, thus decreasing the risk of toxic accumulation of phytanic acid. The objective of this study was to determine, for the first time, the effects of a high phytol diet on female C57/Bl6/N L-FABP, SCP-2/SCP-x triple gene ablated mice. Wild type (WT) control and triple knock out (TKO) mice were placed on a defined, phytol-free, phytoestrogen-free control diet versus a defined 0.5% phytol diet and mouse body weight and composition, liver weight, and liver and serum lipids and proteins were measured. TKO and phytol fed mice displayed loss of lean tissue mass, higher liver weights, mid-zonal hepatic necrosis histologically and metabolic and potential cardiovascular effects observed through qRT-PCR and Western blot analysis of serum and liver lipid and proteins. Thus, ablation of the major lipid binding proteins L-FABP, SCP-2 and SCP-x significantly affects branched chain fatty acid metabolic pathways in mice fed a high phytanic acid diet.

P288 Novel Rat Models for Primary Genital Herpes Simplex Virus-2 Infection Study

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We describe 6 rat models (SD, WIST, LEW, BN, F344, and DA) that are susceptible to intravaginal herpes simplex virus-2 (HSV-2) infection after pretreatment with progesterone. At a virus dose of 5 × 10(6) PFU of HSV-2, all rat models (n = 9 per group) were infected presenting anti-HSV-2 antibodies, infectious virus in vaginal washes,

and HSV-2 DNA genome copies in lumbosacral dorsal root ganglia and the spinal cord. Most of the LEW, BN, F344, and DA rats succumbed in systemic progressive symptoms at day 8-14 postinfection, but presented no or mild genital inflammation while SD and WIST rats were mostly infected asymptomatically. Infected SD rats did not reactivate HSV-2 spontaneously or after cortisone treatment. In an HSV-2 virus dose reduction study, F344 rats were shown to be most susceptible. We also investigated whether an attenuated HSV-1 strain (KOS321) given intravaginally, could protect from a subsequent HSV-2 infection. All LEW, BN, and F344 rats survived a primary HSV-1 infection and no neuronal infection was established. In BN and F344 rats, anti-HSV-1 antibodies were readily detected while LEW rats were seronegative. In contrast to naïve LEW, BN, and F344 rats where only 3 of 18 animals survived 5 × 10(6) PFU of HSV-2, 23 of 25 previously HSV-1 infected rats survived a challenge with HSV-2. The described models provide a new approach to investigate protective effects of antiviral microbicides and vaccine candidates, as well as to study asymptomatic primary genital HSV-2 infection. This is the first published study to describe HSV-2 susceptibility in common rat models. While mice have long been used as the first alternative to study the effects of HSV-2 prophylactic vaccine candidates, they do come with some drawbacks that rat models do not have. Limitations include that the genital mucosa in mice is susceptible to HSV-2 infection only in diestrous phase. Synchronization of the mucosa therefore requires progesterone treatment before infection or challenge. Moreover, the mouse model cannot be used to study latency and spontaneous reactivation and genital shedding. Finally, although protective immunity against HSV-2 has been described for prophylactic vaccine candidates, the effect poorly predicted the outcome in vaccine clinical trials. We have shown that 6 rat models are susceptible to genital HSV-2 infection but the outcome varies between different stocks or strains. Technology improvements have facilitated the development of transgenic rat strains, and the models discussed in this investigation could be used to create even better models of human herpes virus infection as well as providing controls with non-susceptible models.

P289 A Pedi Cures All: Toenail Trimming and the Treatment of Ulcerative Dermatitis

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Ulcerative dermatitis (UD) is the most common cause of preventable euthanasia in research mice, with reported prevalence between 4 and 21%. Deep, ulcerative lesions coincident with intense pruritus are characteristic of UD. Lesions may be found over dorsal neck, followed by facial, flank, limb, and other locations in that order of frequency. Despite its prevalence, the pathophysiology of the disease is poorly understood and consequently, treatments are palliative rather than directed at an underlying cause. Emerging evidence suggests that the scratching behavior accompanying the lesions may play a large role in the development of the lesions. We hypothesized that toenail trimming, a mechanical intervention of scratching behavior, would result in lesion improvement. In this study, we assessed the efficacy of toenail trims against our previous standard of care, Tresaderm, the appearance of lesions over time following treatment, and the practical implementation of this treatment in a large biomedical research setting. First, we evaluated the efficacy of toenail trims with a single application of Vetericyn at the time of treatment versus our previous standard of care, topical Tresaderm applied daily. We found that toenail trims were significantly more effective at resolving lesions (n = 39 toenail trims, n = 100 Tresaderm, P < 0.0001) with 93.3% of animals healing by 14 days (median time to lesion resolution). Furthermore, dorsal neck lesions did not recur by 42 days after a single toenail trim (n = 54); however, flank lesions did not resolve and the outcome of the two lesion distributions following treatment were significantly different (P < 0.0001). Finally, we implemented toenail trims at an institutional level using a novel restraint device allowing a single technician to perform treatment and found similar efficacies (approximately 90%) for toenail trims regardless of one-time topical supplement used (triple antibiotic ointment, Tresaderm, and Vetericyn, n = 55, 58, 18, P = 0.63). These findings provide substantial evidence for a simple, effective treatment of UD that can be carried out at any biomedical institution with minimal training, time, research impact, and financial investment.

P290 Anti-Obesity Effects of Mulberry Leaves and Robusta Fermented with *Leuconostoc mesenteroides* in High-Fat Diet-Induced Obese Mice

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Currently there is much interest in the potential for medicinal food products, particularly microbial fermentation products. We investigated the effects of two fungal species, *Hericium erinaceum,* a well-known edible and medical mushroom, and the ascomycetous fungus *Monascus ruber*. These fungi were cultivated on either mulberry or robusta leaves. Following secondary fermentation with the lactobacillus species *Leuconostoc mesenteroides,* extracts were made. These extracts were designated MLHE-LM and RMR-LM. To test the effects of these two extract on lipid metabolism, mice were fed a 60% Kcal fat diet to induce obesity throughout the study (DIO mice). These mice were then given 100% or 50% dose of MLHE-LM extract, 100% dose of RMR-LM extract or a distilled water control. Food intake was not significantly different between the treatment and control groups. We found that animals treated with visceral adipose tissue was significantly decreased in the 100% MLHE-LM and RMR-LM cohorts, leading to a significant reduction in adipocyte size compared to controls. To further examine the effects of the fungal extracts on metabolism, mice were fasted for 6 hours and were injected intraperitoneally with glucose (1g/kg body weight). Blood glucose levels were subsequently determined from blood drawn from the tail vein. After the end of experimental period, the mice were anesthetized and blood samples were drawn from axillary vein for determination of serum biochemical levels. Blood glucose level was significantly decreased in 100% MLHE-LM and RMR-LM group. The serum GOT and GPT levels were significantly decreased in 100% MLHE-LM, 50% MLHE-LM and RMR-LM groups compared to the control group. These findings suggest that 100% MLHE-LM and RMR-LM group can be used for inhibition of obesity and alteration of glucose metabolism.

P291 The Gut Microbiome Is Modulated by Dietary Vitamin D

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Recent studies indicate that diet plays an important role in regulating the microbiome and that the interactions between the gut microbiota and diet may significantly affect inflammatory bowel disease (IBD) development. While the composition of the diet can influence IBD in part through changes in the microbiome, the role of micronutrients in shaping the gut microbiota has not been studied. Our laboratory has recently shown that increased dietary vitamin D is protective against colitis and colitis-associated colon cancer in *Smad3*$^{-/-}$ (*Smad3*^{tm1Par}/J) mice, a model of IBD and inflammation-associated cancer. We hypothesized that these effects may be mediated in part through changes in the microbiota. To determine if changes in dietary vitamin D content are sufficient to alter the microbiome, *Smad3*$^{-/-}$ mice were fed a purified diet with either control (1 IU vitamin D/g diet) or

increased levels of vitamin D (5-10 IU vitamin D/g diet) for 1 week. 16S rRNA sequence analysis on cecal tissue demonstrated significant differences at the phyla level (*Tenericutes* and *Firmicutes*) between the 2 diet groups. To further study this, we examined whether dietary induced changes in the microbiota could be stably transplanted to germ-free mice without feeding high vitamin D diet. Germ-free Swiss Webster mice were colonized with fecal material prepared from SPF mice fed control or high vitamin D diet via oral gavage and then fed a regular chow diet. 16S rRNA sequence analysis was performed using fecal samples collected at 1, 2, and 4 weeks post transplantation. The microbial community structure differed significantly between animals transplanted with microbiota from mice fed control diet versus high vitamin D diet throughout the 4-week period by PERMANOVA ($p < 0.05$). In addition, recipients of high vitamin D diet-associated microbiota had a more diverse community structure than recipients of control diet-associated microbiota by Shannon index. Our data suggests that changes in a dietary micronutrient such as vitamin D can alter the gut microbiome in a relatively short period of time (1 week) and this altered microbiota can be stably transplanted into germ-free mice, potentially providing a means to investigate mechanisms through which the microbiome can influence IBD and colon cancer.

P292 Vitamin D Deficiency Protects against Colitis and Inflammation-Induced Colon Cancer in Dextran Sodium Sulfate-Treated *Smad3^{tm1Par}*/J Mice

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Epidemiologic studies suggest that vitamin D deficiency increases the risk of developing inflammatory bowel disease (IBD) and colon cancer. We have recently shown that increased dietary vitamin D protects *Smad3*$^{-/-}$ (*Smad3*^{tm1Par}/J) mice, a mouse model of inflammation-associated colon cancer which has defective TGFβ signaling, from IBD and colon cancer. Thus, we hypothesized that decreased dietary vitamin D would exacerbate colitis and colon cancer in a similar model. *Smad3*$^{-/-}$ mice were fed a purified diet without vitamin D (0 IU vitamin D/g diet; AIN93Null) to induce chronic vitamin D deficiency or a control level of vitamin D (1 IU vitamin D/g diet; AIN93M) and given dextran sodium sulfate (DSS) in their drinking water to initiate inflammation. Animals were euthanized at 3 separate time points to assess inflammation and tumor development. AIN93Null-fed mice had significantly decreased levels of serum 25-hydroxyvitamin D after 2 weeks on the diet compared to AIN93M-fed mice (8.4 vs 12.2 ng/ml), which further decreased below the limit of detection after 9 weeks on the diet. However, vitamin D deficiency did not change body weight, serum calcium, bone histology, or bone mineral density in the mice. Unexpectedly, mice fed AIN93Null diet showed improved survival ($P = 0.009$) and significant reductions in colon tumor incidence (7.5% vs 37.7%, $P = 0.0003$) and dysplasia (29.6% vs 60.7%, $P = 0.001$) compared to mice fed AIN93M. We next investigated how vitamin D deficiency protected mice from colon cancer by evaluating inflammation and cell proliferation during the acute disease following DSS treatment. At 9 days post DSS treatment, there was no difference in colonic inflammation between the 2 diet groups. However, at 16 days post DSS, mice fed AIN93Null had significantly decreased colitis scores (7.2 vs 13.9, $P = 0.04$) and an increase in epithelial cell proliferation within the colonic crypts ($P = 0.02$) compared to those fed AIN93M. These findings suggest that vitamin D deficiency may suppress DSS-induced colitis and tumor formation by increasing epithelial proliferation and repair early in disease and thus reducing the potential for development of chronic inflammation. Localized vitamin D deficiency may offer a treatment option for early stage IBD.

P293 Successful Islet Isolation from Canine Pancreata Procured Postcirculatory Death and after Extended Cold Ischemia Time

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Over 1 million dogs and cats in America have diabetes, which requires twice daily injections of insulin. Islet transplantation would offer a markedly improved treatment option for owners of diabetic pets. Widespread availability of this treatment will greatly depend on the accessibility of donor tissue. For veterinary applications, pancreata will most likely be procured after circulatory death and/or in more remote locations than with human organs. To date, outcomes of islet isolation from euthanized canines are marginal despite very short periods of cold ischemia during transport. Herein, a method is described for successful islet isolation from euthanized canine donor pancreata with cold ischemia times (CIT) of up to 20 hours pancreata after overnight air shipment. Briefly, donors were heparinized prior to euthanasia and the pancreas was excised. The organ was then flushed with and stored in a modified HTK cold preservation solution for transport to the processing facility. After collagenase digestion of the pancreas, islets were purified from the digest by density gradient centrifugation in a custom high osmolality medium containing the modified HTK solution and iodixanol. This method yields approximately 1000 islet equivalents (IEQ) per gram of digested tissue at an islet purity of 60% or higher. Islet are > 90% viable and show dose dependent glucose stimulated insulin secretion. Typical islet yields from human pancreata at clinical islet isolation centers are approximately 3000-4000 IEQ/gram, but are obtained from heart-beating donors and drastically shorter CIT, usually < 8 hours. For veterinary applications, human-level organ procurement conditions are likely not feasible at a large scale. Thus, the method reported herein could have tremendous value in bringing islet transplantation to pets and their owners.

P294 Chronic Eosinophilic Esophagitis in SHARPIN-Deficient Mice

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An increased number of eosinophils in the esophagus is common in several esophageal and systemic diseases, and a prominent feature of eosinophilic esophagitis. Mouse models can provide insight into the mechanisms of eosinophil infiltration and their pathogenic role. SHARPIN (SHANK-associated RH domain interacting protein) -deficient mice (C57BL/KaLawRij-*Sharpin*^{cpdm}/RijSunJ) develop a chronic proliferative dermatitis and an esophagitis characterized by epithelial hyperplasia and the accumulation of eosinophils in the serosal, submucosal and epithelial layers of the esophagus. We conducted a detailed investigation of the changes in the esophagus by light microscopy and immunohistochemistry as the mice aged from 4, 6, 8, and 10 weeks, each group had 3 male and 3 female mice, and investigated the expression of cytokines. The thickness of the esophageal epithelium and the number of eosinophils in the esophagus both increased with age. Similar to the epidermis, there were scattered apoptotic epithelial cells in mice at 6–10 weeks of age that reacted with antibodies to activated caspase 3 and caspase 9. The expression of CCL11 (eotaxin-1), IL4, IL13, and TSLP was increased ($p < 0.05$) and there was no or only transient expression of CCL24 (eotaxin-2), IL5, and IL33. This suggests a role for CCL11 in the accumulation of eosinophils and a possible role of TSLP, but not IL33 in inciting the inflammation. There was greatly increased expression of chitinase 3-like 3 and 4 (YM1 and YM2) proteins in the epithelial cells of the esophagus of SHARPIN-deficient mice ($p < 0.05$) consistent with type 2 inflammation. Crosses of SHARPIN-deficient mice with lymphocyte-deficient *Rag1* null mice did not affect the severity of the esophagitis, indicating that the inflammation is lymphocyte-independent.

P295 Evaluation of Arsenic and Lead in Calcium Bentonite Clay Applied Topically as a Treatment for Ulcerative Dermatitis in Mice

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Ulcerative dermatitis (UCD) in laboratory mice continues to be an ongoing clinical problem and animal welfare issue. Over the years, many products have been used to treat UCD in mice with limited success. More recently, the topical administration of calcium bentonite (green) clay has been explored as a viable natural treatment for UCD. Experimental samples of bentonite clay were analyzed for heavy metal content and were found to contain high levels of arsenic and lead. We sought to determine if the topical administration of this product posed a health risk to mice or introduced an unwanted research variable. The study objectives were to measure total lead and arsenic levels in blood and tissues from CD-1 mice exposed to clay via dermal absorption and/or oral ingestion due to grooming post application. Two groups of 10 singly housed, adult, female CD-1 mice were treated daily for 2 weeks via topical application with either a 100% natural calcium nentonite clay paste (0.1-0.4g) or 0.9% saline solution. After the 2-week treatment, all 20 mice were euthanized using CO_2 inhalation. Liver, kidney, and whole blood samples were collected and submitted for total lead and arsenic analysis via atomic absorption spectrophotometry. Results indicated significantly elevated levels of arsenic in the blood of treated animals but no differences in the tissues whereas, lead levels were significantly elevated in the kidney and liver of the clay-treated mice. Based on our findings, these bentonite clay products should not be used in arsenic or lead studies and used with caution in others to minimize the risk of introducing an unwanted variable.

P296 Telemetry for Continuous Glucose Monitoring in Rats

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Metabolic syndrome is a cluster of abnormalities often associated with obesity and diabetes. The hallmark of diabetes is insulin resistance, an impairment of insulin action within tissues at the level of the insulin receptor and subsequent cellular events. Type 2 diabetes arises from a combination of insulin resistance and a relative impairment of insulin secretion in response to meal ingestion. The glucose tolerance test (GTT) is vital for the characterization of metabolic syndrome, the natural progression of type 2 diabetes. In metabolic syndrome research, routine glucose measurements, including GTT, are often accomplished using glucometers and test strips. This method has significant limitations, as it is inaccurate, is time-consuming, requires frequent sampling, and induces animal stress. However, with newly developed implantable continuous glucose telemetry, we now are able to obtain continuous, real-time, blood glucose measurements in conscious laboratory animals without disturbing them. The present study evaluates the use and the performance of this system in hyperglycemic Goto-Kakizaki (GK) rats and Zucker Diabetic Fatty (ZDF) rats. Glucose telemetry was implanted in the abdominal aorta and the transmitter body in the peritoneum via an aseptic surgery. The system enabled monitoring of disease progression over time as well as repeatedly glucose tolerance test on same animal. Two weeks after postsurgical recovery, detectable differences of treatment effects on prandial and fasting blood glucose levels were evident. Telemetry glucose monitoring data, in parallel with measurements of food intake, body weight, and locomotor activity validated this platform technology as a refinement in aiding our understanding of anti-diabetic therapeutic agents.

P297 A Method to Isolate Primary Rat Pancreatic Islets to Study Secretion of Insulin in the Search for Diabetes Treatments

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Diabetes is a chronic disease which affects >300 million people worldwide. A common feature of both Type 1 and Type 2 diabetes is destruction and/or dysfunction of the insulin producing β cells. Insulin is a hormone that is important in the regulation of blood sugar which is produced and secreted from the islets of Langerhans in the pancreas. To study diabetes, primary isolated pancreatic islets are useful tools. Here we describe a method to isolate pancreatic islets from rat and how to measure glucose-stimulated insulin secretion (GSIS) in vitro. GSIS is a standard method to measure the release of insulin when glucose concentration is elevated. This is the way the pancreatic beta cells respond to the demand for insulin to facilitate the uptake of glucose in for instance the muscles. The same method is used in patients when an oral glucose tolerance test (OGTT) is performed and insulin is measured in the blood after an intake of glucose. The rat was anesthetized and the pancreas was perfused with collagenase via the bile duct. The pancreas was then dissected out and incubated at 37°C to let the collagenase enzyme digest the tissues surrounding the islets. Purified islets were kept in cell culture medium and after over-night recovery a GSIS was performed using different glucose concentrations. In addition Exenatide, a glucagon-like peptide-1 agonist (GLP-1 agonist), was tested. Exenatide is a commonly used Type 2 diabetes drug known to further stimulate the release of insulin. To be able to measure the insulin concentrations an enzyme linked immunosorbent assay (ELISA) was used. Approximately 500 islets could be isolated from each rat using this method. The islets responded very well to the glucose stimulation and an even higher release of insulin could be seen when using the GLP-1 agonist (Exenatide). This method to isolate primary rat islets and be able to test if compounds can increase the glucose-stimulated insulin secretion provides a useful tool in the search for better diabetes treatments. Furthermore, the use of isolated islets makes it possible to test several compounds using a single animal.

P298 Intratracheal Instillation in Rat with a Cassette of Compounds for Pharmacokinetic Screening

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Respiratory disease is a general term for a complex set of conditions that affect the lungs and respiratory tract. Chronic respiratory diseases, including asthma and chronic obstructive pulmonary disease, are responsible for 5 million deaths globally every year. Pharmacokinetic evaluation is an essential component of drug discovery to validate the tools that will be used to predict human kinetics. Intratracheal instillations deliver solutes directly into the lung and is an effective method for studying local effects of drugs in the lung. To measure lung retention a single compound can be administered directly in the lung to several animals, followed by analysis of lung homogenate collected at specific time intervals. However, this requires a large number of animals. To decrease the number of animals we use cassette dosing, a common technique in drug discovery screening, which involves the simultaneous administration of several compounds (4 compounds in 1 formulation) to a single animal. Thanks to the casette dosage method, we have decreased the number of animals by 75%. The rat is anesthetized and placed on its back on a slanting position. With a spatula the tongue is lifted up and a lamp is placed towards the throat, which makes it possible to see the epiglottis. A metal gavage needle is taken down past epiglottis to trachea. To make sure that the gavage needle has reached trachea and not esophagus, the gavage needle is gently moved over the cartilage rings and a finger is carefully put over the throat/trachea on the rat to feel the cartilage rings. Thereafter the compound is instilled just above the bifurcation of the trachea to get an optimal exposure in both lung lobes. A positive control is included

in each experiment to ensure correct measurements over time. In conclusion, pulmonary drug delivery allows local drug targeting, and thereby administration of low doses and decreased drug concentrations systemically, resulting in reduced systemic side effects.

Platform Sessions

PS1 Cotton Rats: A New Model with Unique Challenges

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We required a unique animal model to support their respiratory syncytial virus work. The comparative medicine department was asked to bring a new species, the cotton rat (*Sigmodon hispidus*), into the vivarium to support this work. Many challenges were faced bringing this species into the vivarium, including understanding the behavioral characteristics of the cotton rat, learning new housing and husbandry requirements, and bleeding techniques. Other areas of focus included developing training materials, updating standard operating procedures, and developing an animal use protocol for approval by the IACUC prior to the arrival of the cotton rats. By leveraging the expertise of colleagues who were experienced in handling cotton rats, we were able to adapt many of their processes and procedures, and make further modifications to accommodate the study work and increase efficiencies. This allowed us to successfully integrate the cotton rat into our vivarium.

PS2 Too Much Turnover: Sustaining the Workload and Minimizing Deficiencies while Training Staff

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Our animal program has exponentially expanded over the course of a year. The number of active animal housing rooms increased from 12 to 35 and the staff increased from 43 to 74 employees. This kind of growth is hard on any facility and can be a huge pill to swallow for the training department. Most other established facilities may never see this kind of expansion, but may experience a large intake of new employees due to turnover. In either case, it makes provision of training and proficiency challenging, especially with only one dedicated trainer. To meet this challenge, a three way split in initial training was established by combining peer-shadowing, direct training by management, and instruction with a trainer. This strategy required collaborative efforts from the whole staff, but promoted an atmosphere of support and quickly integrated new employees into the workplace. The initial demonstration of the standard operating procedures (SOPs) is done by either the trainer or the trainee's direct supervisor. The trainee is then matched with an established level 2 employee within his/her department for shadowing. Shadowing reinforces the initial training and allows the new employee to gain hands-on knowledge from peers that preform the task on a daily basis. Proficiency is then evaluated by the management/training department in a three check system. This system prevented any one person from being overwhelmed with the increase of new staffing while effectively staying on track with a 90 day training period. Training was completed for up to 7 employees per month.

PS3 Training of Husbandry Personnel to Use Simple Operant Tactics to Transfer Laboratory Dogs—Minimizing Time and Frustration: Maximizing Human–Animal Interactions Following Exercise

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In the Center for Comparative Medicine, Massachusetts General Hospital, dogs are provided group exercise opportunities in shared areas that are anywhere from 20-200 feet from their primary housing rooms. Returning dogs from these exercise areas, especially early in their tenure in the facility, involves a lengthy, frustrating, and sometimes comical process of luring dogs through the facility hallways and occasionally a dog must be picked up and carried back to their housing room. Once in the housing room, an additional lengthy process often follows to maneuver the correct dog into the correct pen. It generally takes ~15 minutes to return dogs from the exercise area to their home pen. In response to this, a simple operant conditioning tactic was implemented by the care staff. Husbandry personnel were involved in the creation of a standardized approach that included 1) an audible cue, 2) a hand gesture, and 3) a treat reward upon successful return to the home cage. Used consistently, this tactic resulted in the ability of naive dogs, within 12 days of arrival, to be returned from the exercise area to the home cage in less than 20 seconds (90+% time reduction). This approach enabled dogs to be effectively and accurately guided back to their assigned home cage with little time and effort. This simple operant technique, that can be applied to other institutions and to other species, requires minimal staff investment, saves time, spares frustration, and provides staff with the ability to engage in beneficial human-animal interactions while maximizing time spent exercising outside of the home cage.

PS4 An Accelerated Staff Husbandry and Technical Training Program for Working with Nonhuman Primates

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We have an extensive onboarding program for new staff that is generally completed within the first 90 days from hire. The program is structured with an intermix of daily instructive sessions conducted by the training program staff and hands-on competency-building sessions overseen by the facility management staff. The highest level of success in getting new staff to an independent working state in this 90 day window was seen with rodent training. Based on the success of the rodent onboarding program, managers, facility staff, enrichment personnel, and veterinarians working specifically with nonhuman primates (NHPs) established a training program that develops proficiency of these tasks within a 6 week time course. Components of the program include: 1) a list of the mandatory processes that a new NHP employee needs to be trained on; 2) unique visual standard operating processes (SOP) and associated instructional guidance tools; 3) work schedule that focuses on skill-building and practice; and 4) a formal competency assessment timeline with responsible parties identified. With this new training curriculum, time to proficiency has been reduced from approximately 12 months to 6 weeks, which is an 89% reduction overall. With the implementation of this program, we are able to more efficiently and proactively train employees and document such proficiency on all relevant techniques instead of waiting for an opportunity to present itself. Successful training results in successful employees, which in turn, leads to well cared for NHPs and high quality research.

PS5 A Working Model of Veterinary Verification and Consultation to Reduce Regulatory Burden

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In 2014 the Office of Laboratory Animal Welfare issued guidance on significant changes to animal activities previously approved by the IACUC. This guidance document introduced the opportunity for IACUCs to establish a mechanism for some significant changes to be handled administratively according to IACUC established policies.

The IACUC at Colorado State University developed a policy to define significant changes to be handled administratively through the veterinary verification and consultation (VVC) process. This policy allows the attending veterinarian to administratively handle via VVC the following protocol changes: anesthesia, analgesia or sedation; experimental substance administration; euthanasia method; duration, frequency, type of procedures; number of procedure performed on an animal; and additional strain or source all based on previously approved IACUC guidance documents. The most significant guidance document developed was the "Performance of Repeat Procedures" that outlined the duration, frequency, and type of procedures that are commonly used to collect biologic samples from laboratory animals including oral gavage, blood collection, subcutaneous administration, cerebral spinal fluid, arthrocentesis, cystocentesis, and rumen collection. Using the VVC method of protocol amendment review for the past 6 months, we have been able to administratively handle 24 of 102 amendments with a turnaround time of 8 days, which would have otherwise gone to designated member review or full committee review with turnaround times of 23 days. We've found the VVC method of review to be an effective and efficient process.

PS78 A GMP Vivarium: What in the World Does that Look Like?

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In 2013, 2 major U.S. universities were affected by outbreaks of meningitis within their student population. This devastating disease has a high mortality rate and survivors may have hearing loss, neurologic and cognitive disability, limb loss, and scarring of skin. Unfortunately, the FDA had not yet approved a Meningococcal-B vaccine in the U.S. In response to these epidemics, the FDA announced that it would accelerate approval of such a vaccine, practically 2-3 years ahead of the standard approval timeline, but only if certain supportive studies could be conducted in an efficient manner so that the vaccine could make its way into the targeted patient population. In early 2014, our vaccines research partners requested that we support this tremendous effort by conducting in vivo potency studies to meet the FDA requirements for stability and release of each manufactured batch of the meningococcal-B vaccine. However, there was one little hitch. In vivo studies would have to be conducted in compliance with good manufacturing practices (GMPs). So, how do you run a vivarium in accordance with GMP standards when GMP regulations do not cover animal work per se? We wholeheartedly accepted this challenge. In order to prepare the facility, we performed a thorough risk assessment of vivarium areas. We reached out to various subject matter experts in GMPs to supplement our colleagues' solid knowledge of good laboratory practices (GLPs) and the similarities or differences between the two regulations. Punch-lists were generated that identified potential vulnerabilities or process improvements, including: job descriptions and curriculum vitae; signature registers; training on GMPs and GDPs (good documentation practices); updates of more than 30 standard operating procedures (SOPs); quality assurance (QA) review; ongoing quality control (QC) for study-related documents; enhancement of operational processes; deviation reports for environmental issues; certification of equipment and materials; and the list goes on. In the end, our willingness to unselfishly partner with our research units and efforts to expeditiously learn about compliance with GMP regulations paid off. In November 2014, the meningococcal-B vaccine was approved by the FDA for use in the U.S.

PS7 Refinement of Postoperative Social Housing for Nonhuman Primates and Canines

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Our team refined the process for resumption of social housing postoperatively for nonhuman primates and canines. A team of experts was gathered including representatives from safety pharmacology, animal operations, and the veterinary team to determine the ideal timeline for return to social housing in our facility. Previously, social housing was suspended for 14 days postoperatively in nonhuman primates and canines undergoing various surgical procedures to allow for healing. The most common surgical procedures addressed in this project included telemetry device implantation, venous cannulation with vascular access port placement, JET-BP (jacketed external telemetry with blood pressure implant), and laparoscopic liver biopsy. Our goal was to decrease the time animals spent without social housing postoperatively while maintaining the integrity of our surgical models. We utilized multiple pilot studies to assess animal behavior/interactions, incision site healing, and stability of surgical models. Given that telemetry device implantation was the most invasive and complex surgical procedure under evaluation, we reviewed telemetry data approximately monthly to verify the surgical model integrity was not adversely impacted. We observed animals interacting positively and intentionally during postoperative social housing without detriment to the incision sites or instrumentation integrity. Following our initial evaluations, we developed a plan for return to social housing after 1 day for all nonhuman primate surgeries as well as canine liver biopsy procedures, and after 7 days for all other canine surgeries. We found that canine interactions postoperatively were generally more rigorous than nonhuman primates, so additional time in single housing was warranted with more invasive procedures. Final outcomes included reduction in postoperative single housing by 93% (decreased from 14 days to 1 day) for all nonhuman primate surgeries and canine liver biopsies and by 50% (decreased from 14 days to 7 days) for all other canine surgeries. This process refinement accomplished enhanced animal welfare through significantly faster return to social housing while upholding surgical model integrity.

PS8 Brownbanded Bamboo Shark (*Chiloscyllium punctatum*) as a Teaching Model of Developmental Biology

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The Brownbanded bamboo shark (*Chiloscyllium punctatum)* is a small and hardy chondrichthyan that breeds well in captivity, making it an ideal teaching model for marine biology and embryology. This teaching model is a unique way to encourage student interest in ichthyology, marine care, and development biology. One captive-bred *Chiloscyllium punctatum* egg was procured when the embryo was approximately 9-10 weeks into development, and allowed to acclimate to a 90 gallon marine display aquarium. At 10-11 weeks of development, the fibrous layer of one side of the egg was carefully removed to allow better visualization of the developing embryo. This allowed students to monitor changes in gill formation, yolk sac depletion, and other signs of embryo maturation throughout the semester. At 15-16 weeks of development, the embryo had grown very large but was unable to hatch naturally; therefore, the shark was manually hatched without incident. The shark remained in the display aquarium, allowing students to further track her growth and development.

PS9 Pigs at Play: Swine Enrichment Beyond the Chain

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The goal with this swine enrichment toy was to add inexpensive enrichment beyond the typical chain to encourage natural behaviors and engage the swine's intelligence and curiosity in the laboratory setting. The toy is made of 4 vertical metal rods that are bolted to diamond-grate flooring. Each rod has a rubber top for stability, and in the middle of the rods is a hard plastic ball that rests on a stain-

less steel bowl. The materials were purchased at a local hardware store or already on hand and should work on most types of coated, diamond-grate flooring. Materials had to be inexpensive, identified as sanitizable, and durable enough to withstand up to 60-80kg pigs. The metal rods had to fit in the flooring in the animal rooms and be strong enough to withstand play without bending. Finding a sanitizable top platform that was sturdy enough for pigs to push on, yet not a potential hazard to them, was a challenge. The solution was a 5/8″ thick rubber mat cut to a 12″ x 12″ top. Once assembled, the enrichment toy can be easily moved from cage to cage. Multiple pigs were observed with the toy. While the pigs enjoyed the basic design, adding a small amount of feed in the stainless steel bowl increased the pigs' interactions with the toy. Natural behaviors such as rooting, shoving, and putting front legs on the toy were observed and considered to be a success.

PS10 Platelets Are Activated in Response to Serial Sedation and Blood Collection in Pigtailed Macaques

GLAS: Yes
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Handling and sedation of nonhuman primates (NHPs) has the potential to cause stress, alter physiologic function, and introduce variability into a study. Stress in humans is associated with increased platelet activation and altered immune function, but these outcomes have not been studied in the context of handling-induced stress in NHPs. We hypothesized that serial handling and sedation of NHPs for venipuncture would result in an increase in platelet activation and a corresponding increase in hair cortisol. Twenty-four pigtailed macaques were sedated with ketamine hydrochloride for blood collection at days 0, 2, 4, 7, 10, 21, and 30 and hair collection at days 0 and 21. Flow cytometry was used to quantify platelet-monocyte aggregates (PMAs) as a measure of platelet activation, as well as platelet-lymphocyte aggregates (PLAs) and surface expression of MHC Class I and Class II molecules on unbound platelets. Hair cortisol was measured by ELISA. Our results show a cumulative increase in platelet activation following serial handling and sedation; after 21 days, there is a significant increase in CD14+CD16+ PMAs ($P < 0.0001$), CD4+ PLAs ($P < 0.0001$), and CD8+ PLAs ($P < 0.0001$), and a corresponding increase in platelet expression of both MHC Class I ($P = 0.0027$) and Class II ($P < 0.0001$), but no change in hair cortisol as compared to baseline ($P = 0.1373$). Platelet activation in the absence of a change in hair cortisol suggests it may be a more sensitive marker of stress in this context. The parallel increase in surface MHC expression with increased aggregation with lymphocytes is suggestive of the platelet modulation of the immune response. Activation occurs in synchrony with the platelet life cycle, peaking after complete turnover of the circulating platelet pool. This suggests the changes in platelet phenotype reflect earlier changes within megakaryocytes. Together, these data show that handling, sedation, and venipuncture in NHPs are sources of nonexperimental physiologic variability that may affect animal health and confound research results, and these physiologic changes occur independent of changes in hair cortisol. Care should be taken to use handling techniques that minimize stress to animals to reduce these potential variables.

PS11 Intraperitoneal Continuous Rate Infusion for Maintenance of Anesthesia in Laboratory Mice

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Intraperitoneal injectable anesthetics are commonly used to achieve surgical anesthesia in laboratory mice. Our previous work has shown that bolus redosing of injectable anesthetics to maintain a continuous surgical plane of anesthesia resulted in variable efficacy and unacceptably high mortality. We investigated the use of intraperitoneal (IP) continuous rate infusion (CRI) to maintain a surgical plane of anesthesia using ketamine (K), xylazine (X), and acepromazine (A). Anesthesia was induced in male C57BL/6J mice with 1 of 3 anesthetic combinations: (1) 80K/8X mg/kg, (2) 80K/8X/0.1A mg/kg, or (3) 80K/8X/0.5A mg/kg. Following induction, mice were given 1 of 4 CRIs for 90 min (n = 108 trials): (1) 20K mg/kg/hr, (2) 40K mg/kg/hr, (3) 80K mg/kg/hr, or (4) 40K and 4X mg/kg/hr. Drugs for CRIs were diluted in 0.9% NaCl and administered at a rate of 1.00 mL fluid per hour using a syringe pump and a butterfly catheter inserted IP. The protocols were compared based on mortality, ability to maintain immobility, and ability to maintain a surgical plane of anesthesia as determined by pedal withdrawal reflex. Consistent with previous studies, response to anesthetic protocols was highly variable. Statistical analysis showed that induction dose and CRI dose significantly affected duration of immobility and duration of surgical plane of anesthesia. The following protocols provided the longest continuous surgical plane of anesthesia while minimizing mortality: induction with 80K/8X/0.1A mg/kg and CRI of 80K mg/kg/hr (surgical plane 69.3 ± 14.4 min), induction with 80K/8X/0.1A mg/kg and CRI of 40K/4X mg/kg/hr (surgical plane 61.9 ± 18.2 min), or induction with 80K/8X/0.5A mg/kg and CRI of 40K/4X mg/kg/hr (surgical plane 84.4 ± 14.1 min). Postmortem examinations performed on mice that died under anesthesia revealed no gross evidence of trauma or other adverse effects of IP drug administration. These protocols are recommended for procedures requiring long periods of anesthesia and may be modified to suit different strains, procedures, and specific experimental needs. We conclude that intraperitoneal CRI anesthesia in mice may be used to extend the duration of injectable anesthesia with lower mortality and a more consistent plane of anesthesia than with bolus dosing.

PS12 CO_2 Induced Pulmonary Hemorrhage—Strain Differences

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Carbon dioxide (CO_2) is the most commonly used method of euthanasia for rodents. AVMA Guidelines recommend CO_2 displacement rate of 10-30% per minute (slow-fill), and recommend against placing conscious animals in prefilled chambers (pre-fill). An investigator reported pulmonary hemorrhage in BALB/c mice euthanized by slow-fill that were not previously observed using pre-fill. This study aimed to determine if CO_2 displacement method rate influences development of pulmonary hemorrhage in 2 mouse strains. Male and female BALB/c (14 6-week-old and 7, 6-month-old) and C57BL/6 (16, 6-week-old and 7, 6-month-old) were euthanized by slow-fill or pre-fill methods followed by cervical dislocation. Lung and nasal turbinates were collected for gross and histologic evaluation and scored from no hemorrhage to severe hemorrhage (0-3). There was no difference in the severity of hemorrhage based on age or sex. BALB/c mice euthanized by slow-fill had severe pulmonary hemorrhage in 11/12 mice, and severe nasal hemorrhage in 4/12 mice. BALB/c mice euthanized by pre-fill had severe pulmonary hemorrhage in 7/9 mice, and severe nasal hemorrhage in 2/9 mice. All 21 BALB/c mice showed pulmonary hemorrhage, and most showed nasal hemorrhage regardless of CO_2 displacement rate. C57BL/6 mice by slow-fill had severe pulmonary hemorrhage in 2/12 mice, and moderate nasal hemorrhage in 1/12 mouse. C57BL/6 mice euthanized by pre-fill showed severe pulmonary hemorrhage in 1/11 mice, and severe nasal hemorrhage in 2/11 mice. Regardless of displacement rate, CO_2 induced more severe pulmonary hemorrhage in BALB/c than in C57BL/6 ($p < 0.0001$, Fishers Exact Test). Further studies are planned to evaluate other factors contributing to the differences in response.

PS13 Factors Influencing the Rabbit Fecal Microbiota

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The gastrointestinal (GI) microbiota is known to play a significant role in health and disease of all mammals. In addition to assisting with optimal absorption of nutrients, it also aids in the development of mucosal immunity, including providing protection from invading pathogenic organisms and providing tolerance to common food antigens, as well as influencing the central nervous system, and therefore, behavior. Changes in the GI microbiota can result in profound changes in immunity and wellbeing. By studying the GI microbiota of rabbits, we can begin to identify how flora changes can influence the health of research animals and better interpret research results. This study aimed to characterize the fecal microbiota of rabbits and to identify how factors such as age, source, disease status, diet, seasonality, and antimicrobial use influenced its composition. Pooled fecal samples were collected from laboratory (n = 12), commercial meat (n = 101), pet (n = 61), and shelter (n = 15) rabbits, and were extracted and processed using 16s rRNA next-generation sequencing to identify the components of the fecal microbiota. The microbiota composition was consistent across all sources, with Firmicutes predominating, comprising 65-79% of the total phyla identified. No differences were apparent between recently weaned rabbits and adults; however, farmed and shelter rabbits had much higher proportions of Proteobacteria, a phylum composed predominantly of pathogenic bacteria species, compared to pet and lab rabbits (13-14% versus 2-4%, respectively). Dietary composition also influenced the fecal microbiota, in that diets enriched with hay and vegetables resulted in an increased proportion of Verrucomicrobia (18-20% versus 7%). Season was also important, with increases in Proteobacteria in the summer versus winter months (18% versus 9%). This is the first study to examine the rabbit fecal microbiota between sources and to examine how various factors influence its composition. These findings raise a number of interesting considerations regarding the impact that the rabbit fecal microbiota has on predisposition to disease development, as well as model phenotypes and reproducibility of results in rabbit-based research under different enrichment and management conditions.

PS14 Prevalence and Characterization of Methicillin-Resistant *Staphylococcus aureus* Carriage in a Colony of Rhesus and Cynomolgus Macaques

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Methicillin-resistant *Staphylococcus aureus* (MRSA) was isolated from infected wounds in nonhuman primates 4 times over a 6-month period. Because MRSA is known to be carried asymptomatically in human and animal populations it was suspected that the bacteria were present in a carrier state within the colony. However, while the emergence of MRSA has been well documented in the human and veterinary literature, no information is available on the prevalence of MRSA in laboratory nonhuman primate populations. The objective of this study was to characterize the prevalence of MRSA carriage in an academic research colony of rhesus and cynomolgus macaques using a cross-sectional analysis of 300 animals. MRSA carriage was determined by deep nasal culture with characterization of isolates using selective media, oxacillin sensitivity testing, and PCR amplification of the *mecA* gene. MRSA isolates underwent antimicrobial sensitivity testing and representative isolates were typed to determine bacterial strain origin. Culture results indicate that *S. aureus* is carried in the deep nasal passages of 59.3% of colony animals with 6.3% carrying MRSA. Sensitivity results indicate that MRSA isolates were highly resistant to beta-lactam antibiotics, which are not recommended for the treatment of MRSA infections, regardless of sensitivity results. Isolates were also highly resistant to macrolides and lincosamides with 76.1% resistant to erythromycin and 100% resistant to clindamycin. 90.5% of isolates were resistant to chloramphenicol, and 23.8% of isolates were resistant to tetracycline, both of which have been successful at treating MRSA in other species. However, 100% of isolates were susceptible to both rifampin and vancomycin. MRSA infections have the potential to confound research outcomes, particularly in animals that are immunosuppressed or have indwelling devices. Additionally, there is evidence that MRSA can be transmitted between animal and human populations, having both zoonotic and anthropozoonotic potential. This study is the first survey of MRSA carriage in laboratory macaques, providing information to guide husbandry and clinical management, and emphasizing the emergence of antimicrobial resistant bacteria in these species.

PS15 Accuracy of Human and Veterinary Point-of-Care Glucometers in Nonhuman Primate Species

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Data from animal studies indicate that point-of-care glucometers should be species specific due to differences in red blood cell glucose concentrations. Hematocrit levels also influence glucometer readings. Given that these parameters vary across NHP species, it is currently unknown whether human or veterinary glucometers are more appropriate for use in particular NHP species. Therefore, the first aim of this study was to compare the accuracy of 2 human and 2 veterinary glucometers in 3 NHP species with different hematocrit ranges. Subsequent aims assessed the impact of hypoglycemia, hyperglycemia, and sampling site on glucometer accuracy. Subjects were 80 rhesus macaques (*Macaca mulatta)*, 50 sooty mangabeys (*Cercocebus atys*), and 12 chimpanzees (*Pan troglodytes*). Human glucometers and companion animal glucometers were evaluated. Accuracy was defined as the mean difference in blood glucose (BG) between each glucometer and a laboratory glucose analyzer. In all NHP species, mean differences in BG between the veterinary glucometers and analyzer were significantly different from the human glucometers. Specifically, the veterinary glucometers overestimated the true BG value by 26-75 mg/dl in all species, whereas both human glucometers were within 7 mg/dl. The two human glucometers performed similarly in all 3 NHP species in a euglycemic state. When assessing each human glucometer in hypoglycemic and hyperglycemic mangabeys and macaques, only one glucometer significantly underestimated the true BG value in hyperglycemic (diabetic) mangabeys compared to euglycemic mangabeys. This finding was not shared by the other human glucometer, suggesting that 1 version maybe more accurate than the other glucometers in diabetic mangabeys. Venous-derived BG values were significantly lower than those derived from capillary samples, emphasizing the importance of sampling site during glucometer interpretation. Overall, findings from this study show that glucometers intended for companion animals are inappropriate for use in these NHP species, whereas the human glucometers showed clinically acceptable accuracy.

PS16 Postoperative Analgesic Efficacy in the Male Guinea Pig *(Cavia porcellus)*

GLAS: Yes

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Despite the relative popularity of the guinea pig (*Cavia porcellus*) in the laboratory and as household pets, there is a lack of species-specific, evidence-based analgesic recommendations. Our goal was to determine clinically efficacious doses and duration of actions for frequently prescribed analgesics in guinea pigs. Analgesiometry

included electronic von Frey (eVF) and behavioral ethogram analysis of remote captured videos at baseline, and after anesthesia and analgesia with or without castration surgery at 4, 8, and 24 hours postcondition. Perioperative treatment included SQ buprenorphine, carprofen, buprenorphine, and carprofen (multimodal), or sterile water, or a testicular block with bupivacaine. The anesthesia/analgesia only condition served as a control for the potentially distressing but nonpainful components of the surgical experience. We hypothesized that adequate analgesia would result in no significant difference in eVF measurements or frequency of pain-specific behaviors when comparing postanesthesia and postsurgical conditions. All results are comparisons of the postanesthesia versus surgery conditions. Animals in the control group demonstrated significant decreases in nociceptive threshold, as measured with eVF, and increases in pain behaviors, as measured by ethogram, for 24 hours after surgery. In contrast, all groups that received analgesia, with the exception of the buprenorphine group at 24 hours, demonstrated no significant changes in nociceptive thresholds after surgery. Animals that received buprenorphine alone or as part of a multimodal treatment had no significant differences in frequency of pain-specific behaviors after surgery. In contrast, bupivacaine and carprofen groups had an increased frequency of pain-specific behaviors after surgery. Based on the eVF data alone, all analgesics appeared efficacious for up to 24 hours, with the exception of buprenorphine at the 24 hour time point. The behavioral data, however, suggests that only the buprenorphine containing treatments provide adequate analgesia. As only the multimodal group demonstrated adequate analgesia for 24 hours after surgery by both measures, we recommend the combination of preemptive buprenorphine and carprofen for guinea pigs undergoing castration or similar surgeries.

PS17 Novel Methods of Evaluation of Postsurgical Pain in Female Guinea Pigs

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Guinea pigs are the most commonly used USDA covered species, with a significant percentage expected to experience pain or distress. Yet our ability to accurately identify and subsequently alleviate pain is severely lacking. In an effort to effect change, we created and validated an ethogram to identify pain-specific behaviors in male guinea pigs (*Cavia porcellus*). Behavioral ethograms may better mirror clinical pain by reflecting spontaneous responses versus the evoked responses of traditional nociceptive measures. In response to an NIH call to represent both male and female research subjects, we repeated and refined the study in female guinea pigs, creating a more robust ethogram. In this study, 10 female albino guinea pigs were assessed by electronic von Frey (eVF) and spontaneous behaviors recorded by remote video across 3 conditions, baseline, postanesthesia, and posthysterectomy at 5 different time points between 2 and 48 hours postanesthesia or surgery. We hypothesized that, as with the males, females would demonstrate an increase in pain-associated behaviors via ethogram, corresponding to a decrease in nociceptive threshold measured by eVF, in the post-surgery condition only. Mechanical pressure sensitivity was significantly increased after surgery compared to anesthesia alone at all time points, indicating posthysterectomy pain lasted for at least 2 days. Early analysis of spontaneous behavior data indicates a progressive decrease in active behaviors after anesthesia and to an even greater degree after surgery. Correspondingly, there was an increase in pain behaviors, particularly after surgery. Comparison with postcastration male guinea pigs identified significant differences in response to mechanical pressure in both baseline and postanesthesia conditions. Additionally, females had significantly greater nociceptive responses in early time points compared to males. Evaluation of females for postsurgical pain assessment completes this behavioral platform which can now serve as a tool for direct clinical application evaluating sex differences in pain behaviors and analgesia efficacy in this species.

PS18 The Effects of Transportation and Institutional Drift on the Mouse Microbiota

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Using animal models, the gut microbiota has been shown to play a critical role in the health and disease of the gastrointestinal tract as well as many other organ systems. Unfortunately, animal model studies often lack reproducibility when performed at different institutions. Previous studies in our laboratory have shown that gut microbiota of mice can vary with a number of factors including strain, vendor, caging, and diet, leading us to speculate that differing environments may alter gut microbiota which in turn may influence animal model phenotypes. In an extension of these studies, we hypothesized that the shipping of mice from a vendor to an institution results in changes in gut microbiota. Furthermore, we hypothesized that mice will develop a microbiota unique to the facility in which they are housed. To test these hypotheses, 8 C57BL/6 and 8 BALB/c mice were shipped. Fecal samples were collected the day prior to shipping, immediately upon arrival, and then on days 2, 5, and 7 postarrival. Thereafter, samples were collected once weekly. Following the first postarrival fecal collection, mice were separated into 2 groups and housed at different facilities while keeping their caging, diet, and husbandry practices the same. DNA was extracted from the collected fecal pellets and samples were sequenced for analysis. Results demonstrated that shipping altered the gut microbiota in both strains of mice. The results also demonstrated that with time the microbiota normalizes to one unique to the strain and facility (strain/facility interaction). These changes may influence disease phenotypes of mice and influence reproducibility of studies at different institutions.

PS19 Evaluation of Traditional and Contemporary Methods for Detecting *Syphacia obvelata* and *Aspiculuris tetraptera* in Laboratory Mice

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Murine pinworm infections remain a concern as highlighted by outbreaks at many institutions in 2014 after receiving infected mice from a well-respected and commonly used vendor. Our renewed interest in this topic was stimulated by inconsistent diagnostic test results during this outbreak. Currently, there is no consensus on best practice for murine pinworm diagnosis. To determine the most sensitive pinworm detection method(s), Swiss Webster mice (Tac:SW) were infected with either *Syphacia obvelata* (N = 60) or *Aspiculuris tetraptera* (N = 60) and the following methods were compared at days 0, 30, and 90, starting 21 or 28 days postinfection, respectively: fecal concentration method (*A. tetraptera* only), anal tape test (*S. obvelata* only), direct examination of cecal and colonic contents, Swiss Roll histology (cecum and colon), and PCR (pooled fur swab and feces). The optimum fecal concentration method was also evaluated prior to comparing all methods by comparing detection rates for feces infected with *A. tetraptera* using sodium nitrate (S.G. = 1.20) flotation, sodium nitrate centrifugation, sugar (S.G. = 1.27) flotation, sugar centrifugation, zinc sulfate (S.G. = 1.35) flotation, zinc sulfate

centrifugation, and water sedimentation. Sodium nitrate flotation was as good as or better than the other fecal concentration methods for detection of *A. tetraptera* ova as it had a 100% detection rate. PCR was superior to the other methods evaluated at detecting *S. obvelata. S. obvelata* detection rates were: PCR = 88%, tape tests = 48%, Swiss Roll histology = 46%, and direct examination of intestinal contents = 36%. This is the first study to compare direct examination of intestinal contents to Swiss Roll histology. Surprisingly, for *S. obvelata,* tape tests have had a better detection rate than either postmortem detection method evaluated. Importantly, no single test detected all positive animals.We recommend a combination of tests that should enable personnel designing biosecurity programs to optimize the detection of these insidious laboratory parasites. In accordance with the 3Rs, increased confidence in ante-mortem test methods may reduce the number of or need for postmortem sentinel analysis.

PS30 Evaluation of Academic Core Facility Sustainability: Assessment of Service Value versus Sustainable Costs

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Effective assessment of an academic core facility includes the identification of metrics by which to measure core success and an assessment of financial sustainability. The Unit for Laboratory Animal Medicine's In-vivo Animal Core (ULAM-IVAC) at the University of Michigan is a centralized academic resource supporting preclinical and translational research involving animal models. As with other core facilities, our challenge is to provide services that are useful and financially accessible to investigators while supporting operational costs. We assessed the efficacy of our core by defining metrics for measuring the value of services to users and by assessing measures of financial sustainability. User metrics deemed useful included number of service requests (2800 annually, representing ~100% increase over 3 years), number of investigators assisted (205 annually), publications with core personnel coauthorship (mean of 24 annually), and grant applications supported (~25 over 2 years). Metrics of lesser value included publications that utilized core-generated data without co-authorship or acknowledgment, owing to difficulty in identifying or tracking these publications. We assessed financial sustainability by comparing recoverable fees to expenditures, both as a whole and within different service centers. Obstacles to sustainability included costs that were not directly attributable to rechargeable services, costs of equipment depreciation, and the necessity to precisely balance costs and fees for each individual service rather than across service centers as a whole. We identified such obstacles and possible ameliorative strategies such as consideration of fee increases, cost savings, and possible core subsidies for certain nonrecoverable fixed costs. In summary, our core services provide high value, as determined by the user metrics we identified, but sustainability requires accurate and ongoing assessment of both these metrics and cost parameters, and this exercise should be regularly integrated into short and long-term core management.

PS31 Optimizing NIH Support for Animal Research Facilities: Lessons Learned

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The NIH Office of Research Infrastructure Programs (ORIP) provides G20 grant support to modernize animal research facilities. ORIP faces challenges in optimizing the program's structure and requirements to ensure that it reaches the broadest community of NIH-supported animal model researchers. Last year, ORIP did a retrospective study of applicants and awardees from the last 10 years drawing data from NIH databases. We examined types and geographical distribution of institutions which were the most successful in securing G20 awards; animal models and research areas which benefitted the most from these awards; and scope of awards. We measured success based on number of awards and their amounts compared to NIH R01 grants. In parallel, we issued a Request for Information (RFI) to seek input from stakeholders about future directions of the program and on how to best prioritize support for technologies, types of facilities, and animal models. Our retrospective analysis showed that ORIP made 170 G20 grants to 101 institutions in 38 states, DC, and Puerto Rico, but that the program's reach was limited as less than 18% of institutions where NIH-funded animal model research is conducted received G20 support. Top animal models supported by the program were rodents, nonhuman primates, and aquatic models. Over a third of scientific projects which used G20-supported animal facilities were in cancer and infectious diseases. Lastly, RFI responses showed need of novel tools and devices to modernize facilities and enhance biomedical research. Based on our findings, we plan outreach to broaden applicant and awardee communities and increase program utility and viability to benefit more animal models, research institutions, and groups of NIH-supported investigators. We also plan new initiatives to promote technological developments relevant to facility management, animal care, rigor of research protocols, and animal wellbeing. Moreover, we intend to revisit program requirements to better target institutional needs for equipment, renovations, and modernization of animal research facilities. Evaluating past program outcomes and stakeholder input allowed us to develop strategies for extending the program's reach and increasing its nationwide impact on research using animal models.

PS32 Promoting Veterinary Student Understanding of Laboratory Animal Medicine and Research through a Role Playing Assignment

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Veterinarians play a key role in assessing animal care and use in the biomedical research setting. Building an understanding of the oversight process in the veterinary profession is valuable. The veterinarian's first exposure to laboratory animals may be during veterinary school when animal research is introduced as part of the ethics course or later during an elective course in laboratory animal medicine. Beginning in 1995 at The Ohio State University and now with Western University of Health Sciences, I have used a role playing assignment to promote understanding of laboratory animal medicine and research in 3rd year veterinary students. Initially students are asked to identify a biomedical research issue. Students are asked to play the scientist role by first writing an essay comparing two or more models that could be used to address the research issue. The models can be cell culture or other alternatives, but least one model must be a vertebrate animal model. Next, students write an IACUC protocol application. The final role is IACUC member. Students exchange and perform an IACUC review of a peer's protocol. The essay rubric and example essays will demonstrate the work products ability to reach the intended goals. This multiphase assignment provides context and promotes appreciation of the process that scientists must go through prior to using animal models. This role playing approach creates resonance by reinforcing stakeholder perspective. Students complain about workload associated with this assignment, but repeatedly confirm they understand why they are given it. You never truly comprehend what the process is like until you try it yourself, even in this mock setting. As one student commented, "I'll never read a research paper the same way again." This result meets the intended goal of promoting a deeper understanding and appreciation of the various stakeholders in an animal care and use program.

PS33 The Role of the Animal Care Technician: Redefined with Empowerment and Capability Building

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Most animal care programs employ dedicated technicians for animal husbandry and veterinary care respectively. Our facility is no different. In this traditional approach, clinical cases were reported by husbandry technicians that are followed up by veterinary technicians for decision making and or consult with veterinarians. This approach required at least one full time veterinary technician evaluating clinical cases on a daily basis. As an alternative, we took the example of the nurse practitioner from human health care settings and trained our husbandry technicians for basic skills of clinical evaluations and treatments for the most common issues, especially in our rodent colonies. The list of common clinical issues were predetermined and a clinical triage was established and posted in all facilities. All husbandry technicians were empowered to make decisions and treat animals for all scenarios that they were trained for. Our husbandry technicians impressed us not only with their interest, but they also delivered quality clinical care irrespective of educational background. This new approach delivered clinical care more promptly and efficiently by eliminating decision making layers. Turn round time for clinical issues from identification to treatment application was reduced from 72 hours at most to 6 hours at most. We have saved a minimum of 4 hours daily for our veterinary technicians that can be used for other value added projects. With increased capability we redefined the role of our husbandry techs, who are now called animal care techs. Empowerment and capability building not only delivered "more with less" but also created employee satisfaction and growth trajectory. There are strategies that programs can embrace to improve productivity. It starts with putting detailed processes in place that employees can turn to when navigating challenges, allowing each member to do the work they are trained to do, and freeing up time for others and veterinarian for better projects.

PS34 Spread the Wealth: Effectively Reaching a Mass Audience of Varying Cultures, Work Experience, Departments, and Education Levels

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In order to maintain any animal facility, it takes a team. That team of people can vary in size, education, cultural, and work experience backgrounds. Credentials range from high school diplomas to bachelor degrees, from ALAT to CMAR certifications. So how do you train such a diverse audience? How do you make sure you convey your message to as many individuals as possible? In the largest small animal facility at the National Institutes of Health, anywhere from 1 to 75 people are trained on a weekly basis. With such a wide audience, ensuring policy and procedures are communicated and understood can prove to be a challenge. In order to overcome this obstacle, several factors have to be considered during each training. Some of these factors include word choice, job relatability, life relatability, etc. Careful attention to these factors increases job knowledge and prevents deficiencies in the quality of animal care.

PS35 Developing Clinical Standard of Care Guidelines for Rodents at a Large Research Institution: Objectives and Challenges

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Rodent clinical cases are frequently managed by licensed veterinary technicians at the University of Michigan. Management strategies are determined relatively autonomously and vary between technicians. Differing approaches to veterinary care and oversight can present regulatory, welfare, or scientific concerns within a program. In response, we are developing standard of care guidelines for the most commonly reported rodent health conditions. A collection of related documents will each include a brief description of a clinical condition including susceptible strains or models, potential causes, expected physical exam findings, and clinical images. In addition, they will outline condition-specific criteria for intervention, veterinary consult, and euthanasia by veterinary technicians. Challenges in guideline development include considerations of an animal's intended use and the associated consequences; a lack of evidence-based veterinary medical literature in laboratory animal medicine; providing clear guidance without limiting one's ability to exercise professional medical judgement; and transitioning to a new system of oversight. We plan to address these challenges by drafting clear, definitive criteria for specific interventions; practicing incremental implementation that incorporates training and feedback; and developing a system for early and ongoing, outcome-based evaluation of the standard of care guidelines. This approach satisfies the needs for veterinary oversight and consistent veterinary care, and allows for the potential development and publication of practice standards.

PS36 Veganism in Biomedical Research: Managing Public Perception

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Public perception of the use of animals in biomedical research has become increasingly less favorable, due primarily to engaged, active animal welfare activists and a silent, insular scientific community. This disconnect has allowed for the spread of misinformation, enabling misguided and exaggerated views of veganism and biomedical research alike. Many in the biomedical research community relegate vegans to the same status of animal rights extremists of the 1980s and 1990s. Some vegans consider biomedical animal research as unethical and unrestrained by moral or civic authority. When these misrepresented views are adhered to, animal and human welfare stagnates. Close examination and thoughtful discussion reveals underlying commonalities between the two communities, i.e., a shared desire for human and animal welfare founded upon the 3R's, and the promotion of health and advancement for both.The increasingly progressive and nonviolent animal welfare community accepts the value of biomedical research with an understanding of the current need for animal models. The biomedical research community is regulated and many scientists actively seek to replace animals with alternative models. In this presentation, I draw upon my concurrent experiences as a vegan and an animal care professional to present these shared values through accurate portrayals of each groups' beliefs. I stress the need for communication now more than ever, ensuring exposure of our shared goals and the dangers of silence, and suggest immediate and personal methods of opening this dialogue.

PS37 The Veterinary Refinement Initiative—Strategic Implementation of Refinements in a UK Academic Institution

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Refinement of scientific procedures is a legal and ethical requirement under various legislation, including the European Directive 2010/63/EU and Animals (Scientific Procedures) Act 1986. Designated veterinarians are legally obliged to provide advice on experimental projects with regard to the application of the 3R principles, in particular on the aspect of refinements. However, in the academic setting effective implementation of refinements can be impeded by factors such as numerous and independent research groups which make effective communication and dissemination of best practise challenging. In addition, reference data are based on well-established published methodology, thus requiring time consuming and potentially costly validation of novel, "more refined" techniques; high student participation might lead to high staff turnover resulting

in the continual need for new training, and the possibility that valuable skill sets may be lost, with resultant consequences for welfare. Thus, despite intense and ongoing contributions from veterinary surgeons, the effective implementation of refinements remains difficult, with many advances tending to be short lived and wider uptake by the community remaining low. The "veterinary refinement initiative" was developed as a two tier support strategy combining regular, structured, and systematic protocol review with support structures such as training workshops and regular "road show" lectures on relevant topics such as aseptic surgical technique. Veterinary advice covered all levels of refinement, suitability of species and animal models, procedural refinements, staff training, recognition and management of adverse effects, and identifying suitable intervention points and humane endpoints. Researchers are encouraged to develop written method sheets to facilitate regular protocol review, staff training, and optimize planning. Recommendations are shared in termly departmental meetings and published in an institutional 3Rs newsletter. The "veterinary refinement initiative" is a strategic and novel approach and has been instrumental in facilitating effective promotion and dissemination of refinements to benefit both animal welfare and science alike.

PS38 Trainer-Student Relationships Significantly Impact the Promotion of Interprofessional Collaboration: A Mixed Method Study

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Funding organizations and institutions of higher education have placed renewed emphasis on One Health and other initiatives that seek to promote interprofessional collaboration. Investigation into the effectiveness of teaching approaches in fostering positive relationships across disciplines is particularly critical in light of recent research indicating that nearly half of the inconsistencies in effectiveness of teaching are related to the quality of the student-teacher relationship. The tension that may exist as a result of perceived differences between animal welfare goals and research goals places this relationship at particular risk; yet little attention has been given to this aspect of adult education within the context of laboratory animal science. Here we report the findings of a mixed-method study that sought to explore relationships between trainers and researchers participating in a hands-on training program at an academic institution. Electronic surveys (pre-class survey $n = 45$, post-class survey $n = 35$) and semi-structured interviews ($n = 10$) were used to examine the perceptions of researchers of their relationships with trainers as result of participating in a two hour rodent handling class. Pedagogical features of the in situ training experience that contributed to fostering collaboration were also analyzed. Quantitative results showed that students were significantly more likely ($P < 0.05$) to contact trainers for assistance after the training session. Analysis of transcripts of interviews using constant comparative method revealed two themes relevant to understanding the development of relationships during the training sessions: a) pre-training obstacles to relationships between trainers and researchers (physical and social separation of workspaces, perception of training staff as regulatory enforcers), and b) factors essential to fostering collegial relationships during training (creating comfortable social environments, teacher accessibility and spending time getting to know one another). This study is the first to report on the significance of the trainer-student relationship on promoting interprofessional collaboration.

PS39 Daytime Blue Light Spectral Transmittance Enhances Nighttime Circadian Melatonin Regulatory Dynamics of Rodent Metabolism and Physiology

GLAS: Yes

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Light entrains the master biologic clock within the suprachiasmatic nucleus (SCN), which tightly controls the nocturnal pineal melatonin signal that temporally coordinates circadian rhythms in metabolism and physiology of all mammals, including laboratory animals. Previous studies from our laboratory demonstrated that the spectral transmittance (color) of light passing through standard laboratory rodent caging impacts these responses in rats. Here, in conjunction with our GLAS-supported studies, we examined how transmission of light in the blue-appearing portion (460-480 nm) of the visible spectrum during daytime can enhance laboratory rodents' daily rhythmic nocturnal melatonin signal, thereby beneficially influencing normal metabolic and physiologic functions. Male, pigmented nude rats (Crl:NIH-*Foxn1*^{rnu}; n = 12/group) were maintained on a lighting regimen 12L(300 lux; 123.0 mW/cm^2; lights on 0600):12D in either standard polycarbonate translucent clear (A, Controls) or blue-tinted (B, Experimental) rodent cages in an AAALAC-accredited facility. After 1 week, animals were subjected to a series of 6 low-volume blood draws via an IACUC-approved survival cardiocentesis technique (0400, 0800, 1200, 1600, 2000, and 2400) over a 4-week period to assess arterial blood melatonin, total fatty acid (TFA), glucose, lactic acid, pO_2, pCO_2, insulin, leptin, and corticosterone concentrations. Results showed no differences in dietary and water intake or body growth rates between the groups. Plasma melatonin levels in pg/mL (mean ± 1 SD) were low (>2.0 ± 0.3) in the light phase (1200 h) in both groups, but significantly higher during dark phase (2400 h) in B (915.8 ± 29.1), compared to A (161.4 ± 16.5) ($P < 0.001$). Arterial blood diurnal rhythms of TFA, glucose, lactic acid, pO_2, pCO_2, leptin, insulin, and corticosterone levels were significantly disrupted in B, compared to A ($P < 0.05$). Together with our previous results, the present findings indicate that a wide variety of light spectral transmittances through differently colored cages have a profound impact on the circadian regulation of neuroendocrine, metabolic, and physiologic parameters that influence laboratory animal health and wellbeing, and ultimately the outcome of scientific investigations.

PS40 Mammalian Target of Rapamycin Signaling Regulates B Cell Survival and Development at 2 Distinct Stages

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Mammalian target of rapamycin (mTOR) is a protein kinase that controls cell growth and division in response to nutrient availability. Inhibition of mTOR with the immunosuppresent rapamycin is a major mode of preventing transplant rejection in humans. However, the mechanisms of how mTOR inhibition suppresses the immune system are incompletely understood. Previous studies elucidated essential roles of mTOR signaling in T-cell proliferation and differentiation. We hypothesized that mTOR signaling is also integral for B-cell development. To test this, we utilized the Cre/LoxP system to disrupt mTOR signaling in murine B-cells at 2 distinct stages of B cell development in the bone marrow and spleen. Bone marrow and spleen cells from 6-8-week old mTOR-signaling deficient mice and wildtype controls (n = 5-10 mice/group) were analyzed using flow cytometry to evaluate the representation of B-cells in each stage of B cell development. Statistical differences were determined using the student's two-tailed t-test. Inhibition of mTOR signaling in early B-cell development resulted in a block in development at the pro-B-cell to pre-B-cell stage, leading to a complete loss of peripheral B-cells and antigen-specific antibody production. Pro-B/Pre-B-cell survival, measured by AnnexinV staining, and proliferation, measured by BrdU incorporation, were reduced in mTOR-signaling deficient mice. Inhibition of mTOR-signaling reduced B cell metabolism, as defined by significant

decreases in oxidative phosphorylation and glycolysis measured by Seahorse extracellular flux analysis. Disruption of mTOR signaling later in B cell development resulted in increased representation of marginal zone B-cells, an "innate"-like B-cell population that produces T-cell-independent antibodies in response to bacterial antigens. Our data reveal novel roles for mTOR signaling in B-cell development, and suggest that mTOR inhibitors may have clinical efficacy in B-cell-mediated autoimmune diseases and B-cell lymphomas by inhibiting B-cell production, survival, and metabolism.

PS41 A Novel Genetic Cell Ablation Technology to Study Cellular Patterning in Vertebrate Models

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The ability to selectively ablate cell types in model organisms is a powerful tool for understanding developmental patterning and disease mechanisms. Current methodologies have limitations that can include lack of cell-type specificity, off–target effects, and the relatively slow onset of cell death. We aimed to create a novel genetic cell ablation methodology for use in multiple species, including zebrafish and rats. In zebrafish, a method for rapidly ablating genetically defined cell populations would enable studies of transient cellular interactions during embryogenesis and later stages. Our novel system utilizes human CD59 (hCD59), a membrane receptor, and intermedilysin (ILY), a toxin produced by *Streptococcus intermedius*, which binds specifically to hCD59 inducing cell lysis. We generated a rat anemia model which expresses hCD59 on erythrocytes. In vitro, complete lysis of erythrocytes expressing hCD59 was observed at and above 250 pM ILY. No lysis was observed in wild type erythrocytes at any ILY concentration (8-1000 pM). In vivo, ILY intravenous injection (100 ng/g body weight) dramatically reduced hematocrit within 10 minutes, with a mean hematocrit reduction of 43% compared to 1.4% in saline control group. To test ablation in multiple cell types, we generated the iCD59 rat strain consisting of ZsGreen and hCD59 expressed constitutively when tissue-specific Cre recombinase is present. The iCD59 rat was then crossed to neuron-specific Cre rats. Brain immunohistochemistry revealed colocalization of ZsGreen and hCD59, demonstrating this system's utility in solid organs. Extending these experiments to zebrafish, we treated hCD59 RNA-injected embryos with 190 pM ILY for 30 minutes. Rapid extensive tissue lysis was observed, resulting in a 70% mortality rate. The hCD59-ILY ablation system induces rapid and dose-dependent lysis of specific cell types in rats and zebrafish, providing a useful tool with wide applications for researchers.

PS42 Impact of Degree of Immunosuppression on Tumor Growth in Murine Models of Prostate Cancer Bone Metastasis

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Immunocompromised mice are frequently used in tumor xenograft studies to avoid host:graft response that would inhibit xenograft growth. Multiple types of immunocompromised mouse models are used for xenograft studies. These models are not used consistently among different research groups and due to the differing degree of immunosuppression may result in different results on tumor biology. This creates a challenge to compare results among investigations. Bone metastasis is a frequent event in multiple cancers and is modeled often in mice. However, it is not known if and how the different immunogenicity of differing immunocompromised mouse models affects tumor growth in bone. Accordingly, we assessed prostate cancer (PCa) growth in several immunocompromised mouse models of bone metastasis. Listed in increasing magnitude of immunodeficiency, we used nude, NOD/SCID, NOD/Rag1$^{-/-}$, and NOD/LtSz-scid, IL2Rg^{null} mice. Single cell suspensions of PCa tumor cell lines (PC3-luc (rapid growing and osteolytic; 2x10^5 cells) and C42B (slow growing and osteoblastic; 1x10^6 cells)) were injected percutaneously into the marrow space of the proximal tibiae of mice through the tibial crest (n = 10/group). Tibiae were radiographed and subjected to bioluminescent imaging (for PC3-luc) every 2 weeks. At 6 weeks postinjection of tumor, mice were euthanized and tumor was subjected to immunohistochemistry to evaluate for apoptosis (Apoptag) and angiogenesis (CD31). PC3-luc tumor growth was greatest in the NOD/LtSz-scid, IL2Rg^{null}, followed by NOD/SCID and NOD/Rag1$^{-/-}$ which were equivalent, and then nude mice which had the least tumor growth. Bone osteolysis, number of apoptotic cells, and tumor-associated microvessels paralleled the degree of tumor growth. In the case of C42B cells, the greatest osteoblastic activity was associated with NOD/LtSz-scid, IL2Rg^{null}, followed by SCID and NOD/Rag1$^{-/-}$ which were equivalent, and then nude mice. These results demonstrate that intraosseous PCa tumor growth and tumor-induced bone remodeling parallels the degree of immunosuppression of the mouse model used. This work indicates that selection of the degree of immunosuppression of the mouse model used requires careful consideration and should be consistent to compare results among different experiments.

PS43 IL-22 Regulates the Early Innate Immune Response to *Helicobacter hepaticus* Infection

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Interleukin-22 (IL-22) plays an essential role in the innate immunity to enteric pathogens, at least in part through induction of anti-microbial peptides. *Helicobacter hepaticus* is an enterohepatic *Helicobacter* species associated with the development of chronic colitis and cancer in susceptible mouse strains. Previous studies have described high levels of IL-22 mRNA within the lower bowel of RAG mice chronically infected with *H. hepaticus*. However, the role of IL-22 in modulating the early innate immune response to *H. hepaticus* has not been fully explored. In this study we explored the early innate response to *H. hepaticus* by infecting sex-mixed 129S6/SvEvTac-*Rag2*^{tm1Fwa} mice with *H. hepaticus* and evaluating the inflammatory response in the lower bowel 2 weeks later. *H. hepaticus* infection rapidly induced inflammation that was significantly more severe in the cecum than either the proximal or distal colon, associated with increases in the percentages of CD45$^+$CD11b$^+$CD64$^+$Ly6c$^+$MHCII$^+$ inflammatory monocytes and Ly6G$^+$Ly6c$^+$ neutrophils, as well as increases in absolute numbers of Thy^{High} type-3 innate lymphoid cells (ILCs). In addition to inflammation, *H. hepaticus* infection induced dysbiosis characterized by reduced species diversity and expansion of the phylum proteobacteria. Interestingly, infected mice also exhibited strong staining of intestinal crypt epithelial cells with γH2AX suggesting the accumulation of DNA double stranded breaks. As anticipated, infection induced marked elevation in expression of IL-22, and the antimicrobial peptides RegIIIβ and RegIIIγ. In addition to markedly inhibiting antimicrobial peptide expression, depletion of IL-22 reduced epithelial staining with γH2AX but did not interfere with *H. hepaticus*-induced inflammatory changes in the cecum. Thus, induction of IL-22 appears to mediate the rapid accumulation of DNA double stranded breaks observed within crypt epithelial cells following *H. hepaticus* infection. These observations could have important implications for our understanding of the relationship between inflammation and neoplasia within the lower bowel.

PS44 Development of a Multiplex PCR-Based Hybridization Chip System for Screening of Multiple Infectious Agents in Feces of Laboratory Mice

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Murine norovirus, parvoviruses, and helicobacters are among the highly prevalent infectious pathogens in contemporary laboratory mice. Periodic health monitoring of laboratory animals is indispensable in control of laboratory animal diseases. Fecal RT-PCR/PCR is an efficient method for routine surveillance of fecal-oral transmitted infectious agents, but it is sometimes challenging in confirming the sensitivity and specificity of multiplex PCR through gel electrophoresis. In this study, a multiplex RT-PCR/PCR-based hybridization chip has been developed to simultaneously detect several infectious agents, including mouse parvoviruses (minute virus of mice [MVM] and mouse parvovirus [MPV]), murine norovirus (MNV) and several helicobacters. Four pairs of generic-specific primers were designed to target mouse parvoviruses, MNV, helicobacters and a house-keeping gene, β-actin, respectively. Species- or strain-specific oligomers were designed and dotted on the plastic chips. The multiplex RT-PCR/PCR products were applied to oligo-anchored chips for hybridization analysis. Six different mouse infectious agents, including MNV, MVM, MPV, *H. hepaticus*, *H. bilis*, and *H. typhlonius* in mouse fecal samples can be simultaneously and specifically detected on the chip by visualized signals. Besides these 6 pathogens, this assay could also monitor the existence of non-MVM/MPV rodent parvovirus and other helicobacters in feces and a mouse housekeeping gene. This gene chip assay is a sensitive and specific assay with detection limits of 10 copies for all target agents. This system also successfully and correctly revealed the infection statuses of 14 mice from 6 different laboratory colonies. The gene chip assay developed here could be a very useful tool to monitor infectious diseases of laboratory mice without sacrificing them or causing any stress/discomforts in animals.

PS45 Optimized Feeding Program Promotes Ideal Body Condition in a Diverse Nonhuman Primate Colony

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Obesity in cage housed research nonhuman primates is prevalent and not only impacts the health of the animal, but may also affect research model suitability in certain therapeutic areas. Our facility maintains a diverse colony of nonhuman primates, ranging in age from 2 to 30 years with varying metabolic needs. As the colony profile changed, a revision to the feeding program was implemented in order to maintain animals in healthy body condition across multiple age ranges and reduce wasted food. The first iteration of the modified program employed a formula derived from *Nutrient Requirements for Nonhuman Primates* for three age brackets with decreasing metabolic needs: juvenile, adult, and geriatric. We used an estimated metabolizable energy (ME) requirement of 70 kcal x $BW_{kg}^{0.75}$, which was multiplied by a basal metabolic rate (BMR) factor specific to that age bracket. This value was then divided by the physiologic fuel value of the diet (kcal/g) and the weight of the food unit (biscuits, in g) to calculate the number of biscuits necessary. The formula for juvenile animals under 7 years used a BMR multiplier of 2, the formula for adult animals between 7 and 20 years applied a multiplier of 1.5 and for geriatric animals over 20 years a multiplier of 1.25 was used. In the second revision to the program, we applied body condition assessments collected at semi-annual health screenings along with weights to determine optimal feeding. We included four additional diet plans to account for age bracket metabolic needs in conjunction with body condition. New plans included BMR multipliers for underweight juvenile animals, underweight adults, overweight animals, and overweight diet resistant animals. The colony as of February 2015 consisted of 92.6% of animals with body condition scores between 2.5-3.5, with 4.7% of colony routinely monitored for underweight condition and 2.7% routinely monitored for overweight condition. This program provided a data driven mechanism to adjust diet plans in order to reduce waste, reduce the risk of spontaneous obesity, and achieve optimal colony health.

PS46 Task-Directed Assessment of Allergen Exposure in Contemporary Mouse Barrier Vivaria

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Allergies to animals are among the most common occupational hazards associated with laboratory animals. Many vivaria engineering and equipment improvements help mitigate risk and improve the work environment. To assess staff safety and guide PPE standards, we examined mouse urinary protein (MUP) levels resulting from work process within a contemporary mouse barrier facility. Using benchmark of an OEL of 5ng/m^3 as an 8-hour time weighted average, we measured MUP exposure to animal care staff, researchers, and visitors. The facility housed approximately 14,000 cages in positive pressure autowater IVC racks. HEPA filtered rack blowers were designed with a 1:3 supply/exhaust ratio. Mice were housed on corncob bedding and changed every 7-10 days. Animal transfer stations were used in positive pressure housing rooms for cage management, while negative pressure procedural rooms were available for more complex procedures. Personal breathing zone samples were collected from selected staff during line checks, cage changing, mouse euthanasia, lab coat processing, and cage dumping/scraping in cagewash operations. In addition, air samples were analyzed to evaluate the effectiveness of ambient allergen control in facility hallways, animal rooms, cage wash and the employee break room/administrative space. Results indicated very low prevalence of allergens in all environmental air samples (range 0.07-2.75ng/m^3). Two routine tasks were identified as very high risk of exposure (mouse euthanasia (159.7ng/m^3) and cage dumping/scraping (41.69ng/m^3). These levels were surprising as controls were in place, such as use of a customized bedding disposal unit, BioBubble, to help mitigate exposure. Program modifications such as respirators and workstation redesigns were instituted which adequately captured high-risk allergens. All other monitored tasks and samples were below the benchmark. Traditional PPE standards are very effective and serve as the last line of defense for protecting staff. Changing these standards should only be considered with quality benchmark data and a thorough risk assessment.

PS47 Development of a Drought Preparedness Plan for Laboratory Animal Facilities

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The multiyear drought in California prompted The Jackson Laboratory to take proactive measures to develop a drought preparedness plan. While institutions in California have an immediate need to develop drought preparedness plans, any institution can implement water conservation efforts which may decrease overall operating costs. An initial investment of $1500 allowed installation of water meters on various water lines to determine specific water usage. Processing equipment and HVAC accounted for >85% of water usage at JAX Sacramento. Conversely, animal drinking water accounted for <2% of the water used. The cooling tower used the greatest percentage of water at 34% and the tunnel washer used the second highest amount at 28% percent. From here, prioritization of water conservation efforts that have the largest impact factor can be examined. With a net investment of $5000, switching from a pulse-power technology cooling tower system to a chemical system will yield an immediate 10% water reduction. We found that a reduction in the air changes per hour from 60ACH to 45ACH within the ventilated caging system would only yield a 1% savings in water. The minimal impact to water conservation coupled with a potential high impact to the health and wellbeing of the mice determined that this was not a viable option at the present time. Processing equipment less often can also reduce water usage via an increased cage change interval or through the use of cage liners. However this type of change would require a detailed

study to assess the health and welfare of mice within the cages. A reduced cage change interval as well as liners could potentially provide a 30% reduction in water usage from the processing equipment. We will discuss our efforts to develop a drought preparedness plan and provide examples of solutions that can be addressed in the near term as well as water conservation efforts that can be planned with new construction.

PS48 Decontamination of Valuable Stocks in a Barrier Facility Using Embryo Transfer Rederivation Procedures in a Class 100 Environment

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The number of genetically engineered mice models (GEMs) is growing rapidly due to high-throughput production strategies employed by the International Mouse Phenotyping Consortium (IMPC). Mouse repositories play an important role in promoting and distributing the models produced by such a large international project. The microbiological quality of these distributed models is critical as health status can alter animal welfare, validity and reproducibility of research data. As one of these mouse repositories, we faced endemic infection with opportunistic agents (*Helicobacter, Pasteurella and Norovirus*) that represent importation issues for researchers requesting strains from our collection. The solution to get rid of these undesired agents is to conduct embryo transfer rederivation procedures. Typically, decontaminations are performed in a different barrier with a higher health status. Isolators within the parent facility are a viable alternative as they provide an elevated level of animal protection from surrounding environment. However isolators are operationally intensive, and not convenient for mice procedures. Clean rooms operating under positive pressure using HEPA filtration to provide protection for both housing and procedural areas, represent an option that is less labor intensive, and reducing operating costs. We report here the outcome of a 24-month program to rederive GEMs internally with a customized class 100 environment. This enclosure has been installed within our contaminated facility. Embryo-recipient females and rederived mice were health-screened to assess their microbiological status according to the FELASA guidelines. More than 50 strains so far has been successfully rederived free of *Helicobacter, Pasteurella and Norovirus*, the 3 agents endemically present in our facility with a high prevalence (76, 21 and 94% respectively). To date, 24 months of follow-up mice screening indicates that we achieved our goal of eradicating endemic infectious agents, for strains to be distributed from our repository, based on the use of clean room enclosure. Besides the outcome of this rederivation program we also present a time/cost analysis between solutions to upgrade mice health status, which drive us in these times of financial limitations.

PS49 A Risk-Based Approach to Reducing Exposure of Staff to Laboratory Animal Allergens

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Within the biomedical research industry, people who work with laboratory animals are potentially at risk of developing laboratory animal allergy, which can lead to occupational asthma. Under UK and EU laws, employers must prevent or adequately control exposure to any hazardous substance, which includes animal allergens, so far as reasonably practicable, for the protection of all people on the premises. This can be achieved by assessing the risk of allergen exposure in specific areas or activities of an animal facility and implementing appropriate infrastructure, environmental, and performance controls to minimize that risk. Although not a legal requirement in the UK, monitoring allergen levels in different parts of the animal facility has proved a valuable approach in determining the effectiveness of the different controls in place. It also highlights effectiveness of any refinements that have been put in place to improve on existing allergen levels and prompts review and updating of risk assessments. There is no common agreement as to what corresponds to a "safe" level of allergen protein (Mus m1) in the working environment, but regular monitoring of the animal facility at my institute has allowed a conservative figure of 2.5ng/m3 to be adopted as the tolerable limit for wearing respiratory protective equipment (RPE) rather than insisting on a blanket cover approach for use of RPE often used elsewhere. Here is presented the risk based approach used for determining what controls are needed to minimize risk of exposure and to provide a safe working environment for all our staff.

PS50 Eliminating Preventable Harm for Animals in Research and Teaching by Implementing a "Zero Hero" Program

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Working with animals in research is a privilege. As researchers, technicians, and veterinarians, it is our job to ensure that they are treated humanely and are taken care of to the best of our abilities. They are our patients. Unfortunately, as is seen in both human and animal clinical care situations, patient care is not infallible. Animals under our care may unintentionally get hurt or die due to a multitude of reasons such as equipment malfunctions or human error. In 2009, Nationwide Children's Hospital (NCH) implemented a program called "Zero Hero" with the goal being to provide perfect, harm-free care. The name "Zero Hero" was to empower employees to be heroes for their patients by eliminating preventable harm. The program has been so successful it is now used both statewide and nationally for patient safety, and has been expanded to encompass employee safety at NCH. Inspired by this model, the Animal Resources Core (ARC) at the Research Institute of Nationwide Children's Hospital implemented a "Zero Hero" program for the animals used in research and teaching. Five focus areas of preventable harm for the animals were identified: flooded cages, expired materials/drugs, inadequate animal observations, rodents left in cages destined for cage wash, and insufficient postanesthetic monitoring. Programs to address each of these areas were designed using some of the same patient and employee "Zero Hero" tools combined with Business Process Improvement (BPI) methodologies. This poster will provide details on the nature of the preventable harms identified, the method(s) used to either evaluate and/or ameliorate the issues, and the animal safety outcome for each focus area.

PS51 The Lean Transformation of Our Mouse Facility: A Four Year Journey

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Modern laboratory animal facilities aim to provide high standards of animal care and scientific support within a framework of regulatory compliance and with a strong focus on efficiency of operations. Meeting these objectives with limited resources is a challenge for facility managers and requires the application of a range of traditional and innovative management techniques. Lean production is a management philosophy derived from the Toyota Production System, which considers the expenditure of resources for any goal, other than the creation of value for the end customer, to be wasteful, and thus a target for elimination. Lean has been successfully applied to many activities of laboratory animal facilities from the design phase to the operation of animal care programs. The authors manage a 600 m^2 (6,500 ft^2) mouse facility housing around 6,000 ventilated

cages. Over the years, several upgrades (ranging from the introduction of dirty side automation systems, to optimization of the logistics) have been implemented in order to increase the efficiency of the operations. In order to further improve, the main animal care activities were reevaluated and reorganized using a lean approach during the summer of 2011. The initial transformation was carried out with the support of a team of consultants and involved mainly cages and bottles processing, both in the animal rooms and in the washing area. Activities were balanced and new workflows defined together with new process layouts and time schedules. The authors, together with the animal care staff, subsequently spread lean also to the areas not involved in the initial transformation, such as supplies management, daily cages and health checks, and breeding activities, etc. The results of the application of lean management were striking, both initially and over the 4 years of continuous implementation, and will be discussed together with the challenges encountered during the process.

PS52 Green Principles of Steam Sterilization

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Autoclaves are notorious for their consumption of water due to vacuum systems that call for water and hot steam condensate that must be cooled. Typically these challenges are addressed through water conservation systems that are purchased for the autoclave. However, consideration should be given to how sterilization cycles can be optimized for more efficient cycles. More efficient cycles require less time, less steam, less cooling water, and ultimately less energy. In this presentation, a brief overview will be given to lay the ground work for how autoclaves use water, but most of the emphasis will be on practical examples of ways to optimize cycles to reduce cycle times and thus water and energy consumption. Attention will be given to liquid cycles and "red bag" waste cycles, both of which present challenges to sterilization and require long cycle times. As a result, not only is water conservation realized, but good sterilization principles are addressed to ensure decontamination and sterilization.

PS53 Developing Behavioral Performance Standards for Laboratory Animals

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Performance standards are increasingly being emphasized to improve the quality of care and welfare programs for laboratory animals. Defining performance standards can be challenging especially when using behaviors as a measure of success. We developed species specific behavioral performance standards for mice, rats, rabbits, minipigs, macaques, and dogs. Our goal was to identify key behaviors to encourage using knowledge about and experience with the species (for example: natural environment, biology, social structure, species typical behaviors) and thus we identified 5-7 key behaviors for each species. Summaries were written to help define each behavior, explain why the behavior is important, and how to encourage the behavior. Information about each standard was distributed to our animal care staff, IACUC, investigator community, and management to help educate and gain support for implementing recommended improvements in our care and welfare programs. As a result of this work, we were able to make changes to our environmental enrichment program that promoted behavioral enhancements (for example: some strains of mice built better nests) and were able to provide more enrichment opportunities (for example: exercise and socialization sessions for rabbits) for animals to exhibit additional behaviors. Identifying key behaviors to encourage has helped us get support for designing new housing and improving our enrichment programs for dogs and macaques. Subsequently, we have seen an expanded range of species typical behaviors in dogs and monkeys housed in our new kennels and cages. Developing performance standards has also helped us gain resources for our enrichment programs, ensure enrichment consistency, and help prioritize enrichment strategies to improve animal welfare for our animal colonies.

PS94 Viral Vector Biosafety in Animal Research: A Systematic Risk Assessment Approach

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Viral vectors are molecular tools used to introduce nucleic acids into cells to alter gene expression. In animal research, they have been used to develop animal models of metabolic, neoplastic, and degenerative diseases and to assess potential gene therapies. While there are clear regulatory guidelines for in vitro usage, containment procedures for their use in animals is less clear. Therefore, our Institutional Biosafety Committee (IBC) developed guidelines for biocontainment practices 8 years ago. Given advancements in viral vector usage and novel viral vectors developed since then, an update of our current version was needed with the goal of producing a comprehensive guideline. A literature search was performed in collaboration with our research librarian. The goal was to identify potential pathogenicity, immunomodulation, cytotoxicity, insertional mutagenesis, replicative ability, environmental stability, and biodistribution/shedding for viral vectors used in research. Also risk reduction practices for viral vector work in animals were assessed. Data was difficult to retrieve since studies with relevant information did not always have expected key words in the title or abstract. For example, a PubMed search using the terms "viral vector" and "biosafety" in the title or abstract, identified 4 articles from 2010–2015. In contrast, a search with "viral vector" alone in the title or abstract yielded over 1,000 articles for the same period. In the end, 10 viral classes and 20 specific viral vectors were evaluated after review of over 120 reference materials. The prior guideline had no references cited to support the risks or associated recommendations and 4 classes of viral vectors (autonomous parvoviruses, poxviruses, baculoviruses, and paramyxoviruses) identified as absent from the guideline completely. Our review identified inconsistencies in our current institutional guideline, including over and under classification for containment requirements based on risk assessment. Our report was provided to the IBC to allow for revision and amendment of the current containment recommendations and development of appropriate risk mitigation strategies. We believe the review we conducted would have value to other institutions attempting to address similar concerns.

PS54 Pharmacokinetics and Efficacy of Sustained-Release Buprenorphine in Guinea Pigs

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Guinea pigs are common animal models used for biomedical research due to similarities in symptoms and immune responses of human diseases, often requiring analgesic support. Buprenorphine hydrochloride (Bup-HCl) is one of the most common opioids given to laboratory animals and requires dosing every 6 to 12 hours, demanding repeated animal handling and increased animal stress. Sustained-release formulations of Buprenorphine (SR-Bup) have been shown to provide adequate analgesia for 48-72 hours in other rodent species, eliminating the need for repeated dosing and reducing animal stress. Fourteen guinea pigs separated into 2 groups were either given Bup-HCl (0.05 mg/kg) subcutaneously twice daily for 3 days or SR-Bup (0.3 mg/kg) subcutaneously once. Plasma collection and paw pressure pain analysis (PP) was conducted at 0, 1, 3, 6, 12, 26, 48, and 72 hours. The data shows SR-Bup and Bup-HCl PP coincided with the plasma concentrations averages over the 72 hours

postinjection. Both groups PP were higher than base line for the 72-hour period. SR-Bup PP were significantly higher than Bup-HCl PP at 6- and 12-hour time points ($P < 0.075$). These results suggest that SR-Bup provides equal and consistent analgesia for a prolonged period of time compared to Bup-HCl and that SR-Bup may be an alternate method for analgesia in guinea pigs.

PS55 Antimicrobial Use and Resistance in Zoonotic Bacteria Recovered from Nonhuman Primates

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Antimicrobial resistance (AMR) has become a central topic as it is a growing threat in human and animal health. Major surveillance systems, such as the National Antimicrobial Resistance Monitoring System, are now established to monitor AMR and provide physicians, veterinarians, and scientists with valuable information to make informed decisions on policy and therapeutic treatment. However, multiinstitutional AMR surveillance among nonhuman primates (NHPs) is not well established. This study aims to provide preliminary data on the prevalence of AMR among biomedical research institutions with NHPs, and data on current antimicrobial use strategies. We focused on four zoonotic enteric bacteria: *Shigella flexneri, Yersinia enterocolitica, Yersinia pseudotuberculosis,* and *Campylobacter jejuni*. Twelve veterinarians and 6 biomedical research institutions participated, with 4 institutions providing susceptibility test results across a 3-year period (1/2012 – 1/2015). Seventy-five percent (9/12) of participating veterinarians reported enrofloxacin as their primary antimicrobial for treating suspected *S. flexneri*-caused diarrhea cases. *S. flexneri* isolates were most frequently resistant to erythromycin (87.5%, 21/24), doxycycline (73.7%, 14/19), amoxicillin/clavulanic acid (63.2%, 12/19), tetracycline (38.2%, 157/411), and gentamicin (24.0%, 7/29). Seventy-five percent (9/12) of veterinarians reported enrofloxacin as their primary antimicrobial for treating suspected *Y. enterocolitica* cases as well. All *Y. enterocolitica* cases were resistant to ampicillin (49/49) and amoxicillin/clavulanic acid (5/5), and 93.6% (44/47) were resistant to cefazolin. No AMR was observed for *Y. pseudotuberculosis* (0/57). Finally, 50% (6/12) of participating veterinarians reported azithromycin as their primary antimicrobial for treating suspected *C. jejuni* cases, with tylosin and enrofloxacin identified by other veterinarians. Ninety-nine and a half percent (569/572) and 97.5% (557/571) of *C. jejuni* isolates were resistant to methicillin and cephalothin, respectively. These results suggest that AMR is highly prevalent among zoonotic pathogens from NHPs, but isolates are primarily susceptible to antimicrobials most frequently chosen for therapy, including fluoroquinolones such as enrofloxacin.

PS56 Validation of a Multiplex Antibody Diagnostic Test for Tuberculosis in Nonhuman Primates

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Tuberculosis (TB) in nonhuman primates is highly contagious and often produces rapid disease. Identification of animals infected with *Mycobacterium tuberculosis* (*M. tb*.) in a timely manner is therefore critical. Animals in breeding colonies are periodically tested using the tuberculin skin test (TST) and/or a blood assay. However, these tests lack desirable sensitivity, specificity, efficiency, and/or throughput. We have developed a blood-based multiplex immunoassay based on antibody profiling in *M. tb*.-infected animals that can be used potentially for routine colony surveillance. In continuation of our previously published proof-of-concept studies we have used a bead based multiplex panel with 28 *M. tb* antigens/assays in the current validation study. Antibody levels were examined in plasma samples from several cohorts of well-characterized specific pathogen-free (SPF) colonies at several facilities. A total of 684 healthy macaques were used in the validation study to determine specificity of the test. Sera from 2 different rhesus macaque (*Macaca mulatta*) colonies (n = 460) and two cynomolgus macaque (*Macacca fasicularis*) colonies (n = 224) were used in the study. The cut-offs were calculated and overall specificity of the test was found to be 90%. The panel was screened for sensitivity using experimentally infected rhesus (*M. tb*. strains: Erdman, n = 6 and H37RV, n = 4) and cynomolgus macaques (Erdman, n = 9). The panel sensitivity was between 80-100% at various time points (8, 12, 16, and 24 weeks) during seroconversion. In conclusion, we have validated a blood-based test which is highly sensitive and specific for screening of *M. tb.* in nonhuman primates and can be performed in a user friendly and high throughput format.

PS57 Postoperative Analgesia of Sustained-Release Buprenorphine, Sustained-Release Meloxicam, and Carprofen Gel in a Model of Incisional Pain in Rats (*Rattus norvegicus*)

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Postoperative analgesia in laboratory rats is complicated by frequent handling due to analgesic dosing requirements. Here, we refined postoperative analgesia by using sustained-release buprenorphine (Bup-SR), sustained-release meloxicam (Melox-SR), and carprofen gel (CG). The aim of this study was to investigate whether postoperative analgesia of Bup-SR, Melox-SR, or CG is effective to control behavioral mechanical and thermal hypersensitivity in a model of incisional pain in rats. Rats (n = 33) were randomly placed into 5 treatment groups: 1) saline (1 mL/kg, BID); 2) Buprenorphine HCl (Bup HCl; 0.05 mg/kg, BID); 3) Bup-SR (1.2 mg/kg, once); 4) Melox-SR (4 mg/kg, once); 5) CG (2 oz, SID). Mechanical (Von Frey method) and thermal (Hargreaves method) hypersensitivity were tested daily from day 1 through 4. Bup HCl and Bup-SR attenuated mechanical and thermal hypersensitivity on day 1 through 4. Melox-SR and CG attenuated mechanical, not thermal, hypersensitivity on day 1 through 4. In a second experiment, rats were randomly assigned to groups 2-5 above ($n = 48$) for daily blood collection for 4 days. Plasma concentrations were consistent for both buprenorphine formulations. There were no signs of toxicity in any group on gross pathologic examination. These findings suggest that postoperative analgesia of Bup HCl and Bup-SR, but not Melox-SR or CG, is effective to attenuate mechanical and thermal hypersensitivity in a model of incisional pain in rats.

PS58 Evaluating the Effects of a Selective and Nonselective NSAID in Axenic Mice by Clinical and Anatomic Pathology Parameters

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Axenic mice are valuable models in biomedical research, particularly in the study of inflammatory bowel disease and the role of microbiota in disease processes and immune system development. Significant physiologic differences have been described in the gastrointestinal tract of axenic mice as compared to mice with microbiota. Currently, no analgesics have been tested for gastrointestinal effects in axenic mice. Challenges associated with administering an analgesic in germ-free conditions include maintaining a sterile isolator while entering and administering, as well as determining potential confounding study factors. Injectable nonsteroidal

antiinflammatories (NSAIDs) have been used extensively for analgesia in laboratory mice. However, a well-documented side effect of NSAID use is gastric and small intestinal ulceration. We hypothesize that injectable NSAIDs can be safely administered to axenic mice for use as an analgesic. We evaluated clinical chemistry and anatomic pathology parameters following administration of saline, or a cyclooxygenase (COX)-2 selective NSAID (meloxicam), or a non-selective NSAID (ketoprofen). Meloxicam and ketoprofen were used at recommended doses (2mg/kg and 5mg/kg respectively) subcutaneously for 5 days in 9-12 week old female axenic C57BL/6J and IL-10 knockout on a C57BL/6J background. We selected IL-10 knockout (IL-10 KO) mice because chronic intestinal inflammation has been described in IL-10 KO mice under various conditions. On day 6, mice were euthanized for blood and tissue collection. Histology and isolator culture results revealed injectable analgesics could be entered and administered to axenic mice while maintaining sterile conditions. Data suggests while there are no significant clinical chemistry alterations, axenic IL-10 KO mice develop gastric erosion or ulceration while axenic C57BL/6J mice develop only mild crypt dilatation when given either meloxicam or ketoprofen. Further studies should be conducted to determine if altering the dose, duration, or NSAID formulation will prevent gastrointestinal lesions in axenic IL-10 KO mice.

PS59 Real-Time Application of the Rat Grimace Scale for Cage-Side Pain Assessment

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The rat grimace scale (RGS) is a validated pain scale for assessing spontaneous pain behavior in rats via facial expressions. The following 4 action units are each assigned a score of 0 (normal), 1 (moderate change), or 2 (painful) and a total score calculated from an average of the 4 scores (possible range of 0-2): orbital tightening, ear, nose/cheek, and whisker changes. The standard method of video recording and image grabbing is time consuming, laborious, and limits timely intervention. This study assessed if the RGS can be used in real time (RT) to assess pain using an inflammatory pain model. Twenty-four male and female 8-week-old Sprague–Dawley rats (12/group) were randomized to receive a subcutaneous injection of either buprenorphine (0.03 mg/kg) or saline, followed by intraplantar 0.1% carrageenan 30 minutes later. Observations were made at baseline, 3, 6, 9 hour postcarrageenan injection. Observation periods lasted 15 minutes, with an observer performing RT scoring (every 30 seconds). Concurrent video recording for offline image capture (every 30 seconds) and scoring (IMG) was performed for comparison. Videos and images were randomized and blinded. In both treatment groups the majority of time point comparisons did not differ significantly between RT and IMG methods. In the buprenorphine group no significant differences were observed at baseline (RT 0.25 [0-0.75], IMG 0.5 [0.25-1.0], P = 0.97), 6h (RT 0.5 [0-1.75], IMG 0.5 [0-1.5], P > 0.99), and 9 hours (RT 0.75 [0-1.25], IMG 0.75 [0-1.25], P = 0.41). A significant difference was observed at 3 hours (RT 0.25 [0-1.5], IMG 0.25 [0-1.25], P = 0.04). In the saline group no significant differences were observed at 3 hours (RT 0.25 [0-0.75], IMG 0.5 [0.25-1.0], P = 0.38), 6 hours (RT 0.5 [0-1.5], IMG1.0 [0.25-1.33], P > 0.99), and 9 hours (RT 1.0 [0.5-1.5], IMG 0.71 [0.25-1.67], P = 0.38). A significant difference was observed at baseline (RT 0.25 [0.25-0.5], IMG 0.5 [0-1.5], P < 0.001). There were no significant differences between treatment groups at any time point (P > 0.05) with each scoring method (P > 0.05). These data suggest that the RGS has the potential to be used in real time. This has significance for the practical application of the RGS in both basic science and clinical settings. The observed lack of difference between treatment groups requires further investigation.

PS60 Severity and Distribution of Wounds in Rhesus Macaques (*Macaca mulatta)* Correlate with Observed Self-Injurious Behavior

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Self-injurious behavior (SIB) occurs within laboratory housed nonhuman primates (NHPs) at low frequencies, but can have a devastating impact on animal research and welfare. One barrier to the study and clinical management of these cases is the cost of equipment and personnel time to quantify the behavior according to the current standard of observation and scoring remotely obtained video recordings. In studies of human SIB where direct observation is difficult or prohibited, researchers have demonstrated that quantification of tissue damage resulting from SIB may be a useful proxy to represent the underlying behavior. We hypothesized that the nature of wounds resulting from SIB in NHPs could be used in a similar manner to measure the abnormal behavior. Using a cohort of rhesus macaques with high incidence SIB, we examined wound severity, distribution, and number and compared them to observed incidences of SIB over the course of a 12-week experiment. We found the number, severity, and distribution of physical wounds were associated with incidences of biting behavior observed during the 2 weeks prior to measurement. We also found greater number of wounds was associated with greater severity. Animals with moderate wounds were also more likely to have severe wounds than those individuals with less severe ones. This research is the first representative study in NHPs to find behavioral SIB correlates to physical wounds and increased frequency and number of the body regions affected correlates with severity of wounding.

PS61 Six-Minute Walk Test: Evaluation in a Canine Model of Chronic Heart Failure

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It is estimated that 5 million Americans are affected with heart failure and half will go on to die from the disease. The syndrome of heart failure is associated with multiple pathophysiological alterations and adaptations, often manifested by exercise intolerance. The six-minute walk test (6MWT) has become a safe and reliable predictor of morbidity and mortality in human patients with heart failure, and a predictive measure of canine quality of life and wellbeing in veterinary medicine. We wanted to assess if the 6MWT would be a suitable addition to our canine model of chronic heart failure (CMCHF) to evaluate exercise intolerance and also animal wellbeing. Our CMCHF is produced by multiple sequential intracoronary embolizations with microspheres. After each embolization a cardiac echo and ventriculogram was performed to calculate left ventricular end diastolic volume (LVEDV) and dogs were categorized in heart failure when the LVEDV was < 30%. We hypothesized that dogs in chronic heart failure would show exercise intolerance and therefore walk a shorter distance than normal healthy controls. To test this hypothesis, 20 intact male mongrel hounds (8-12 months of age; 25-30 kg body weight) were studied. Control (n = 10) and heart failure dogs (n = 10), previously trained to walk unobstructed on a leash, were walked on a pre-measured indoor path. The distance that each dog walked, regardless of speed or quality, was recorded and the mean calculated. To further assess cardiovascular status, heart rate, respiratory rate, mucus membrane color, capillary refill time, and oxygen saturation by pulse oximitry were also recorded before and after the 6MWT for each dog. Analysis of these results confirmed our hypothesis that dogs in chronic heart failure will walk a significantly shorter distance than their control counterparts. In conclusion, the 6MWT was a safe and reliable adjunct tool for the assessment of exercise intolerance and a predictive measure of quality of life and wellbeing in our CMCHF.

PS62 A Modifiable Risk Factor for Post-Arthroplasty Implant Infection: Evaluation of Vitamin D in a Mouse Model for Joint Infection

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While vitamin D is a known modulator of both innate and adaptive immunity, no studies to date have investigated the role of vitamin D as a modifiable risk factor for postoperative implant infection. This study uses an established in vivo mouse model of arthroplasty infection to 1) evaluate the influence of vitamin D deficiency on periprosthetic infection and 2) assess the modifiability of this factor by perioperative "rescuing" a cohort of mice from their vitamin D-deficient state. To test this, 26 male lysEGFP mice, which have myelomonocytic cells labeled with green fluorescent protein (GFP), were randomized to receive a diet completely deficient of vitamin D (n = 13) or a standard vitamin D sufficient diet (n = 13) for 8 weeks preoperatively. The mice underwent survival surgery in which a titanium implant was placed into the femur in a retrograde fashion and the joint was inoculated with $1x10^3$ colony forming units of a bioluminescent strain of *Staphylococcus aureus*. At 24 hours postoperation, a subset of the vitamin D-deficient mice were "rescued" with an intraperitoneal injection of 3H-25D, a well established method of rapidly restoring active vitamin D, then switched over to a vitamin D sufficient diet (rescue group). Infection and immune response were quantified longitudinally using bioluminescent and florescent imaging, respectively on postoperative days (POD) 0, 1, 3, 5, 7, 10, 14, 18, 21, and 28. The immune response was more robust and the infection burden was significantly lower for mice with vitamin D sufficient diets than those with deficient diets al all time points ($P < 0.05$). The group rescued from vitamin D deficiency perioperatively were less susceptible to infection than the deficient group at all time points, ($P < 0.05$). The rescue group also showed lower infection burden on all days as compared to mice on a deficient diet. The ability to rescue a cohort of deficient mice with an IP injection of 3H-25D underscores the causality of vitamin D deficiency in increasing post-arthroplasty infection, and perhaps more importantly, its modifiability. This is the first evidence which suggests that a period of perioperative supplementation of vitamin D may decrease infection rates.

PS63 "Smart" Coatings: A Novel Implant Coating to Deliver Antibiotics through An Active Trigger Mechanism in a Spine Infection Mouse Model

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Spinal implant infections are clinically and economically devastating. Current methods of local antibiotic delivery are short lived or via passive release from biologic suboptimal vehicles. The goal was to use an in vivo mouse model of spinal implant infection to develop a biodegradable, "smart" polyethylene glycol (PEG) coating that would actively release antibiotics when challenged with bacteria. The polyethylene glycol-polypropylene sulfide (PEG-PPS) vehicle coating delivers antibiotic through passive elution at above minimum inhibitory concentration (MIC) of *Staphylococcus aureus*. We then modified the coating to increase release of antibiotics in response to lowering pH, a product of reactive oxygen species-driven tissue change to infection. We employed an established mouse model of postoperative spinal implant infection, in which 18 lysEGFP mice, which have green-fluorescent myeloid cells, were subjected to survival surgery. Subjects were randomized to receive a PEG-PPS, vancomycin PEG-PPS, or tigecycline-PEG-PPS coated implant press fit into the L4 spinous process and placed longitudinally along the posterior elements. The implants were inoculated with $1x10^3$ colony forming units (CFU) of bioluminescent *Staphylococcus aureus* and bacterial burden and inflammation were tracked longitudinally using bioluminescence and fluorescence imaging up to postoperative day (POD) 21. Ex vivo CFU enumeration was evaluated on POD 21. Bioluminescent imaging results showed significantly lower bacterial signal throughout the entire postoperative period for the vancomycin group ($P < 0.05$) and on POD 1,5,7, and 10 for the tigecycline group. In peri-implant tissue, tigecycline and vancomycin resulted in a marked reduction of CFU when compared to the PEG-PPS group. The antibiotic-eluting coated implants markedly reduced the infection-induced neutrophil recruitment and inflammation in a concentration- and elution-dependent fashion showing that PEG-PPS is an optimal vehicle to deliver antibiotics in the setting of spinal implants. The vancomycin impregnated PEG-PPS coatings prevented implant colonization by bacteria completely. Antibiotic linked implant coatings represent a promising approach to preventing periprosthetic infections.

PS64 Microfluidic Alternatives to Animal Research

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Drug discovery demands the use of high throughput systems in order to handle a large number of biologic samples and chemical combinations. The long conventional process requires the use of animal models for drug candidate efficacy and toxicity evaluation. This process comes at a great monetary cost as many drug candidates tend to pass animal testing only to fail in integral human clinical trials. To minimize costs associated with drug discovery and evaluation, animal use can be decreased by employing animal model alternatives like microfluidics or lab-on-a-chip technology, the science and technology of systems that utilizes small (10^{-9} to 10^{-18} liters) volumes of liquids using channels only micrometers in size. It is a fast growing research area that shows promise in handling large numbers of samples in parallel. Microfluidic devices have been shown to isolate, purify, and manipulate chemicals, cells, and whole organisms. The techniques have been applied to the miniaturization of current gold standard microbiological assays and have mimicked disease states as long-term, functional cell-based models such as tumors models. With further development to overcome barriers of adoption in mainstream biomedical research, microfluidics and lab-on-a-chip based in vitro and in vivo models could provide better models of the human system than current animal-based models. Overall, considerations of alternatives to animal use needs to be performed for any IACUC protocol application and researchers should strive to find alternatives in the form of replacement as part of the 3R principles.

PS65 Antioxidant Therapies for Ulcerative Dermatitis: A Potential Model for Skin-Picking Disorder

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Skin-picking disorder affects 4% of the general population, with serious quality of life impacts and potentially life-threatening complications. Standard psychoactive medications do not help most patients. Similarly, mouse ulcerative dermatitis (skin lesions caused by excessive abnormal grooming behavior) is very common in widely used inbred strains of mice, and represents a serious animal welfare issue and cause of mortality. Treatment options for ulcerative dermatitis are largely palliative and ineffective. We have proposed mouse ulcerative dermatitis as a model for human skin-picking disorder based on similar epidemiology, behavior, and its comorbidity and mechanistic overlap with hair pulling (trichotillomania). We predicted that mouse ulcerative dermatitis would be treated by

N-Acetylcysteine, as this compound is highly effective in treating both skin-picking disorder and trichotillomania. Furthermore, we hypothesized that N-Acetylcysteine's mode of action is as a precursor to the production of the endogenous antioxidant glutathione in the brain, and therefore intranasal glutathione would also treat ulcerative dermatitis. Accordingly, we show in a heterogenous prospective trial, the significant reduction in ulcerative dermatitis lesion severity in mice receiving either N-acetylcysteine (oral administration) or glutathione (intranasal). The majority of mice treated with N-acetylcysteine improved slowly throughout the course of the study. Roughly half of the mice treated with glutathione showed complete resolution of lesion within 2-4 weeks, while the remainder did not respond. These findings are the first to show that the use of N-acetylcysteine and glutathione can be curative for mouse ulcerative dermatitis. These findings lend additional support for mouse ulcerative dermatitis as a model of skin-picking disorder and also support oxidative stress and glutathione synthesis as the mechanism of action for these compounds. As N-acetylcysteine is poorly tolerated by many patients, intranasal glutathione warrants further study as potential therapy in skin picking, trichotillomania, and other body-focused repetitive behavior disorders.

PS66 Use of Novel Automated Feeders to Control Obesity of Socially Housed Rhesus Macaques

GLAS: Yes

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The high prevalence of obesity in captive nonhuman primate (NHP) colonies has generated interest in new feeding systems that monitor individual food intake in a social setting. Recently, an innovative automated computer-controlled feeding system was developed for research purposes that reliably records individual calorie consumption in socially-housed rhesus macaques (*Macaca mulatta*) by means of detecting unique radio frequency identification (RFID) microchips implanted in each hand. Via these RFID microchips, the system has the ability to limit individual food availability, thereby identifying a possible method to treat obesity in socially housed monkeys. In addition, animals eat what they obtain, suggesting that use of this system may lead to less food wastage compared to a traditional bin system. Using rhesus macaques (*Macaca mulatta*) housed in large breeding groups, the primary aims of this study were to compare food wastage associated with automated feeders to traditional bin feeders and to determine if automated feeder enabled caloric restriction (CR) is a feasible method to reduce adiposity without inducing food competition. Food waste was collected in 2 compounds using either bins or automated feeders. Based on dry weight, animals feeding from bins discard 29% of total food offered, while animals using automated feeders waste only 3%. Due to marked hyperphagia noted at baseline, 16 overweight adult female macaques were only allowed to consume up to 60% of their baseline calorie intake. Consequently, CR females experienced a safe reduction in adiposity over 12 weeks compared to baseline and free-feeding controls. Behavioral observations revealed that obese female macaques do not displace or steal food from lower ranking animals while under CR. In addition, CR does not appear to increase anxiety-like behaviors in both high and low-ranking females. Together, these findings suggest that automated feeders offer several colony health and management benefits compared to traditional bin feeders.

PS67 Preventing the Perpetuation of *Corynebacterium bovis* Infections from Contaminated Patient-Derived Xenograft Tissue

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Human patient-derived xenograft (PDX) tumor use in cancer research has increased dramatically over the last several years. Immunodeficient strains of mice needed to propagate these tissues are very susceptible to opportunistic pathogens like *Corynebacterium bovis,* which can cause tumor growth perturbations and strain dependent morbidity and mortality. To successfully eliminate *C. bovis* from a large athymic nude mouse colony, a procedure was needed to safely passage PDX tumors maintained in infected donor mice to naïve recipient mice without *C. bovis* cross-contamination. To evaluate if the current PDX tumor harvest technique was effective at preventing *C. bovis* contamination of tumor tissue, 5 randomly selected cryopreserved PDX samples were evaluated for *C. bovis* DNA by qPCR. Results indicated a 20% (1/5) prevalence of contamination within the cryopreserved bank. To eliminate *C. bovis* contamination during tumor harvest for both cryopreservation and in vivo horizontal tumor passage, a standard operating procedure was developed in combination with retraining on aseptic surgical technique. Following implementation, representative PDX tissue collected from the first 17 harvested tumors were evaluated by qPCR for *C. bovis* DNA. All samples were negative. To date, we have successfully transferred 56 unique PDX tumors from confirmed *C. bovis* positive nude mice into hundreds of naïve recipient mice without horizontal transmission of *C. bovis*. However, in 2 separate cases we have documented the horizontal transmission of *C. bovis* through contaminated, cryopreserved PDX tissue harvested prior to implementing aseptic training and the new harvest procedure. These tissues were implicated after ruling out other routes of cross-contamination and after qPCR analysis of identically cryopreserved, paired tumor samples which confirmed the presence of *C. bovis* DNA among the tumor tissues. We conclude that the threat of *C. bovis* transmission lies within nonaseptically harvested, cryopreserved and in vivo horizontally passaged PDX tissue originating from *C. bovis* infected mice.

PS68 Chronic Gastritis and *Helicobacter* Species Identified in Sooty Magabeys (*Cercocebus atys)*

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Helicobacter pylori is a gram-negative microaerophilic bacterium residing in the stomach and duodenum of humans. This microorganism is known to cause chronic gastritis, peptic ulcers, and gastric carcinoma. While *H. pylori* prevalence and pathogenesis has been well characterized in humans, relatively little is known about spontaneous disease, including gastritis and gastric carcinoma caused by gastric *Helicobacter* spp. (*H.* spp.) in sooty mangabeys. We previously reported a high incidence of spontaneous gastric carcinoma in captive sooty mangabeys at the Yerkes National Primate Research Center. In addition, we previously reported that this colony had 70% prevalence for gastric *H.* spp. infection. Given gastritis is a precursor for gastric carcinoma, we performed a retrospective analysis on formalin-fixed paraffin-embedded stomach tissue of mangabeys to determine the relationship between gastric *H.* spp. and chronic gastritis. Of these, 17 mangabeys had chronic gastritis. Four nongastritis clinical cases were used as negative controls for detection of *H.* spp. Of the 17 gastritis cases, 6 also had gastric adenocarcinoma. Hematoxylin and eosin stain and immunohistochemistry (IHC) using a commercially available *H. pylori* test kit was performed on all the tissue samples. Of the 17 chronic gastritis cases, 12 tested positive for *H. spp.* by IHC. Of the animals tested 8/9

females (89%) and 4/8 males (50%) were *H.* spp. positive. Three of 6 (50%) gastric carcinoma cases were positive for *H.* spp. by IHC. Fluorescent in situ hybridization confirmed *H.* spp. infection in 4/6 (67%) animals with gastric carcinoma. Animals lacking histologic evidence of gastritis showed none to rare *H.* spp. by IHC. Speciation of *Helicobacter* infection was performed by fluorescent in situ hybridization. Electron microscopy was also performed to visualize large non-*H. pylori* spiral gastric bacteria. This data supports our contention that the *H. pylori* test kit cross reacts with gastric non-*H. pylori Helicobacter* spp. These results suggest a role for *H.* spp. in the development of chronic gastritis and gastric carcinoma in sooty mangabeys.

PS69 *Bordetella hinzii*: A Confounding Organism in Murine Models of Pulmonary Disease

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An investigative group studying acute lung injury observed increased numbers of polymorphonuclear (PMN) cells in the bronchoalveolar lavage (BAL) fluid of mice. Bronchoalveolar lavage was performed and lung samples were collected sterily from five C57BL/6 mice that had been bred inhouse. Pure colonies of *Bordetella hinzii* were cultured from 2 of the 5 mice and these 2 mice had the highest PMN percentages (21% and 26%) in the BAL fluid. Ten mice were selected from the investigator's colony to determine the best antemortem test to screen for *B. hinzii* in the facility. A BAL was performed, the left lung lobe was collected for culture and PCR, the right lungs and nasal passages were collected for histology, an oral swab was collected for culture, and an oral swab and fecal pellets were collected for PCR. Eight of the 10 mice had *B. hinzii* cultured from the oral cavity, lung, or both. All 8 of these mice also had fecal pellets that were PCR positive for *B. hinzii*. Seven of the 8 mice had increased BAL PMN percentages (greater than 4% and some as high as 20%) and the eighth mouse had a normal PMN percent (2%). Histology demonstrated no abnormal pulmonary changes, but infected mice had mild to moderate rhinitis. Colony wide surveillance was performed by pooling fecal pellets from each mouse room and *B. hinzii* was found to be endemic. *Bordetella hinzii* appears to be an emerging microbial agent in mouse colonies that can confound pulmonary research. Institutions with a pulmonary research focus should consider colony screening for *B. hinzii* and exclusion of this agent.

PS70 Preventing the Spread of *Corynebacterium bovis* in Nude Mouse Colonies through Rapid Detection

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PCR evaluation of the exhaust air dust (EAD) from individually ventilated caging (IVC) racks is a sensitive method for the detection of *Corynebacterium bovis* in athymic nude mouse colonies. Weekly IVC rack EAD surveillance was initiated for 6 *C. bovis*-free nude mouse colonies established within facilities housing a larger number of infected colonies. In July 2014, 1 of 5 IVC racks in a *C. bovis*-free housing room tested *C. bovis* positive by EAD sampling after 11 months of infection-free status. This rack held 36 cages of athymic nude mice with unique human patient-derived xenograft tumors from an in vivo tumor bank. Following the initial discovery, all experimental manipulations were stopped and only essential husbandry procedures were performed while under quarantine. Within 72 hours of the initial and confirmatory positive tests, the positive rack was moved out of the *C. bovis*-free room into a housing room without immunodeficient mice. Once removed, all husbandry and research equipment were surface cleaned with a germicidal detergent then a chlorine dioxide disinfectant and all consumables, such as nitrile gloves and rodent feed, were purged from the *C. bovis*-free room. All cages from the positive rack were transferred to a new autoclaved rack. Twenty-four hours later, EAD samples from individual row exhaust air manifolds were tested for *C. bovis* by qPCR to localize the infection. Individual row sampling indicated 1 of 10 rows was positive. Following those results, individual cage sampling on the positive row revealed 1 of 7 cages was positive. The infected cage was removed, the remaining 35 cages were again transferred to a new autoclaved rack, and the rack was retested after 7 days along with all remaining racks in the *C. bovis*-free housing room. All racks tested negative for *C. bovis*, ending 19 days of quarantine. The 35 *C. bovis* negative cages were returned to the *C. bovis*-free room and all subsequent weekly rack surveillance samples have remained negative to date. We conclude that if *C. bovis* infected mice are discovered by a rapid surveillance technique and an aggressive quarantine is performed, focal decontamination can prevent the spread of infection.

PS71 Spontaneous Dilated Cardiomyopathy and Right-Sided Heart Failure in a Production Pig: Important Differential Diagnosis for Hepatosis Dietetica

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A 37.7 kg singly housed Yorkshire cross gilt was found dead unexpectedly 2 days after arrival in her indoor pen. Except for a 24-hour fast in preparation for a nonrecovery experimental surgical procedure, she was a naïve animal. No abnormalities were identified on veterinary intake exam or subsequent daily observation by husbandry and veterinary staff. Investigation into the production vendor's herd health and most recent feed analysis, necropsy and histopathology of the affected animal, and micronutrient analysis of formalin fixed liver were pursued. Necropsy revealed severe dilated cardiomyopathy characterized grossly by markedly dilated ventricles and thinned ventricular walls and interventricular septum. Histologically, there was multifocal myofiber attenuation and patchy loss of myofiber cross striations within the heart. In the liver, there was submassive to massive, diffuse hepatic centrilobular hemorrhage and degeneration. Micronutrient analysis revealed normal hepatic selenium levels, and the production facility had no recent herd health problems. The most recent feed analysis performed for the vendor was normal. The cause of death was determined to be due to dilated cardiomyopathy with right-sided heart failure and secondary hepatic degeneration due to marked acute passive congestion. The diagnosis of dilated cardiomyopathy and right-sided heart failure rather than hepatosis dietetica was supported by the gross cardiac chamber dilation, the absence of pathologic lesions typical of mulberry heart disease, the character of the hepatic lesions, and the normal hepatic selenium levels. To our knowledge, this case is the first report of spontaneous dilated cardiomyopathy in swine, and represents a potential diagnostic challenge in the differentiation of the liver lesion from hepatosis dietetica.

PS72 Comparison of Anesthetic Activity of Alfaxalone to Ketamine in Mice

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Alfaxalone encased in hydroxypropyl-beta-cyclodextrin (Alfaxan®), is a neuroactive steroid compound that has recently been FDA approved in the United States as a dog and cat anesthetic and is labelled as a Class IV controlled substance by the DEA. We performed a pilot evaluation in C57BL/6 mice and compared it against ketamine, both alone and in combination with xylazine, for intraperitoneal injection. Onset, duration of action, reflex responses, respiratory rate and clinical signs were recorded and analyzed. Time after drug injection until loss of righting reflex was used as the onset of action time. The immobilized mice were tested via tail pinch reflex, muscle tone of rear leg, toe pinch reflex and respiratory rate to determine depth of anesthesia. Alfaxan® at 80 mg/kg induced a light surgical plane of anesthesia in 100% of the mice, with an onset of 2.22±0.15 minutes and a duration of 57.11±3.77 minutes, whereas ketamine at 80 mg/kg did not provide a surgical plane of anesthesia, with an onset of 5.44±0.41 minutes and a duration of 6.89±0.82 minutes for the sedative effects. Clinically, Alfaxan® was noted to cause a spectrum of seizure-like activities including popcorn-like jumping movements after injection, intense scratching of the face, hyperresponsiveness to noise/touch even when recumbent, and significant limb jerking during recovery. The addition of 10 mg/kg of xylazine induced both the Alfaxan® and ketamine groups to reach a deep surgical plane of anesthesia, with mean durations of 80.29±17.8 and 37.43±8.2 minutes, respectively. Adding xylazine also ameliorated the adverse clinical signs seen with Alfaxan® alone. Female mice had a longer duration of anesthetic effect of Alfaxan® than males in both the single-dose and xylazine combination studies. Necropsy revealed that intraperitoneal injections of the product caused no apparent intra-abdominal effects in the mice, in spite of the post-injection reactions. From our preliminary analysis it appears that Alfaxan® may not be a viable single-agent option for mouse anesthesia, although further studies looking at different routes of administration or drug combinations may be warranted. Alfaxan® combined with xylazine appeared to be a more viable option, although mild seizure-like activity was still present in some mice and the long duration of action could be problematic in terms of body temperature maintenance and recovery monitoring, so these factors would need to be carefully considered before choosing this regimen.

PS73 Fibrosarcoma Associated with Long-Term Telemetry Implant in an Aged Sprague–Dawley Rat

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A 13-month-old male Sprague–Dawley rat that was a part of pharmacologic challenge studies influencing sleep/awake architecture presented with a rapidly growing subcutaneous mass on the dorsal neck and back (intrascapular). A telemetric transmitter using silicone elastomer leads for EEG and EMG recordings had been implanted subcutaneously between the scapulae 10 months prior to presentation. Due to the rapidly developing mass, the animal was euthanized and necropsied. The subcutaneous mass (7.4 x 2.5 x 3.0 cm) was lobulated, white, firm, and encapsulated. An impression smear showed a few scattered spindloid cells with large nuclei and multiple nucleoli. Histopathology showed partial encapsulation by compressed adjacent fibrous connective tissue and adipose tissue. Neoplastic cells were closely packed and arranged in interlacing bundles and streams of spindle cells separated by a fibrovascular stroma. Spindled cells range from 10-20 microns with large central vesicular nucleus and one to several basophilic nucleoli. The cells had indistinct cell borders and varying amounts of a fibrillar, eosinophilic cytoplasm. Marked anisokaryosis was evident along with multinucleated tumor cells. Mitoses averaged 3 per 40x field. Intratumoral hemorrhage and necrosis were noted. Tumor cells extended past margins of section submitted. Depending on the sections examined, there were 4 or 5 (2-4 mm diameter empty spaces) surrounded by fibrous connective tissue, tumor cells, and neovascular tissue. These spaces were consistent with the location of the lead wires. Trichrome stains of the mass revealed blue cytoplasmic staining of the neoplastic cells. These characteristics are consistent with a fibrosarcoma. The authors believe this is the first report of tumor development in a Sprague–Dawley rat associated with a chronically implanted telemetry unit.

PS74 Treatment Approaches and Clinical Parameters Predictive of Cytomegalovirus and Resolution in Immunodeficient Cynomolgus Macaques

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Cytomegalovirus (CMV) infection is a common complication in immunocompromised patients. CMV targets the gastrointestinal, respiratory, immune (bone marrow), and central nervous systems. We have studied the immunologic responses of CMV disease before and after treatment in immunodeficient Mauritian cynomolgus macaques (cynos) (n = 10). All underwent a bone marrow transplant (BMT) with a nonmyeloablative lymphodepleting preparatory regimen including irradiation and T cell depletion. Six animals received a 28-day course of cyclosporine A (CyA) and 4 received a rapamycin (RAPA). A CMV PCR was developed to monitor for CMV viremia. Immune reconstitution was monitored by immunophenotyping the peripheral blood. All animals developed CMV viremia between days 10 and 34 post-BMT. When viral counts surpassed 10,000 copies/mL clinical CMV symptoms were observed. Only ganciclovir (GCV) with a dose of 12.5mg/kg IV BID was able to resolve viremias. Two animals died of uncontrollable CMV disease. Valganciclovir and forcarnet alone were not protective. Successful resolution of CMV disease required early treatment before the viremias reached 10,000 copies/mL. CNS morbidity and mortality increased with viremias above 10,000 copies/mL. Long protracted use of antivirals induced BM suppression causing severe cytopenias which further delayed the recovery of protective T cell responses. CD4, CD8, and CD4CD8 double positive(DP) T cell counts under 250, 200 and 100/uL respectively were associated with CMV viremia (and disease). Recovery of CMV (without the requirement of antivirals) was achieved when CD4, CD8, and CD4CD8 DP cells were above 350, 250, and 100 cells/uL respectively. Unlike cyclosporine A, mTOR inhibitors (such as RAPA) protected lymphodepleted animals from CMV during the 28 days of treatment post-BMT. RAPA required levels to be maintained above 10ng/mL in the serum and CMV reactivated upon RAPA discontinuation. In conclusion, cyno CMV can be reliably induced with lymphodepleting strategies in 100% of carriers. Like humans, T cell responses are required to clear the infection. Furthermore, GCV is protective albeit at high (bone marrow suppressive) doses. We also demonstrated that CMV viremias can be prevented with RAPA during the most vulnerable lymphopenic phase.

PS75 The Effect of 2 Different Housing Systems on Germ-Free Mice Colonized with a Complex Gut Microbiota

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Translational animal models are essential prerequisites in exploring functions and causality of the microbiome in human health and disease. Animal models targeted at microbiome research can be germ-free mice inoculated either with a monoculture or with defined (gnotobiotic) or undefined bacterial communities of varying complexity. Traditionally, gnotobiotic mice are housed in isolators, which is costly both in labor and footprint. With rigorous cage handling procedures, it is possible to maintain mice germ-free in

individually ventilated cages (IVCs) for shorter periods of weeks or a few months, but there is a lack of knowledge on the stability of complex bacterial communities in IVCs. Germ-free SW mice were inoculated with a complex murine microbiota, housed in an isolator or in IVCs, and bred for two generations, corresponding to a time course of 5 months. The gut microbiota was characterized by 16S ribosomal RNA sequencing, and the community structure of the different generations was compared to the inoculum to see the effect of housing and time on the relative bacterial abundances and the appearance of contaminants and their ability to change the overall community picture. The results indicate that the stability over time is as good in IVCs as in the isolator, but that both the isolator housed mice and IVC mice differ slightly from the inoculum. The possibility of keeping a complex microbiota stable over time without using strict gnotobiotic techniques is discussed.

PS76 Preventative Drinking Valve Refurbishment Reduces Cage Flood Rates in the BSL-2 Animal Facility

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Automatic watering systems have allowed research facilities to house a substantially larger number of animals as they are much less labor-intensive than conventional water bottles. However, these systems must be carefully monitored in order to prevent cages from flooding with water. This is especially true in BSL-2 facilities that regularly autoclave automatic drinking valves, as valves have been shown to degrade over time when exposed to the harsh temperatures, pressure, and steam in the autoclave. In order to prevent cage floods associated with drinking valve failure, we have implemented an ongoing refurbishment and replacement process in our facility. Failing drinking valves are defined as those which 1) allow water to pass through when not actively actuated by the animals and 2) have undergone more than the recommended number of autoclave cycles as defined by the manufacturer. Once identified, failing valves are sorted by date of manufacture and returned to the vendor for refurbishment. When refurbished valves arrive from the vendor, they are mounted back onto cages and returned to circulation. By using this method we have decreased cage flooding rates to 1.58% in our facility and expect this figure to approach the manufacturer valve failure rate of 0.1% as we monitor census and cage flooding data. Preliminary results suggest that preventative drinking valve refurbishment is an effective method of reducing cage flood rates, particularly in BSL-2 facilities relying primarily on autoclave sterilization.

PS77 Renovating Cage Wash: Strategies to Minimize the Impact on Research, Staff, and Resources

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Cage wash renovations present a variety of operational challenges that can significantly impact many facets of an institution's research activities. Projects of such scale require the logistical coordination of equipment, machines, and people to avoid the interruption of valuable research. Vanderbilt University recently completed a 120-day cage wash renovation project that included the complete structural and mechanical renovation of a large cage wash facility. Several strategies and tools were implemented during the planning, preparation, and execution phases to minimize ineffective utilization of resources, unpredictable work flows, unreliable inventory levels, and added stress to divisional and research staff. During the planning phase, facility profiles that outline cage, bedding, and feed types were developed to help managers identify how and where each type of equipment would need to be processed and assembled. Daily cage counts were recorded to create daily throughput profiles for each affected facility. Combining these profiles with high, medium, and low throughput rates for each cage type for each machine allowed the facility manager to create best, expected, and worst case scenarios for the future state based on machine run times. These run times were used to create Gantt charts that represent the predicted cage wash operational requirements and schedules. This information was communicated to all affected work groups to coordinate staffing, alternative work flows, and operational support. The communication of reliable expectations to operations and research staff in advance of major change effectively reduced anxiety and helped to ensure consistent and reliable operations while adhering to procedural and regulatory standards. By combining basic data analysis, process modeling, and effective communication, facility managers were also able to generate confidence and the critical buy-in necessary across all affected work groups to implement major process change while maintaining the integrity of all research being conducted. This model could be implemented across facilities and institutions to minimize the impact of cage wash renovations on research and related activities.

PS6 Toys on Chains Encourages Swine Enrichment Gains

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Enrichment is an integral part of any research animal environment, but is especially important for animals as intelligent and playful as swine. We house our animals on elevated, perforated flooring, rendering us unable to provide straw which is traditionally used as bedding but also a main source of enrichment. While the pigs do enjoy the hard rubber or plastic toys we provide, complications inevitably arise. First, these toys must not be too small for the larger pigs to choke on them or be able to fit down the drain, but also not too big that the smaller pigs are unable to participate. Second, once the toy inevitably makes its way into the pile of feces it is immediately no longer desirable. Third, our animals often root the toys under their J-feeders and are then unable to play until it is physically moved by one of the technicians. For swine, it is necessary for enrichment devices to emulate their natural behaviours, such as the desire to root, or they may turn to tail or ear biting out of desperation. Our animals include various breeds of pig ranging in age from a couple weeks to up to five years old, leading to the need to entertain animals of wildly different sizes and mental states. To address these problems and provide more enjoyable, longer-lasting enrichment opportunities, we took our existing devices, balls and rubber rings, and suspended them nose-height with stainless steel chains. By hanging the toy from a chain, the size is no longer an issue as the toy is stationary and cannot fall into the drain or be choked on. The chain keeps the object elevated and out of feces and hard-to-reach places, allowing it to be enjoyed constantly. Additionally, the animals tend to interact with the toy more once it was elevated. Observations were even made of swine manipulating the toys in novel ways such as playing tug-of-war, something we never saw while the toy was on the floor. By carefully adjusting how the toy is secured to the chain, we have minimized the risk of potential issues arising from animals getting body parts stuck in loops. Although we have not witnessed this situation, we continue to monitor for the animals' safety. Overall, we found hanging toys to be a successful and easy improvement to our swine enrichment program.

PS79 Restructuring to Create a Flexible Workforce in the Husbandry Section

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Facility managers are often asked to do more with less. What do you do when the animal population decreases? Can restructuring units

help bring in additional revenue? Our goal was to create a stronger more diversified workforce that would enable the department to expand services. To do this, goals and duties were clearly delineated. Using the Kotter model, a team was assembled to lead change. Using information from staff, management, and researchers, goals and specific job duties were defined. Managers were tasked with figuring out what workforce was needed to perform all necessary husbandry tasks at each facility. Using this information, an organizational structure was created in which each facility was fully staffed to perform all husbandry duties and additional services were created.

PS80 Animal Room Light Exposure at Night Disrupts Melatonin Circadian Regulatory Dynamics in a New Tissue-Isolated Human Prostate Tumor Xenograft Model in Nude Rats

GLAS: Yes
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Controlled lighting and lighting protocols in animal facilities have long been a concern to both biomedical scientists and animal care personnel. Exposure to light at night (LAN) of sufficient intensity, wavelength, and duration suppresses pineal circadian neurohormone melatonin that influences normal and neoplastic tissue metabolism and physiology. Previously, our laboratory demonstrated that animal room dark phase "light contamination" with as little as 0.2 lux suppressed production of melatonin and stimulated human breast tumor growth and metabolism. This occurs via an MT_1 melatonin receptor-mediated signal transduction pathway resulting in elevated linoleic acid (LA) uptake and conversion to the mitogeneic metabolite 13-hydroxyoctadecadienoic acid (13-HODE), and increased aerobic glycolysis (Warburg Effect). We developed a new tissue-isolated VCaP castration-resistant prostate tumor model in adult male (230 g) athymic nude rats (Hsd:RH-*Fox*^{rnu}) and tested whether nocturnal melatonin level suppresses, while dim LAN (dLAN) stimulates, tumor growth activity. VCaP xenograft-bearing rats (n = 6/group) maintained on either a control LD 12:12 (300 lux; 123 µW/cm^2 light phase intensity) or an experimental LD 12:12dLAN (0.2 lux; 0.08 µW/cm^2 dark phase intensity) in an AAALAC-accredited facility for 8 weeks resulted in a 2-fold decrease in latency-to-onset and over a 2-fold increase in tumor growth rates in experimental, compared to control, animals. In control animals, plasma melatonin levels were high in the mid-dark phase (183.4 ± 12.8 pg/mL) and low (2.2 ± 0.4 pg/mL) in mid-light phase, and low throughout the 24-hr period in experimental animals. Tumors harvested during the mid-dark (2400 h) phases revealed cAMP levels, LA uptake, 13-HODE production, Warburg Effect, and DNA [^{3}H]thymidine incorporation were significantly elevated ($P < 0.001$) in dLAN as compared to the control group during dark phase. Signaling pathways (AKT, MEK, ERK ½, STAT3, GSK3ß, and NFκß) were activated in dLAN experimental tumors as compared to controls. These findings demonstrate that LAN, as sometimes occurs in animal facilities, inhibits the nocturnal melatonin signal, thereby increasing LA metabolism, Warburg Effect, and growth activity in VCaP human prostate cancer in vivo.

PS81 A Comparative Analysis of Cage Processing that Focuses on Maximizing Staff Resources and Operational Throughput

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The cage processing or cagewash area is often called the heart of the animal facility. It is vitally important to ensure that cages are washed, assembled, and adequately sterilized in an efficient manner. Recently the Center for Comparative Medicine at Massachusetts General Hospital evaluated the 30-year-old equipment in one of the multispecies facilities. A decision was needed to either replace the equipment (dump station, rack washer, and cabinet washer) with updated versions of the same or to select new equipment with the goal of maximizing throughput and efficiency. We mapped out the current process of cleaning a cage using these devices: considering the number of steps taken to clean a dirty cage, and how long it took for a cage to complete the overall process. We researched alternatives, including the inclusion of a tunnel washer, and similarly mapped out the processes using that equipment. Through this comparison, we found we should be able to reduce both the overall processing time and number of steps by 50%. A tunnel washer with an integrated dumping station and a new rack washer was installed. After installation, we again mapped out our process flow to evaluate the actual impact of the new equipment. We found that we were able to reduce our number of steps by over 50%, from 9 steps down to 4 while increasing throughput of cages by 75% with the tunnel washer. In conclusion, the new equipment for the washing of rodent cages cuts back on time needed to process the cages and on how many steps staff members have to perform in completing this work.

PS82 Investigation of an In-Line Filter Manifold to Simplify and Improve PCR Detection of Rodent Pathogens on an IVC Rack

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Exhaust air dust (EAD) collected from the plenums of IVC racks is becoming a common PCR sample type for the routine monitoring of pathogens on IVC racks. EAD PCR testing of IVC racks without cage-level filtration provides the advantage of improved pathogen detection and elimination of sentinels and associated labor and maintenance cost. In an attempt to simply the process of EAD testing on an IVC rack that does not use cage level filtration, a prototype manifold was designed to place a filter in the exhaust air stream of the vertical plenum with the intent of being able to use a single sample type for PCR and provide a mechanism for easy filter replacement. Eight pet shop mice (2 mice/cage), were used to simulate a low prevalence infection and were predetermined to be infected with 20 infectious agents. Five percent soiled bedding was provided to 4 sentinel mouse cages (3 SOPF CD-1 mice/cage). One sentinel mouse per cage was evaluated monthly by PCR and traditional screening methods. EAD collected by pooled plenum and hose swabs, sentinel cage filter and the in-line filter were evaluated at 3 months by PCR. At 3 months, direct sentinel screening (combined PCR and serology) detected or partially detected 6 agents. Sentinel filter EAD PCR testing detected or partially detected 17 agents. Plenum and hose EAD (combined) samples detected 15 agents. The in-line filter detected 19 agents. Our investigation demonstrated that EAD PCR analysis of the filter placed in the manifold detected agents not found in sentinel mice by PCR or traditional methods. Additionally, the filter also demonstrated higher nucleic acid copy numbers than the standard adhesive swab technique used to collect EAD from horizontal plenums.

PS83 Persistance of Murine Norovirus RNA after Cleaning and Dry Heat Sterilization of the Housing Rack

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As environmental testing for rodent pathogens through use of PCR-based methods becomes increasingly integrated into rodent disease surveillance programs, new questions arise about the efficacy of cleaning practices in the elimination of pathogen RNA from testing surfaces. While decontamination processes may inactivate these pathogens, residual nucleic acid on the test surface can generate positive PCR test results. To evaluate the efficacy of our rack sanitation practices at eliminating detectable nucleic acid contaminants, ventilated mouse rack exhaust plenums were swabbed for PCR detection of murine norovirus (MNV). Swabbed plenum samples were taken prior to cleaning in a commercial rack washer,

after passage through the rack washer, and again after final processing through our dry heat bulk sterilizers. Results of testing showed that 10/11 (91%) racks positive for MNV prior to washing were negative after washing. One of eleven racks tested positive for MNV prior to washing remained positive after washing. However, the rack tested negative for MNV after dry heat sterilization. This latter finding led to a secondary question of whether use of the dry heat oven alone can effectively destroy viral nucleic acid so that PCR testing returns negative. Subsequently, 5 additional MNV-positive racks were sent straight through the dry heat oven without first passing through the rack washer. Four of five (80%) racks remained PCR positive for MNV after dry heat sterilization. These results reinforce the necessity of reliable mechanical cleaning to remove all sources of viral nucleic acid on testing surfaces. Sterilization through use of dry heat ovens is not effective at altering MNV nucleic acid to a level undetected by PCR. Furthermore, mechanical cleaning is not always failsafe. Therefore, validation of all cleaning processes should be integrated into routine practices to assure that these types of positive PCR test results are not a confounder in an environmental rodent pathogen monitoring program.

PS84 Physiologic Response of C57BL/6 Mice to Different CO_2 Euthanasia Chamber Displacement Rates

GLAS: Yes
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Rodent euthanasia with CO_2 using a gradual displacement rate between 10% to 30% volume/minute is considered acceptable with conditions by the AVMA. Higher flow rates are not acceptable. However, there is no information whether there is a difference in the pain and distress between the different chamber displacement rates. We therefore examined the effect of different CO_2 chamber displacement rates on physiologic, histologic, and behavioral parameters of mice undergoing CO_2 euthanasia. Male C57BL/6 mice were implanted with telemetry probes and euthanized at least 1 week later with 15%, 30%, 50%, or 100% volume/minute CO_2 displacement rates. Heart rate, systolic, mean and diastolic blood pressure, total activity, behavioral responses, and lung histologic changes were analyzed. As expected, mice died significantly faster in the higher CO_2 displacement rates. The total increased heart rate above baseline was significantly increased in the 15% and 30% displacement rate groups compared with the 50% and 100% displacement rates. There was no significant difference in amount of activity or any other cardiovascular parameter. There was no pawing of the face, burying of the nose, breath holding, jumping or any other evidence of pain exhibited by any of the mice except for labored breathing and gasping. Histologic analysis of the incidence and severity of pulmonary perivascular edema, congestion, and alveolar hemorrhage revealed very mild or mild changes in 77% of the lungs. However, there were no significant differences between the 4 groups. Based on these findings there is no evidence that a slow CO_2 chamber replacement rate is better than a fast CO_2 chamber replacement rate.

PS85 Investigations of Murine Astrovirus: Genetic Diversity, Prevalence, and Pathogenesis

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Limited publications exist for mouse astrovirus (MuAst) first reported in mice in 2011. Early reports suggest high prevalence among commercially available mice, including immunodeficient strains B6.Rag1$^{-/-}$, Rag2$^{-/-}$, and NSG, as well as transgenic and nude mice in research facilities. Although astrovirus in other hosts is associated with enteric disease, no disease or research impact has been reported or observed for MuAst. Partial sequencing of the RNA–dependent RNA polymerase gene demonstrated up to 8% genetic diversity for 40 field samples. A conserved region was used to develop a real-time reverse transcriptase PCR (RT-PCR) with which a 44% prevalence was determined for ~8,000 field samples. In endemically infected colonies, fecal genome copies were ~ 10^{5-6} /mg in 3-10 week-old mice, but was lower or absent in six 12 month-old mice. No gross or histologic changes were observed in tissues (brain, lung, heart, liver, spleen, kidneys, GI tract) from NOD-SCID at 2 weeks postinoculation (PI) with genome copies ~10^{5-6}/mg in feces and ileum. Four C57BL/6N and 4 CD-1 mice were evaluated per time point by ileum and fecal RT-PCR and histology (same tissue set). Time points included 3, 7, 10, 14, 21, 28, 35, and 259 days postinoculation with additional fecal RT-PCR at 3-4-week intervals. Soiled bedding (20%) transmission to sentinels occurred by 5 weeks; however, no histologic changes were observed. Peak shedding (10^{5-6} and 10^{6-7} genome copies/mg feces in C57 and CD-1, respectively) occurred 3-10 days PI and waned slowly with intermittent positives appearing beginning at 98 days. The high prevalence among research and commercial colonies and lack of evidence of a histologic changes should be factors in determining the relevance of this virus.

PS86 Improving the Welfare of Estrogen-Responsive Murine Breast Cancer Models

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Breast cancer is the second most common cancer in the world and murine breast cancer models have been developed to better understand its biology, diagnostics, and treatment. Commonly, estrogen pellets are implanted into athymic mice to stimulate breast cancer cells growth, but this can lead to significant urinary side effects (urinary incontinence, struvite urotlithiasis) and high mortality in certain mouse strains. In order to refine this, we investigated translating common treatment regimes used in canine urolithiasis to a murine breast cancer model. Forty 8-week-old female BALB/c athymic mice were divided into 3 control groups (implanted with estrogen pellet only (E), provided urine acidifier only (A) or diet only (D)) and 3 treatment groups that all had estrogen pellets implanted. The treatment involved providing a urinary acidifier (1% ammonium chloride) in drinking water (EA), a commercially available diet for the control of canine urolithiasis (ED), or both (EAD). The mice were observed for clinical signs such as perineal ulceration and weight loss, and urinalysis and microscopy were performed. We observed that perineal ulcers presented earlier in mice in the E group compared to those in the treatment groups and that crystalluria appeared before ulcerations in 85.7 % of mice in the E group and 100% of mice in the EA group. Comparing survival between the E, A and the EA groups, a significant difference was found between the E and EA groups ($P < 0.01$), and the A and E groups ($P < 0.001$) while no significant difference was found between A and EA groups. It was also noted that enlarged bladders and a high urinary pH are strong indicators of imminent death and may be useful humane endpoints for such models. Early results into dietary management similarly showed a delay in clinical signs and reduced severity in the ED group but it was noted that 100% of mice in the EAD group demonstrated significant weight loss of >20% in the first week of treatment. In summary, treatment (with urinary acidifiers or diet) holds promise in improving the welfare of mice implanted with estrogen pellets. Further evaluation of the various treatment regimes will be required to determine the compatibility of such strategies with our research and welfare goals.

PS87 Low-Intensity Electromagnetic Fields and Animal Welfare: Clinical-Pathologic Safety Assessment in C57Bl/6N Mice

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The recent development of rodent cages equipped with sophisticated technologies using low-intensity electromagnetic fields (LEMF), in the range of 5 Hz to 100 Hz, (for the purposes of monitoring both the behavior/welfare of the animals and a number of cage environmental parameters) raises questions on the potential effects of LEMF on the animals, even though LEMF are generally considered to have low biologic activity. The aim of this study was to perform a long-term (up to one year), clinical-pathologic study of mice exposed to LEMF at very low intensities. Three-hundred twenty male and female C57Bl/6N mice were randomly divided into control and exposed groups on a single IVC rack. Throughout the experiment, body weight, water, and diet consumption were recorded at 14-day intervals. At sacrifice (programmed after 60, 120, 180, and 365 days of exposure) hematology, bone marrow analysis, and histology-pathology on major organs was performed. Preliminary results obtained after 60 days of exposure revealed no significant alterations of body weight, water, and diet consumption between exposed mice and the controls. Hematology, bone marrow, and histology were within normal limits. After 60 days of exposure, LEMF determined no relevant clinical-pathologic effects on mice.

PS88 Murine Norovirus Abrogates *Chlamydia pneumoniae* Accelerated Atherosclerosis in ApoE$^{-/-}$ Mice

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*Chlamydia pneumonia*e (*Cpn*), a common human respiratory pathogen, is associated with human cardiovascular disease and acceleration of atherosclerosis in hyperlipidemic animal models. Our laboratory demonstrated recently that murine norovirus (MNV), a highly prevalent infection of laboratory mice, variably alters atherosclerosis in hyperlipidemic B6.129S7-*Ldlr*^{tm1Her}/J (Ldlr$^{-/-}$) and B6.129P2-*Apoe*^{tm1Unc}/J (ApoE$^{-/-}$) mice. Given that MNV has a tropism for macrophages and may exacerbate atherogenesis, we investigated whether coinfection with MNV and *Cpn* might alter macrophage phenotypes in vitro and atherosclerosis in ApoE$^{-/-}$ mice. In the presence of oxidized low-density lipoprotein, MNV/*Cpn* coinfection of ApoE$^{-/-}$ bone marrow derived macrophages (BMDM) resulted in significant increases in gene expression of IL-6, MCP-1, iNOS, and TNF-α ($P \leq 0.001$) as compared with *Cpn* monoinfected BMDM. Based on these findings, we hypothesized that concurrent MNV/*Cpn* coinfection might increase plaque lesion size in vivo. As expected, for ApoE$^{-/-}$ mice, *Cpn* infection alone significantly increased mean plaque size by 64% ($P = 0.04$) as compared with uninfected mice. Surprisingly, MNV/*Cpn* coinfection was associated with a decrease in mean plaque size of 54% ($P = 0.057$) as compared to *Cpn* monoinfected mice. Mechanisms by which MNV might mediate the effect of *Cpn* were investigated. There were no differences in aortic cytokines locally at the site of plaque development, or in peritoneal macrophages 1 week following infection in MNV/*Cpn* coinfected mice as compared to *Cpn* monoinfected mice. MNV was not detected in the aortic tissues of MNV infected mice at 1 or 8 weeks postinfection regardless of *Cpn* status. These data suggest that MNV infection can abrogate the effect of *Cpn* on atherogenesis and that this effect may be through viral modulation of systemic responses rather than through local induction of inflammatory cytokines in the aorta. Moreover, these findings demonstrate that MNV infection may confound studies of other pathogens in ApoE$^{-/-}$ mice.

PS89 MNV Infection Exacerbates Microbiota Driven Intestinal Inflammation in the IL10-deficient Mouse Model of IBD

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Inflammatory bowel disease (IBD) is a multifactorial chronic inflammatory disorder of the gastrointestinal tract. The disease development is driven by a combination of diverse factors such as host genetic susceptibility, inappropriate immune response towards enteric microbiota and environmental factors. The disruption of the intestinal barrier is thought to be a potent trigger of IBD development. However, the exact mechanisms underlying the inflammation development are not well understood yet. We used murine norovirus (MNV) infection to analyze structural and functional barrier changes in an IL10-deficient mouse model of IBD. B6.129P2-*Il10*^{tm1Cgn}/JZtm (B6-*Il10*$^{-/-}$) mice were inoculated with 2.5×10^8 $TCID_{50}$ of MNV. In situ RT-PCR was used to confirm presence of MNV infection in the intestine. 48 hours postinfection (p.i.) flux measurements were performed and expression of tight junction was assessed by qRT-PCR and immunhistology. Inflammatory markers were determined by qRT-PCR and staining for apoptosis by TUNEL assay was performed. MNV was found to be present in epithelial and lamina propria cells at 48 hours after infection. Changes in B6-*Il10*$^{-/-}$ mice induced by MNV infection included increased paracellular permeability indicated by increased mannitol flux, reduced expression of tight junctions and increased rate of epithelial apoptosis. In MNV infected germfree (GF) B6-*Il10*$^{-/-}$ mice, reduction of tight junction gene expression and inflammatory lesions were absent, whereas increased epithelial cell apoptosis was still present. Furthermore, GF B6-*Il10*$^{-/-}$ mice colonized with defined microflora developed similar inflammatory lesions as the MNV infected SPF IL10-deficient mice. Our data provide strong evidence that MNV infection causes epithelial barrier disruption in B6-*Il10*$^{-/-}$ mice. We also confirmed that this colitogenic influence largely depends on the intestinal microbiota. Thus, MNV might trigger mucosal inflammation in susceptible individuals by impairment of the intestinal barrier and contribute to the concept that exposure to virus infection may trigger IBD development in the susceptible host.

PS90 The Influence of Ferret Age on the Production of the Strain-Specific Influenza Antisera

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Seasonal influenza is a common respiratory infection affecting millions of people each year worldwide. Due to their susceptibility to influenza viruses and clinical presentation mimicking human infections, ferrets (*Mustela putorius furo*) are a preferred animal model for influenza studies and generation of strain-specific antiserum. Strain specific antisera are essential reagents for the Hemagglutination Inhibition (HI) assay, the gold standard test for monitoring antigenic changes among circulating influenza viruses. Traditionally, older ferrets (>2 yr) which may produce larger volume of sera were used. However, some older ferrets failed to generate strain-specific antisera with acceptable homologous titers. The rapid production of high quality, specific sera is a vital element of surveillance and vaccine selection programs. To refine the protocol for antisera production, we assessed the effect of ferret age on immune response to seasonal influenza. Seronegative, "young" (6 month to 1 year old) and "old" (>2 year old) male ferrets were inoculated with influenza viruses intranasally. For antisera to influenza B viruses, animals were boosted by subcutaneous injection near the footpad at 2 weeks postinfection if necessary. Viral shedding, antibody kinetics, clinical symptoms of infection (including fever, weight loss, sneezing, and lethargy), and animal health were monitored throughout the course of the study. We found a more consistent and robust antibody response among sera from the "young" animals group. Among the influenza B-infected animals, sera collected from animals 1 week post

boost yielded higher HI antibody titers than sera collected 2 weeks post boost in all ferrets tested. Reduction in time required to generate quality antisera to influenza B viruses coupled with use of animals <2 years of age will allow for refinement of the antisera production protocol. These findings help assure that optimized quality reagents are developed, reduce the number of animals utilized, and shorten the length of time ferrets are on study.

PS91 In Vivo Corneal Confocal Microscopy: A Novel, Noninvasive Imaging Technique to Evaluate Corneal Nerve Density in Rhesus and Pig-Tailed Macaques

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In vivo corneal confocal microscopy is an emerging technique currently used in human medicine for examination of corneal morphology in numerous disease states. Recently, in vivo confocal microscopy has been used to obtain quantitative data on corneal nerves to diagnose and monitor progression of diabetic and chemotherapy induced peripheral neuropathy. This noninvasive imaging modality, which uses laser scanning to produce images of the cornea at various depths, is replacing traditional invasive biopsy techniques for the diagnosis of peripheral neuropathy. Despite the widespread use of nonhuman primates as models of peripheral neuropathy, corneal confocal microscopy to assess corneal nerve morphology has not yet been employed in these species. Our study used in vivo confocal microscopy to obtain normative reference values for corneal nerve fiber density in healthy, adult rhesus and pig-tailed macaques, and is the first to establish an in vivo method for this purpose. Males and females of each species were divided into 4 groups, ranging from 3 to 18 years of age, for a total of 36 animals. Five images of the sub-basal corneal nerve plexus were obtained from each animal in a single imaging session. Corneal nerve fiber density was then measured using a previously validated and reproducible manual counting method. Mean corneal nerve fiber density for rhesus macaques was 9.90 nerves/mm^2 (range: 6.48-15.51 nerves/mm^2). Mean corneal nerve fiber density for pig-tailed macaques was 10.94 nerves/mm^2 (range: 8.35-13.80 nerves/mm^2). Our data revealed no significant difference in corneal nerve fiber density by age, sex, or species. In addition to the normative parameters established in this study, the introduction of this novel imaging modality in nonhuman primates has many future applications in studies of peripheral neuropathy, which to date have been limited to in vitro and invasive techniques. In vivo corneal confocal microscopy provides the ultimate advantage of obtaining longitudinal data from individual animals in a non-invasive manner, and thus embraces the principles of reduction, replacement, and refinement.

PS92 Persistent Neurologic Sequelae in a Mouse Model of Nonfatal Alphavirus Encephalomyelitis

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Recent outbreaks of encephalomyelitis caused by arthropod-borne alphaviruses reveal their importance as an emerging cause of significant human disease and disability. An outbreak of Venezuelan equine encephalitis in the mid 1990s in Venezuela and Columbia affected an estimated 75,000 to 100,000 people, and the number of human cases of eastern equine encephalitis in the northeastern United States has markedly increased in the last decade. Patients that recover from clinical disease, especially infants and children, are often left with lifelong debilitating neurologic defects, such as intellectual disability, impaired motor control, and emotional and behavioral disturbances. Sindbis virus (SINV), the prototypic alphavirus, provides a valuable model for studying alphavirus-induced encephalomyelitis. Previous studies have shown that infectious virus is cleared within 7-8 days, but viral RNA is cleared more slowly and persists in neurons at low levels for the life of the animal. We hypothesized that mice infected with a nonfatal strain of SINV would develop neurologic deficits measurable by behavior tests, and these deficits would persist beyond the period of active virus infection. Five-week-old C57BL/6 mice were intranasally inoculated with SINV or PBS control and underwent a battery of behavioral tests to assess neurocognitive function at different phases of infection. Following behavioral tests, brains were collected, and infectious virus titers, SINV RNA levels, and tissue pathology were assessed. At the height of active virus infection, characterized by peak infectious virus titers, SINV-infected mice demonstrated increased locomotor activity ($P < 0.01$) and decreased anxiety ($P < 0.01$) in open field testing and markedly impaired hippocampal-dependent memory in contextual and cued fear conditioning ($P < 0.0001$). Following recovery from clinical disease, SINV-infected mice continued to show memory deficits in contextual fear conditioning ($P < 0.05$) when only viral RNA persisted in the brain. These findings show that SINV induces long-term neurologic sequelae in mice that persist beyond active virus infection and correlate with viral RNA presence.

PS93 Bisphosphonate Targeted PET Imaging of Breast Cancer in an Aged Female, Retired Breeder, Sprague–Dawley Rat Model

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Early detection of breast cancer is paramount in improving chances of survival by allowing treatment before the cancer can progress. However, numerous recent studies indicate that the decrease in breast cancer mortality due to mammography is miniscule, and there is a serious need for an effective alternative method of early detection. The two major barriers to the progression of breast cancer screening are the lack of sensitive imaging modalities and the absence of effective small animal models to test them. In this study, we sought to validate a novel rat model of human breast cancer imaging as well as develop a breast specific PET imaging agent. Human breast tissue contains structures called microcalcifications, which are visualized with mammography and used in the diagnosis of cancer. We utilized the presence of these microcalcifications by targeting them with a class of drugs known as bisphosphonates, which have a high affinity for calcium crystals. We conjugated two bisphosphonates (Risedronate and Alendronate) to DOTA (1, 4, 7, 10-tetraazacyclododecane- 1, 4, 7, 10-tetraacetic acid) or desferoxamine then radio-labeled with copper (^{64}Cu) or zirconium (^{89}Zr), and performed PET imaging on aged, female, retired breeder, Sprague–Dawley rats. Much like humans, these rats have significantly developed thoracic mammary glands, which have undergone several cycles of pregnancy associated hypertrophy and atrophy, and show similar age related histologic changes and microcalcifications. These rats also develop spontaneous benign tumors, as well as NMU (*N*-Nitroso-*N*-methylurea) induced carcinomas which are similar to those seen in humans. In our model, we observed significant targeting of ^{64}Cu -Alendronate-DOTA to normal and tumor bearing rat mammary tissue. Bisphosphonates were also conjugated to carboxyfluorescein, and 1.4nm nano-gold for use in confocal and electron microscopy to validate microcalcification targeting specificity in our rat model. Our research shows that aged, female, retired breeder Sprague–Dawley rats are effective models for human breast cancer imaging research and bisphosphonates are useful molecules for targeting microcalcifications in breast tissue for use in imaging or therapy.